GLOBAL WATER RESEARCH COALITION

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Global Water Research Coalition

INTERNATIONAL GUIDANCE MANUAL FOR THE MANAGEMENT OF TOXIC CYANOBACTERIA

International Guidance Manual for the Management of Toxic Cyanobacteria

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GLOBAL WATER RESEARCH COALITION

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
- KWR – Watercycle Research Institute (Netherlands)
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These organisations have national research programs addressing different parts of the water cycle. They provide the impetus, credibility, and funding for the GWRC. Each member brings a unique set of skills and knowledge to the Coalition. Through its member organisations GWRC represents the interests and needs of 500 million consumers.

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Best Practice Guidance for Management of Cyanotoxins in Water Supplies. EU project "Barriers against cyanotoxins in drinking water" ("TOXIC" EVK1-CT-2002-00107)

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.

The manual is a user friendly document that can be accessed on several levels, from basic information for the water quality manager who knows very little about cyanobacteria, to those requiring more detailed guidance on, for example, source water management methods, or doses of activated carbon required to reduce toxin concentration to below the WHO guideline. It is hoped this manual will be accessed by water utilities world-wide, and feedback on its application will be used to update and implement revisions to maintain and enhance its usefulness to the international water industry.

HOW TO USE THE MANUAL

The manual has been developed to cover several levels of knowledge. Level 1 is designed to be read as a basic text to help the water manager, or any interested community member, understand the issues surrounding cyanobacteria and the reasoning behind various monitoring and management practices. This level can be downloaded from this package and printed as a stand-alone document if desired (Guidance Manual Level 1, left menu). The entire manual can be found in this package as seven separate chapters (left menu). In each of these chapters there are either two or three levels of information; Levels 2 and 3 are accessed through links in Level 1. Level 2 adds additional details to the basic information in Level 1, in some cases engineering aspects, some more fundamental information, or in Chapter 6 for example, specific details required to implement an alert levels framework as part of an overall cyanobacteria incident management plan. Chapter 3 has a third level, with more detailed information on analytical procedures.

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

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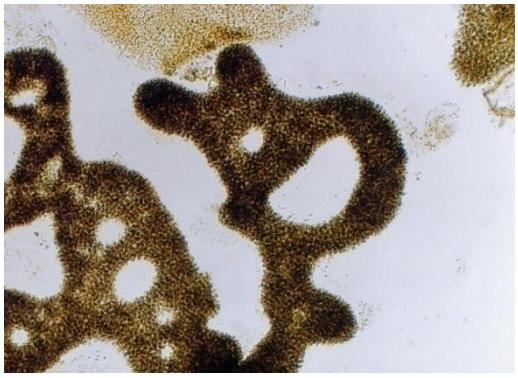
CHAPTER 1 INTRODUCTION (LEVEL 1)

CYANOBACTERIA

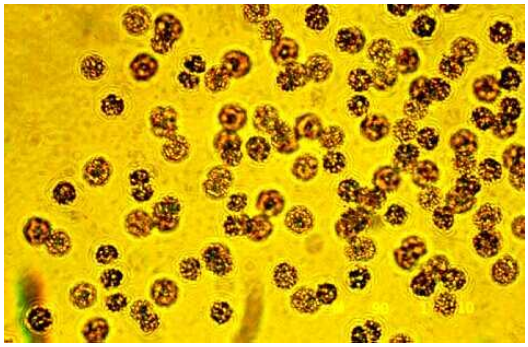
Cyanobacteria, also known as blue-green algae, blue-green bacteria or cyanophytes, are part of a primitive group of organisms which, according to fossil records, have existed for approximately 3.5 billion years [1, 2]. They are not true algae, they are gram-negative bacteria which contain chlorophyll 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

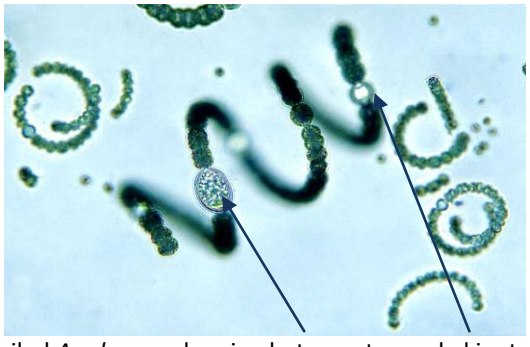
STRAIGHT FILAMENTS

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.

Follow this link for a list of potentially toxic cyanobacteria their toxins, and where they have been found

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.

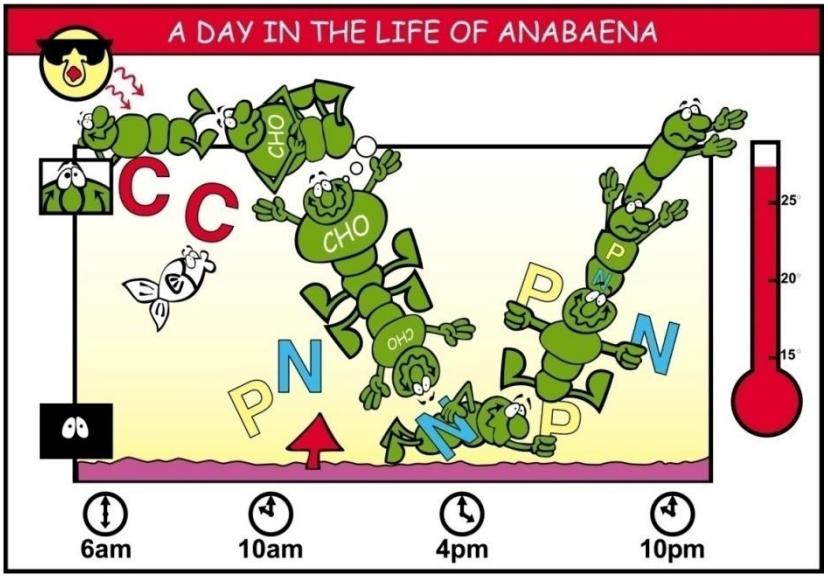


Figure 1-2 A stylised diagram of the daily cycle of buoyancy regulation and vertical migration in a lake by the cyanobacterium *Anabaena*

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.*

For more information on buoyancy regulation by cyanobacteria, click here

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.

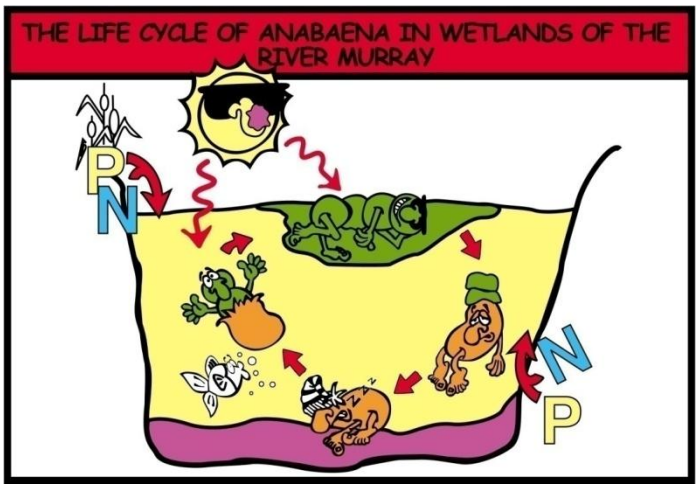


Figure 1-3 The typical life cycle of the cyanobacterium *Anabaena* showing akinete formation and germination

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 two to four cell divisions, which corresponds to a 4 - 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 those 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.

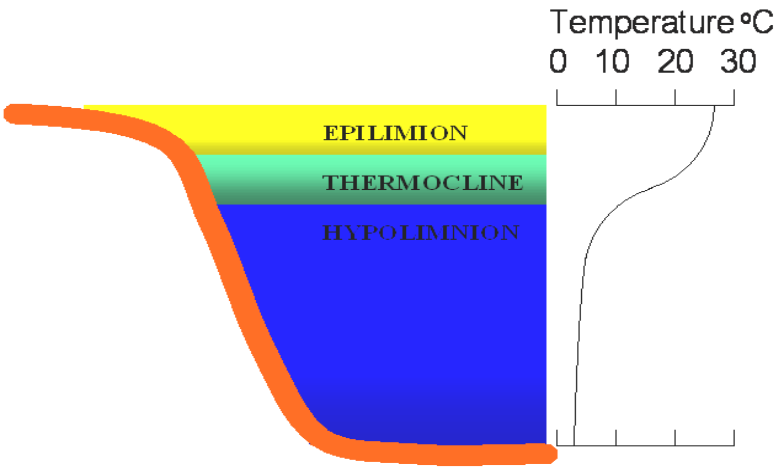


Figure 1-4 Cross section of a thermally stratified lake showing location of the epilimnion and hypolimnion and associated temperature changes.

For more information about stratification follow this link

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].

For more detailed information on the cyanotoxins follow these links:

Peptide hepatotoxins (microcystins and nodularin)

Neurotoxins

Cylindrospermopsin

β-N-methylamino-L-alanine (BMAA)

Lipopolysaccharide endotoxins

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]. Many 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

For some examples of toxicity of benthic cyanobacteria follow this link

For examples of adverse human health effects follow this link

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 Organization 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.

For a detailed summary of guidelines worldwide, and procedures for guideline derivation, go to:

International guidelines for cyanobacterial toxins and

Procedures for guideline derivation

CHAPTER 1 INTRODUCTION (LEVEL 2)

CYANOBACTERIA

POTENTIALLY TOXIC CYANOBACTERIA

Table 1-1(L2) Potentially toxic cyanobacteria, the toxins they can produce, and where they have been found to date.

Algal Species	Cyanotoxin	Location
Anabaena circinalis	Microcystins, Saxitoxins	France, Australia
Anabaena flos-aquae	Microcystins, Anatoxin-a	Canada, Norway
Anabaena lemmermanni, flos-aquae, circinalis	Microcystins, Anatoxin-a	Finland, Denmark
Anabaena spp.	Microcystins, Anatoxin-a	Egypt, Denmark, Finland, Germany, Ireland, Japan
Anabaena planktonica	Anatoxin-a	Italy
Anabaenopsis millerii	Microcystins	Greece
Aphanizomenon flos-aquae	Saxitoxin, Neosaxitoxin	USA
Aphanizomenon ovalisporum	Cylindrospermopsin	Israel, Australia
Aphanizomenon sp.	Anatoxin-a	Finland, Germany
Aphanocapsa cumulus	Microcystins	Brazil
Cylindrospermum	Anatoxin-a	Finland
Cylindrospermopsis raciborskii	Cylindrospermopsin, Saxitoxins	Australia, Brazil, Hungary
Haphalosiphon hibernicus (soil isolate)	Microcystins	USA
Microcystis aeruginosa	Microcystins	Worldwide
Microcystis flos-aquae	Microcystins	Australia
Microcystis botrys	Microcystin	Denmark
Microcystis viridis	Microcystins	Japan
Nodularia spumigena	Nodularins	Australia, Baltic Sea, New Zealand
Nostoc sp.	Microcystins	Finland, England
Oscillatoria limosa	Microcystins	Switzerland
Oscillatoria sp.	Anatoxin-a	Ireland, Scotland
Oscillatoria agardhii/rubescens group (=Planktothrix)	Microcystin	Denmark, China, Finland, Norway
Planktothrix formosa	Homoanatoxin-a	Norway
Planktothrix mougeotii	Microcystins	Denmark
Planktothrix sp.	Anatoxin-a	Finland
Plectonema (syn. *Lyngbya) wollei*	Saxitoxins	USA
Raphidiopsis curvata	Cylindrospermopsin, Deoxycylindrospermopsin	China
Umezakia natans	Cylindrospermopsin	Japan

Note: Most cyanobacteria outer cell wall components are implicated in gastro-intestinal disorders, skin and eye irritation and respiratory symptoms.

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FACTORS INFLUENCING OCCURRENCE

BUOYANCY REGULATION

Bloom forming cyanobacteria possess gas vesicles within cells that are collectively called gas vacuoles. These structures are rigid hollow cylindrical chambers made of protein which contain atmospheric gas [39] and provide cells with buoyancy. Some cyanobacteria can combine this positive buoyancy with the accumulation and loss of carbohydrate which acts as ballast to regulate their buoyancy and enables them to migrate up and down. The way this works is that colonies near the surface are exposed to high light and so have a high rate of photosynthesis and therefore build up carbohydrates within the cells. This makes them heavy and although they contain gas vacuoles the carbohydrate ballast makes them sink at a rate dependent upon their colony size and density of the cell. Large colonies sink faster than small ones. As the colonies sink down into a depth of lower light intensity they stop producing and start consuming carbohydrate by respiration [40]. The colonies then become buoyant again and float back up to the surface euphotic (higher light intensity) zone. 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 [41]. This may be a significant advantage over other phytoplankton algae particularly in stratified lakes where turbulence is low and heavy cells tend to sink.

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FACTORS INFLUENCING GROWTH

STRATIFICATION OF WATER BODIES

Stratification occurs when the surface layer of a water body (epilimnion) is warmed by sunlight. The resulting rise in temperature causes the water to be less dense and so it separates from the denser bottom layer (hypolimnion). The area between the two layers is known as the thermocline (Figure 1-4). As it is separated from the atmosphere, the hypolimnion is oxygen deficient, or anoxic. The upper, warmer, epilimnion can become wind-mixed and, because of its exposure, can freely exchange dissolved gases (such as O_2 and CO_2) with the atmosphere. The density change at the thermocline, caused by the temperature difference, acts as a physical barrier that prevents mixing of the upper and lower layers.

As there is little or no mixing between the surface layer and the hypolimnion, the latter becomes depleted of oxygen, or anoxic, due to microbial activity using up the available oxygen, which is not replenished by normal gaseous diffusion from the water column above. Under oxygenated conditions (i.e. well mixed water body) phosphorus rich sediments are sealed by an oxidised surface layer of an iron-phosphorus complex. However, under anoxic conditions the complex breaks down resulting in phosphorus, iron and manganese release from the sediments. In the case of phosphorous, this causes an increase in the internal nutrient loading to a water body. This, in turn, can result in an increase in cyanobacterial biomass. During stratified conditions, sediment-bound phosphorus can become a major nutrient source for cyanobacteria. The amount of phosphorus released from the sediments is governed by water exchange rates, sediment chemistry, temperature, mixing conditions, and sediment disturbance.

Usually, shallow (e.g. 2-3 m), wind-exposed lakes are non-stratified. Lakes of intermediate depth (e.g. 5-7 m) may develop transient thermal stratification for a few calm and sunny days, which is then disrupted by the next rain or wind event. In temperate climates deeper lakes can exhibit a stable stratification from spring to autumn. Thermal stratification of a water body influences the depth at which cyanobacteria are likely to be found, the light levels they receive, and the concentrations of nutrients in the water body.

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CYANOTOXINS

PEPTIDE HEPATOTOXINS (MICROCYSTINS AND NODULARIN)

The hepatotoxins are cyclic peptides, the most frequently encountered compounds of which are the microcystins. These are cyclic heptapeptides produced most commonly by *Microcystis aeruginosa* but also by other species of *Microcystis* and other genera such as *Planktothrix (Oscillatoria)*, *Anabaena*, *Nostoc, Anabaenopsis,* and *Hapalosiphon* [17]. A similar cyclic pentapeptide, nodularin, which is equally as toxic as the most toxic microcystins is commonly produced by *Nodularia spumigena* which is normally a brackish water cyanobacterium [42]. Other cyclic pentapeptide toxins have been characterised, e.g., motuporin isolated from a marine sponge [43] and [L-Har^2]-nodularin [44]. The structures of the peptide hepatotoxins are shown in Figure 1-1(L2).

Microcystins were initially considered to contain five invariant and two variant amino acids. One of the invariant amino acids is a unique β-amino acid called Adda. A two-letter suffix (XY) is ascribed to each individual toxin to denote the variant amino acids. X is commonly leucine (L), arginine (R) or tyrosine (Y), and Y, arginine, alanine (A) and methionine (M). Variants of all the "invariant" amino acids have now been reported, e.g., desmethyl amino acids and/or replacement of the 9-methoxy group of Adda by an acetyl moiety. Currently there are in excess of 70 variants of microcystin which have been characterised [17]. Of these 70 compounds, microcystin-LR is the microcystin most frequently found in cyanobacteria. Often more than one microcystin is produced by a particular strain of cyanobacterium [45]. The microcystin variants also differ in toxicity [46]. The literature indicates that hepatotoxic blooms of *M. aeruginosa* containing microcystins occur worldwide.

The cyclic pentapeptide nodularin contains amino acids similar or identical to those found in microcystins, namely arginine, glutamic acid, β-methylaspartic acid, N-methyl-dehydrobutyrine and also Adda [42]. Motuporin has arginine replaced by valine [43] and in [L-Har^2]-nodularin, arginine is replaced by homoarginine [44].

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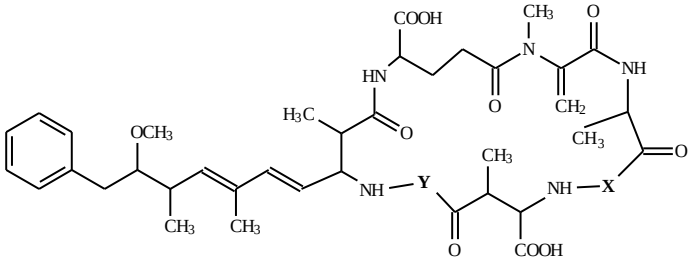

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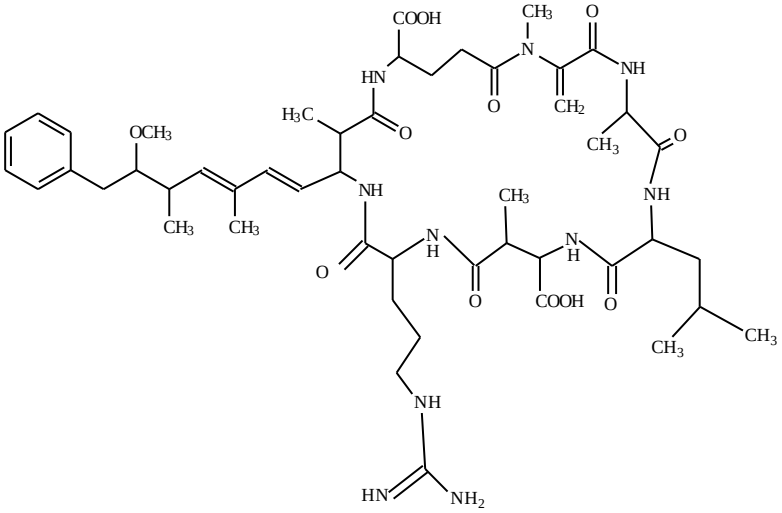

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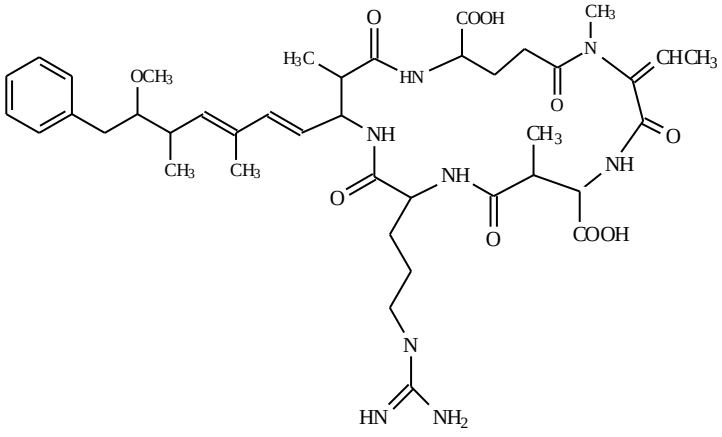


(3)

Figure 1-1(L2) Structures of peptide hepatotoxins; (1) - General structure of microcystins, (2) - microcystin-LR, (3) - nodularin

NEUROTOXINS

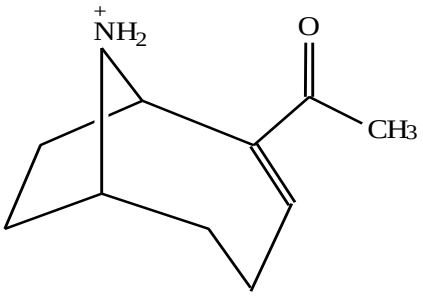
ANATOXINS

Toxins in this class identified to date are the neurotoxic alkaloids anatoxin-a, homoanatoxin-a and anatoxin-a(s). Anatoxins have been shown to be widespread in cyanobacteria in the northern hemisphere; only one report of anatoxin-a in cyanobacteria in the southern hemisphere has been confirmed [47].

SAXITOXINS (PARALYTIC SHELLFISH POISONS, PSPS)

The neurotoxic saxitoxins or paralytic shellfish poisons (PSPs) belong to one of a number of groups of toxins produced by dinoflagellates in the marine environment (Figure 1-2(L2)). Shellfish feeding on toxic dinoflagellates can themselves become toxic and hazardous if consumed, even causing human fatalities [48]. Poisoning incidents usually coincide with the sudden proliferation of these organisms to produce visible blooms, the so-called "red tides" [49].

In freshwater these toxins are produced by a fairly limited number of species of cyanobacteria. To date the only neurotoxic cyanobacterium encountered in Australia is *Anabaena circinalis,* which produces saxitoxins [50]. Elsewhere in the world, saxitoxins have been found to be responsible for neurotoxicity in the cyanobacterial species *Aphanizomenon flos-aquae* [51, 52], *Lyngbya wollei* [36] and *Cylindrospermopsis raciborskii* [53]. Saxitoxins in Danish lakes appear to be produced by *Anabaena lemmermannii* [54]. Toxin profiles are complex and variable, similar to those that have now been found in dinoflagellates and contaminated shellfish.



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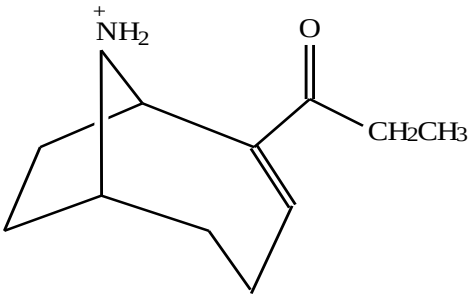

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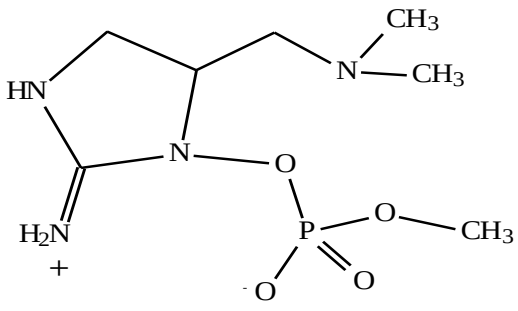

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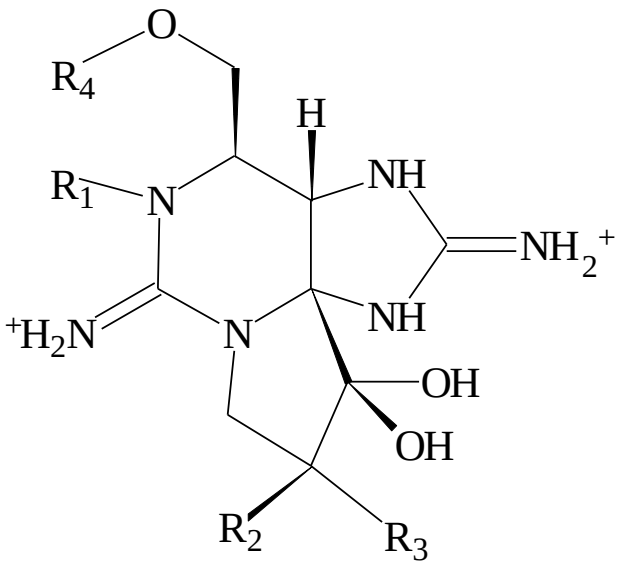
Figure 1-2(L2) Structures of the anatoxins; (1) - anatoxin-a, (2) - homoanatoxin-a, (3) - anatoxin-a(s)



	R1	R2	R3	Net Charge	Relative Toxicity
R4=$CONH_2$ (CARBAMATE TOXINS)					
STX	H	H	H	+2	1
neoSTX	OH	H	H	+2	0.924
GTX1	OH	H	OSO_3^-	+1	0.994
GTX2	H	H	OSO_3^-	+1	0.359
GTX3	H	OSO_3^-	H	+1	0.638
GTX4	OH	OSO_3^-	H	+1	0.726
R4 = $CONHSO_3^-$ (N-SULFOCARBAMOYL (SULFAMATE) TOXINS)					
GTX5 (B1)	H	H	H	+1	0.064
GTX6 (B2)	OH	H	H	+1	-
C1 (epiGTX8)	H	H	OSO_3^-	0	0.006
C2 (GTX8)	H	OSO_3^-	H	0	0.096
C3	OH	H	OSO_3^-	0	0.013
C4	OH	OSO_3^-	H	0	0.058
R4=H (DECARBAMOYL TOXINS)					
dcSTX	H	H	H	+2	0.513
dcneoSTX	OH	H	H	+2	-
dcGTX1	OH	H	OSO_3^-	+1	-
dcGTX2	H	H	OSO_3^-	+1	0.651
dcGTX3	H	OSO_3^-	H	+1	0.754
dcGTX4	OH	OSO_3^-	H	+1	-

Figure 1-3(L2) Structures of the saxitoxins (paralytic shellfish poisons (PSPs)). Toxicity data from [55]

The widespread occurrence of saxitoxins makes them an important class of cyanotoxins. In *A. circinalis* in Australia, toxin profiles appear to be relatively constant and dominated by the C toxins [56, 57]. There is also some limited evidence that this cyanobacterium can produce both neurotoxins and hepatotoxins [58], a phenomenon which has been reported elsewhere with *A. flos-aquae* [59, 60].

The saxitoxins are a relatively complex class of 18 compounds with widely differing toxicities which can be divided into three groups as shown in Figure 1-3(L2). They can also be divided into three groups based on the net charge of the molecule under acidic conditions [61]. This grouping comprises the saxitoxins (saxitoxin (STX), neosaxitoxin (neoSTX) and decarbamoyl derivatives) (charge +2;), the gonyautoxins (GTXs) including decarbamoyl derivatives (charge +1) and C toxins (charge 0). These properties form the basis of analytical methods involving high performance liquid chromatography (HPLC) (Chapter 3).

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CYLINDROSPERMOPSIN

In 1979 at Palm Island, Queensland, Australia there was a severe outbreak of hepatoenteritis in the population supplied with drinking water from a dam which had been treated with copper sulphate to kill a heavy bloom of algae [62]. Subsequent research on *Cylindrospermopsis raciborskii* from this source showed it to produce toxicological effects in animals consistent with the symptoms observed at Palm Island. On this basis it was subsequently suggested that the 1979 outbreak was caused by toxic *C. raciborskii* [63]. This species has also been responsible for cattle deaths in Queensland [64].

A hepatotoxic alkaloid toxin was isolated from *C. raciborskii* and named cylindrospermopsin (Figure 1-4(L2)) [65]. It has also subsequently been isolated from the cyanobacterium *Umezakia natans* in Japan [66] and *Aphanizomenon ovalisporum* in both Australia [67] and Israel [68]. Cylindrospermopsin can be classified as a hepatotoxic alkaloid but toxicological studies have shown that, while the principal organ affected is the liver, other organs such as the kidney are also affected [69]. A report that another toxic compound, 7-epicylindrospermopsin, was isolated from a strain of *Aph. ovalisporum* from Israel [68] suggests that the potential presence of other toxins, some possibly unknown at present, should also be considered when dealing with these cyanobacteria.

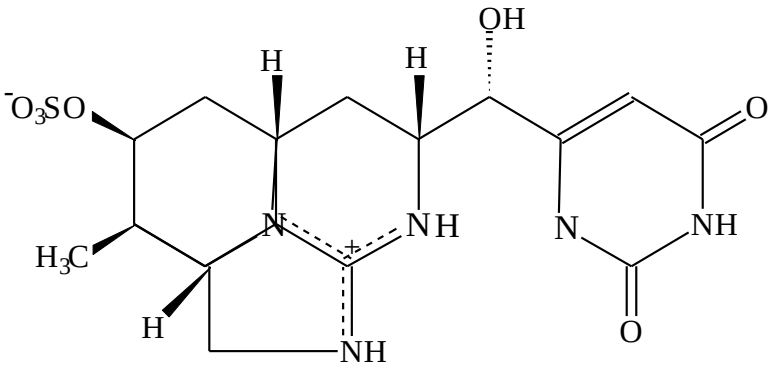

Figure 1-4(L2) Structure of cylindrospermopsin

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B-*N*-METHYLAMINO-L-ALANINE (BMAA)

The neurotoxic amino acid BMAA (β-methylamino-L-alanine) has been associated with a fatal human neurodegenerative disease, with similarities to Alzheimer's and Parkinson's diseases.

The disease (Amyotrophic Lateral Sclerosis/Parkinson's Dementia complex; ALS/PDC) was first described on Guam and BMAA was found to be produced by a symbiotic cyanobacterium living in specialized roots in cycads on the island [70]. BMAA has been reported in the brain tissue of patients who died of ALS/PDC [71] although a subsequent study found no BMAA in the brains of affected individuals [72]. BMAA is concentrated in various parts of the cycad plant [73] including the seeds which are used by the local people to produce flour. The flour is treated to remove toxins resulting in very little BMAA being ingested by this route. Consequently, flour was not considered to be a significant source of exposure to BMAA [74].

It has been hypothesised that, since flying foxes feed on cycad seeds and flying foxes are consumed by the people of Guam, this may be a route of exposure [75]. Subsequent analysis of flying foxes confirmed the accumulation of BMAA [76]. More recently, Cox et al. [70] described the biomagnification of BMAA from the cyanobacterium, through the cycads, in the flying foxes which feed on the cycad seeds and in humans who eat the flying foxes. Thus a plausible route of significant exposure has been identified. This hypothesis is supported by the decline in ALS/PDC in recent years mirroring the decline in flying fox numbers [77].

BMAA is found not only in its free form but also at higher levels bound in proteins, as are other normal protein amino acids, at all levels of the food chain (cyanobacteria, cycad plants including flour, flying foxes and brain tissue). This suggests that these proteins function as an endogenous neurotoxic reservoir slowly releasing free toxin [78].

Neurodegenerative diseases in other areas of the Pacific have also been associated with exposure to cycad material [79] which suggests that BMAA is involved in these disorders as well. A similar amino acid, β-N-oxalylamino-L-alanine (BOAA) is produced by the plant *Lathyrus sativus* and is responsible for a neurological disorder, neurolathyrism, when consumed [80].

Recent studies have suggested that BMAA is also produced widely by free-living cyanobacteria from freshwaters throughout the world. BMAA has also been found in brain tissue of not only people on Guam who had died of ALS/PDC but also Alzheimer's patients in Canada [71] although, as mentioned, a subsequent study could not reproduce these findings [72]. Other sources of BMAA, possibly free-living cyanobacteria, may contribute to these types of neurological disorders [70].

The detection of BMAA in a number of common cyanobacteria and the demonstrated capacity of BMAA to biomagnify raises some concern for the water industry. Research is needed to assess the level of risk of exposure from drinking waters. However, at this point in time, the association between BMAA and neuro-degenerative diseases must be considered tenuous.

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LIPOPOLYSACCHARIDE ENDOTOXINS

The lipopolysaccharide (LPS) endotoxins are perhaps the least understood of the toxins produced by cyanobacteria. These toxins are constituents of the outer wall of both cyanobacteria and heterotrophic gram-negative bacteria [81]. LPS endotoxins produced by cyanobacteria are less toxic than those produced by bacteria; however they may be responsible for illnesses such as gastroenteritis in human populations exposed to cyanobacteria [82]. Consequently the involvement of LPS endotoxins in episodes of human toxicity warrants further attention.

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EXAMPLES OF TOXICITY OF BENTHIC CYANOBACTERIA

Several dog deaths were linked to the presence of *Oscillatoria*-like species [83], *Phormidium favosum* mats containing anatoxin-a [84] and *Phormidium autumnale* containing both anatoxin-a and homoanatoxin-a [85]. Cattle have also died through ingestion of *Oscillatoria limosa* [86]. Baker et al. [87] investigated *Phormidium* aff. *formosum* and *Phormidium* aff. *amoenum* from two reservoirs and a recreational lake in South Australia and found them lethal to mice by intraperitoneal injection. Neuro and hepatotoxic affects have also been reported from *Calothrix parietina* and *Phormidium tenue* [88]. Izaguirre et al., [89] found several microcystin-producing *Phormidium, Oscillatoria* and *Lyngbya* species which were isolated from drinking water reservoirs and Seifert et al. [90] produced the first evidence of cylindrospermopsin and deoxy-cylindrospermopsin production by *Lyngbya wollei*.

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ADVERSE HUMAN HEALTH EPISODES DUE TO CYANOTOXINS

In view of the potential health risks of people drinking water that is contaminated by cyanotoxins, it is important to highlight the more serious suspected human poisonings that have been recorded:

Paulo Afonso gastroenteritis incident in the region of Bahia State in Brazil: In 1988 the people in the surrounding villages, who were supplied with conventional treated water from the newly built Itaparita Dam, experienced severe gastroenteritis (2000 cases were reported, of whom 88 people died). The investigation revealed that the source water from the Itaparita Dam contained very high concentrations (approximately 10^6 per millilitre) of *Anabaena* and *Microcystis* and people became sick after drinking boiled water from the dam [26].

Caruaru dialysis incident in Brazil: In 1996 an outbreak of severe hepatitis occurred at a Brazilian haemodialysis centre in Caruaru, Brazil. One hundred patients developed acute liver failure after receiving routine haemodialysis treatment; 52 of the affected patients died. The clinical symptoms included visual disturbances, nausea, vomiting, muscle weakness and painful hepatomegaly. Microcystins and cylindrospermopsin were found in the source water, in the water delivery tanker, and in the dialysis unit's holding tank as well as in the ion exchange resins and carbon filters from the dialysis centre's in-house treatment system. Microcystins were also detected in the blood sera and liver tissue of both live and deceased patients [28, 91].

Sewickley gastroenteritis incident in the United States of America: In 1975 approximately 62% of the people receiving piped water from the distribution network become ill, experiencing abdominal pains and diarrhoea. Due to a hole in the groundwater intake structure more than 40% of the source water supply came from the Ohio River. Cyanobacteria were found in the open finished-water reservoirs and it was concluded that the contamination of the distribution network was through these reservoirs [92].

Harare seasonal gastroenteritis incidents in Zimbabwe: Seasonal gastroenteritis in children is possibly due to the lysis of the cells of the annual *Microcystis* blooms that occur in the source water reservoir. The naturally-liberated cyanotoxin would probably not be effectively removed during the basic drinking-water purification process [93].

Armidale liver damage incident in Australia: The water in the Malpas Dam, which supplies water for the drinking water treatment plant for the town of Armidale was regularly treated for cyanobacteria with copper sulphate after complaints about taste and odour. In 1981, *Microcystis* scum formation around the abstraction point put additional stress on the drinking water treatment process, resulting in cyanobacteria cells passing through the treatment process

leading to re-growth in the open post-treatment drinking water tanks. Elevated enzyme activity in the sera of some town residents strongly suggests considerable liver damage. The presence of *Microcystis* and subsequent cyanotoxin release during the lysis of the cells may be responsible for the observed liver damage [21].

Palm Island poisoning incident, Queensland, Australia: In 1979, there was a major outbreak of hepato-enteritis amongst the children of the Aboriginal community after drinking water from the treatment works that received its source water from the Solomon Dam. Clinical symptoms included anorexia, vomiting, headache, painful liver enlargement, initial constipation followed by bloody diarrhoea and dehydration. It was concluded that the poisoning was due to the release of cyanotoxins during the lysis of the cyanobacteria cells after treating the surface water of the reservoir with copper sulphate. Subsequent evaluations confirmed that the poisoning was due to the presence of the cyanobacterium *Cylindrospermopsis raciborskii* in the dam [20].

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CYANOTOXIN DRINKING WATER GUIDELINES

INTERNATIONAL GUIDELINES FOR CYANOTOXINS

Guideline values for cyanotoxins are summarised in Table 1-2(L2).

Table 1-2(L2) Guideline values or standards for cyanotoxins in drinking water from various countries. (Information derived from websites and [94] unless otherwise stated).

Country	Guideline Value/Standard	Comments/Explanations
Argentina	Under revision	
Australia	1.3 µg L^{-1} total microcystins, guideline value	Australian Drinking Water Guidelines
Canada	1.5 µg L^{-1} cyanobacterial toxins as microcystin-LR MAC	Canada uses guidelines as the standard of water quality. The guidelines are expressed with the unit of Maximum acceptable concentrations (MAC). These are derived from tolerable daily intake (TDI) which in turn are derived from a calculated no-observed adverse effect level (NOAEL) from data from human or animal studies. To derive a MAC from a TDI adjustments are made for average body weight and drinking water consumption, as well as other considerations. In terms of health the guidelines ensure that the MACs are far below exposure levels at which adverse effects have been observed. For the case of cyanobacterial toxins the guideline is considered protective of human health against exposure to other microcystins (total microcystins) that may also be present
Czech Republic	1 µg L^{-1} microcystin-LR	Value as national legislation, follows WHO provisional guideline value.
China	1 µg L^{-1} microcystin-LR	WHO provisional guideline for microcystin-LR
France	1 µg L^{-1} microcystin-LR	Drinking water decree
Italy	1 µg L^{-1} microcystin-LR	WHO provisional guideline for microcystin-LR used as a reference by local authorities.
Japan	1 µg L^{-1} microcystin-LR	WHO provisional guideline for microcystin-LR
Korea	1 µg L^{-1} microcystin-LR	WHO provisional guideline for microcystin-LR.
New Zealand	MAV for cyanobacterial toxins: Anatoxin: 6.0 µg L^{-1} Anatoxin-a (S): 1.0 µg L^{-1} Cylindrospermopsin: 1.0µg L^{-1} Microcystins: 1.0 µg L^{-1} Nodularin: 1.0 µg L^{-1}	Maximum acceptable values (MAVs) for micro-organisms or organic determinands of health significance. MAVs are based on the WHO 'Guidelines for Drinking Water Quality'. They are the concentration of a determinand, which is not considered to cause any significant risk to the consumer over a lifetime of consumption of water. The method of derivation varies according to NZ conditions and the way in that the determinand presents a risk. However they are derived with the use of a TDI. The MAVs are standards in NZ. The Standards provide compliance criteria and

	Saxitoxins:1.0 µg L^{-1}	compliance is routinely monitored
Norway	1 µg L^{-1} microcystin-LR	Provisional WHO guideline for drinking water adopted
Oceania	None found	Clean drinking water supply to all people main current focus
Poland	1 µg L^{-1} microcystin-LR	National legislation for guideline value in drinking water
South Africa	0-0.8µg L^{-1} for microcystin-LR	Guideline levels for microcystins in potable water as a "Target Water Quality Range"
South America (Brazil)	1.0 µg L^{-1} for microcystins 3.0 µg L^{-1} for saxitoxins (equivalents) 15 µg L^{-1} for cylindrospermopsin	Guideline values for microcystins, saxitoxins and cylindrospermopsin, along with biomass monitoring programs. Guideline value for microcystins adopted as mandatory. Guideline values for equivalents of saxitoxins and for cylindrospermopsin included as recommendations. Use of algicides prohibited and toxicity testing/toxin analysis when cell counts exceed 10,000 cells/mL or 1mm^3 biovolume. .
Spain	1 µg L^{-1} microcystins	National legislation, maximum permissible amount in drinking water
Thailand	No guideline currently	Awareness for need for guidelines
United States of America	None currently known.	Maximum Contaminant Levels (MCLs) are the highest level of a contaminant that is allowed in drinking water. They are enforceable standards. Cyanobacteria and their toxins are listed as microbiological contaminants on the contaminant candidate list (CCL). This means that they are currently recognised as unregulated contaminants, but are known to occur in public water systems and may require regulation under the Safe Drinking Water Act. Contaminants on the CCL are a priority for the US Environmental Protection Agency with the aim to set MCLs
Uruguay	Under revision	
World Health Organisation	1.0 µg L^{-1} for microcystin-LR GV	Refer to World Health Organisation Guidelines for Drinking-Water Quality, 1996 [95]

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PROCEDURES FOR GUIDELINE DERIVATION

The guideline for microcystin–LR was derived using the following equation:

Guideline value ($\mu g\ L^{-1}$) = (TDI x Bw x PI)/DI

where:

TDI = An estimation of the amount of a substance in the drinking water expressed on a body mass basis ($\mu g\ kg^{-1}$), that can be ingested over a lifetime without significant health risks. The TDI ($\mu g\ kg^{-1}day^{-1}$) is calculated as (NOAEL or LOAEL) / Uncertainty factors. The NOAEL is the highest dose or concentration of a substance that causes no detectable adverse health effect. The LOAEL is the lowest observed dose or concentration of a substance at which there is a detectable adverse health effect. The source of uncertainty is from interspecies variation, intraspecies variation, adequacy of studies or databases and the nature and severity of the effect. The uncertainty values (factor of 10) thus ranges from 10 to 10000.

Bw = The average body weight of an adult (60 kg) or child (10 kg) or infant (5 kg).

PI = The portion of intake due to drinking water. This value is usually 10%. However, cyanotoxins intake is mainly via drinking water and is thus taken as 80 to 90%.

DI = The average drinking water consumption per day of an adult (2L) or child (1L) or infant (0.5L).

Therefore:

Guideline value (microcystin–LR as $\mu g\ L^{-1}$) = [(40/1000) x 60 x 0.8)]/2

= 0.96 $\mu g\ L^{-1}$

= 1 $\mu g\ L^{-1}$ microcystin-LR

where:

TDI = NOAEL is 40 $\mu g\ kg^{-1}\ day^{-1}$ and the uncertainty factor is 1000.

Bw = The average body weight of an adult is 60 kg.

PI = The portion of intake due to drinking water is 80%.

DI = The average drinking water consumption per day of an adult is 2L.

It is important to stress that the provisional guideline is only for microcystin–LR and thus excludes the toxicity of other microcystins that may be present [3, 6]. It is therefore advisable for drinking water suppliers not to base their guidelines on microcystin–LR alone. To overcome this problem, it has become common practice to use the 1.0 $\mu g\ L^{-1}$ microcystin-LR guideline value as a surrogate for all microcystin variants (total microcystins) to reduce the exposure risk. Therefore the frequently used guideline is 1.0 $\mu g\ L^{-1}$ microcystin equivalents (equivalent toxicity to microcystin-LR). The microcystin equivalents are calculated from the available microcystins variant toxicity data, assuming equivalent toxicity to microcystin–LR for those with no toxicity data available. Furthermore, the guideline value 1.0 $\mu g\ L^{-1}$ total microcystins is also based on the ELISA bioassay. This approach is frequently used by those water treatment facilities that do not have the capacity to monitor the full spectrum of microcystin variants, or by those that incorporate it as part of their Cyanobacteria Incident Management Framework.

Falconer [3] followed a similar approach to that of the WHO [96] in developing a proposed guideline for cylindrospermopsin:

Guideline value (cylindrospermopsin as $\mu g/L$) = [(30/1000) x 60 x 0.9)]/2

= 0.81 $\mu g/L$

= 1 $\mu g/L$

where:

TD = NOAEL is 30 $\mu g/kg/day$ and the uncertainty factors is 1000 (10 for intraspecies variation, 10 for interspecies variation, 10 for data adequacy).

Bw	=	The average body weight of an adult is 60 kg.
PI	=	The portion of intake due to drinking water is 90%.
DI	=	The average drinking water consumption per day of an adult is 2L.

It must be stressed that the guideline concentrations for both these toxins are not directly applicable to short term exposures as they aim to protect humans over a lifetime of consumption and are thus conservative [3]. This is very important for drinking water suppliers, as they may experience higher concentrations for short periods. Fitzgerald *et al*. [97] recommended that the safety factor of 10 could be omitted from the TDI calculation as the data are mainly based on subchronic exposure duration. The guideline for short-term exposure can thus be increased 10-fold. Subsequently, it was proposed that water utilities in Southern Australia use a guideline value of 10 $\mu g\ L^{-1}$ for microcystins as well as for nodularin as their alert levels. Falconer [3] argued that this value was too high and that a more conservative approach must be followed as people may be exposed to cyanotoxins several times a year. It is thus recommended that a concentration of 5 $\mu g\ L^{-1}$ be used for both the alert level and the drinking water guideline for alerting the health authorities regarding cyanotoxins.

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CHAPTER 2 HAZARD IDENTIFICATION AND RISK ASSESSMENT IN SOURCE WATERS (LEVEL 1)

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 [1].

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.* [1].

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. For this purpose a nutrient load screening tool such as NEAP may be applied. This modeling will indicate the percentage of the total load attributable to background, non-point source and point source nutrient inputs. This information may then be used to prioritize mitigation and management efforts.

For more information about assessment of phosphorus and its relationship with chlorophyll-a and several case studies, follow this link

A valuable web-based tool for the assessment of the eutrophication level of a water body is the Nutrient Enrichment Assessment Protocol, NEAP. The outputs from NEAP can include the phosphorus loading generated by the catchment, the trophic state of the water body, and the expected annual mean and peak chlorophyll-a concentrations.

For information on the Nutrient Enrichment Assessment Protocol (NEAP) model for the prediction of cyanobacterial growth follow this link

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 fertilsers, 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 Composting	Including the treatment works, pumping stations, wastewater overflow structures and the reticulation system (<250 kilolitres/day) 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

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.

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 [2,3, 4]. 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 [5], 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.* [6] 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' [7]. 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 (°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 [8, 9, 10].

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 [11].

More sophisticated deterministic water quality models are also available to predict cyanobacterial growth [12, 13]

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. Model assumptions for the three cases are described in Level 2

			Predicted concentrations of cyanobacteria and their metabolites						
Reservoir nutrient status	Total Phosphorus (µg L^{-1})	Scenario modelled:	Bioavailable Phosphorus (µg L^{-1})	*Microcystis aeruginosa* (cells mL^{-1})	Microcystin (Total) (µg L^{-1})	*Anabaena circinalis* (cells mL^{-1})	Geosmin (Total) (ng L^{-1})	Geosmin (Dissolved) (ng L^{-1})	Saxitoxin (Total) (µg 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

For details about the assumptions and parameters used to derive the information in Table 2-5 click here

An example of a source water risk assessment based on phosphorus limitation can be found here

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 2 HAZARD IDENTIFICATION AND RISK ASSESSMENT IN SOURCE WATERS (LEVEL 2)

FACTORS INFLUENCING CYANOBACTERIAL BLOOM OCCURRENCE

PHOSPHOROUS LEVEL ASSESSMENT

The preferred approach to managing water sources is to aim for control of the frequency with which blooms occur. Provided that adequate historical data are available, this may be achieved by identifying the level of phosphorous at which there occurs a marked increase in the incidence, or percentage occurrence, of algal growth at a specified level, e.g. chlorophyll-a levels exceeding 20 μg L^{-1}.

ANALYSIS

It is generally accepted that chlorophyll-a levels persistently in excess of 20 μg L^{-1} pose problems for the treatment of water. As concentrations increase further above this value, problems pertaining to recreational and direct abstraction uses become more relevant. Algal blooms are generically defined as conditions with chlorophyll-a levels > 40 ug L^{-1}.

In general, a total phosphorus level of 10–25 μg L^{-1} presents a moderate risk in terms of the growth of cyanobacteria. For levels of less than 10 μg L^{-1} there is a low risk of cyanobacteria growth, and a level greater than 25 μg L^{-1} provides high growth potential.

By analyzing available data to produce a similar interpretation for any specific reservoir, the phosphorus concentration-based threshold at which problems of a specific magnitude start to occur can be identified. Based on the management requirements, the identified threshold can be compared with the seasonal mean concentrations of phosphorus and targets set for nutrient load reductions (see following section).

In the example shown in Figure 2-1(L2) it can be seen that for levels of chlorophyll-a in excess of 20 μg L^{-1} there is a rapid increase in the percentage occurrence of blooms at a median phosphorus concentration of approximately 25-27 μg L^{-1} total phosphorus, TP; similarly, for chlorophyll-a > 40 μg L^{-1} the breakpoint at which blooms of this magnitude increase in frequency is slightly in excess of 40 μg L^{-1} and for > 60μg L^{-1} chlorophyll-a, the rise starts at a median TP of between 50 and 60 μg L^{-1}. It may be noticed that this concentration lies within the boundary between meso- and eutrophic lake conditions, as defined by various trophic state boundary values e.g.[3].

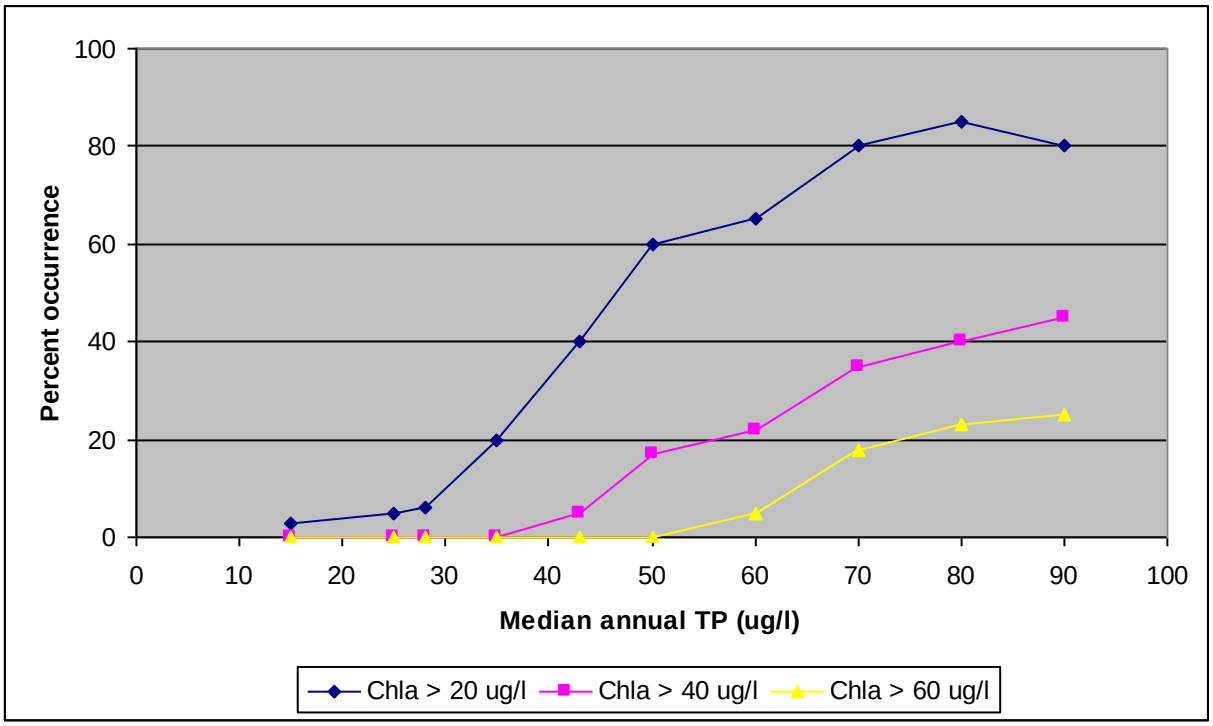

Figure 2-1(L2) Percent occurrence of chlorophyll-a concentrations in excess of specified levels as a function of total phosphorous (example)

By analysing available data to produce a similar interpretation for any specific reservoir, the phosphorus concentration-based threshold at which problems of a specific magnitude start to occur can be identified. Based on the management requirements, the identified threshold can be compared with the seasonal mean concentrations of phosphorus and targets set for nutrient load reductions (see following section).

A large number of South African impoundments are characterized by high ambient levels of phosphorus, with a national median of 55 µg L^{-1} as total phosphorus. This was compared with a combined analysis of the phosphorus:chlorophyll relationships in 40 reservoirs, with the result shown in Figure 2-2(L2).

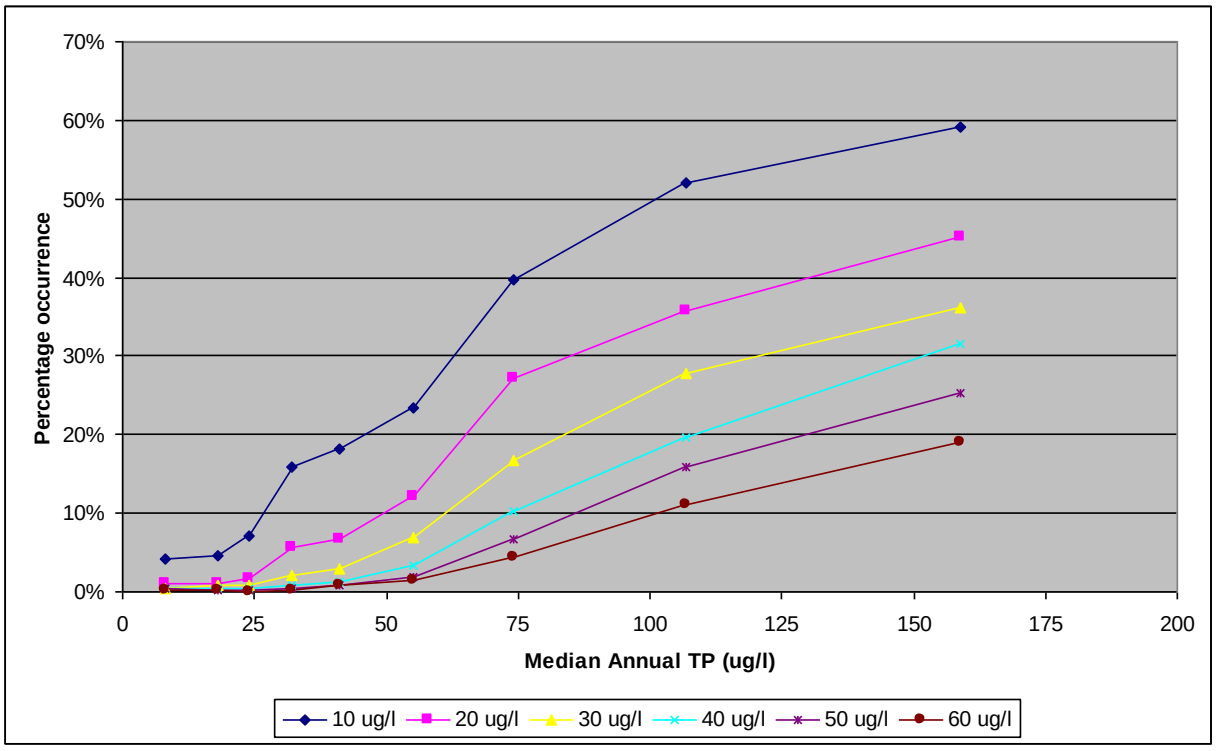

Figure 2-2(L2) Percent occurrence of chlorophyll-a concentrations in excess of specified levels for a set of 40 South African reservoirs.

This analysis reveals that for a median concentration of 55 µg L^{-1} as total phosphorus, problem levels of chlorophyll-a – at the 20 µg L^{-1} threshold - will be experienced 12% of the time (the pink line) and blooms, i.e. chlorophyll-a levels > 40 µg L^{-1}, 5% of the time (the light blue line). This closely reflects the observed situation.

Nutrient-poor reservoirs do not conform to this approach. At the other end of the scale, i.e. high ambient nutrient concentrations, analyses for individual reservoirs reveal a high and persistent level of chlorophyll. For example, Bloemhof Dam (Figure 2-3(L2)) has 50 µg L^{-1} TP as the lower limit of the range of phosphorus concentrations experienced. Under such conditions it can be anticipated that sustained and problematical levels of algal development will be encountered, and the analysis confirms this. In the case of Bloemhof Dam chlorophyll-a levels in excess of 20 µg L^{-1} are encountered 50% of the time, and blooms start to increase in frequency from a concentration of 70 µg L^{-1}. As the median TP for Bloemhof Dam is 86 µg L^{-1} it is clear that the dam will be problematical for much of the year.

A similar situation exists for another dam (Figure 2-4(L2)), with a marked across-the-board increase in chlorophyll-a occurring at a TP level of 60 µg L^{-1}. As there are no "low" TP levels, the precise breakpoints cannot be determined but are likely to lie at the 25 µg L^{-1} TP.

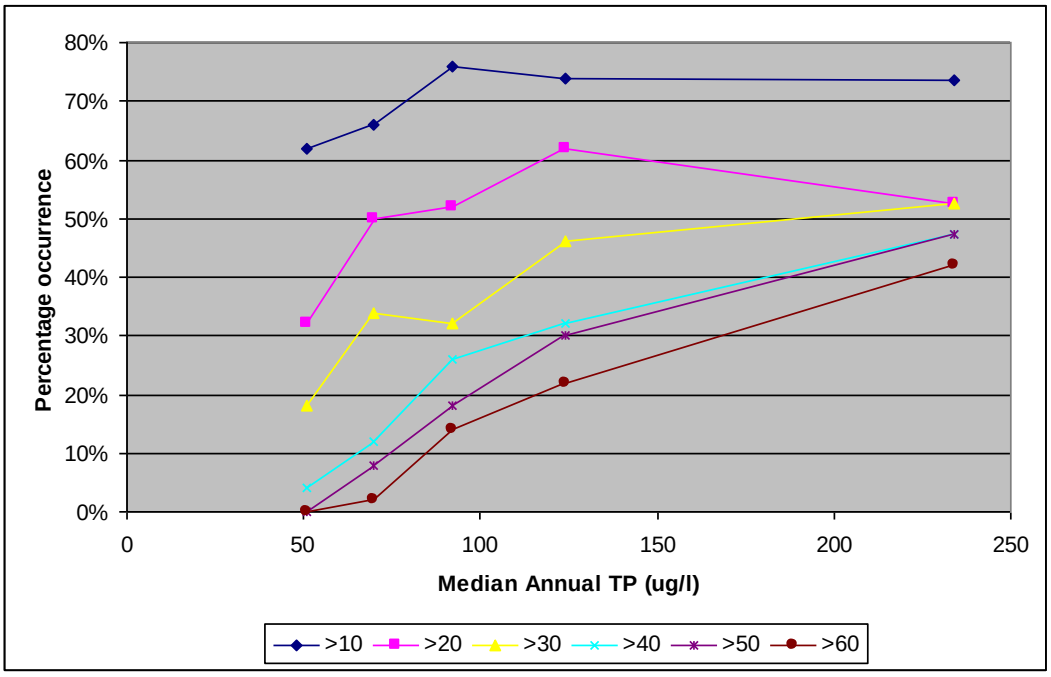

Figure 2-3(L2) Percent occurrence of chlorophyll-a concentrations in excess of specified levels in Bloemhof Dam

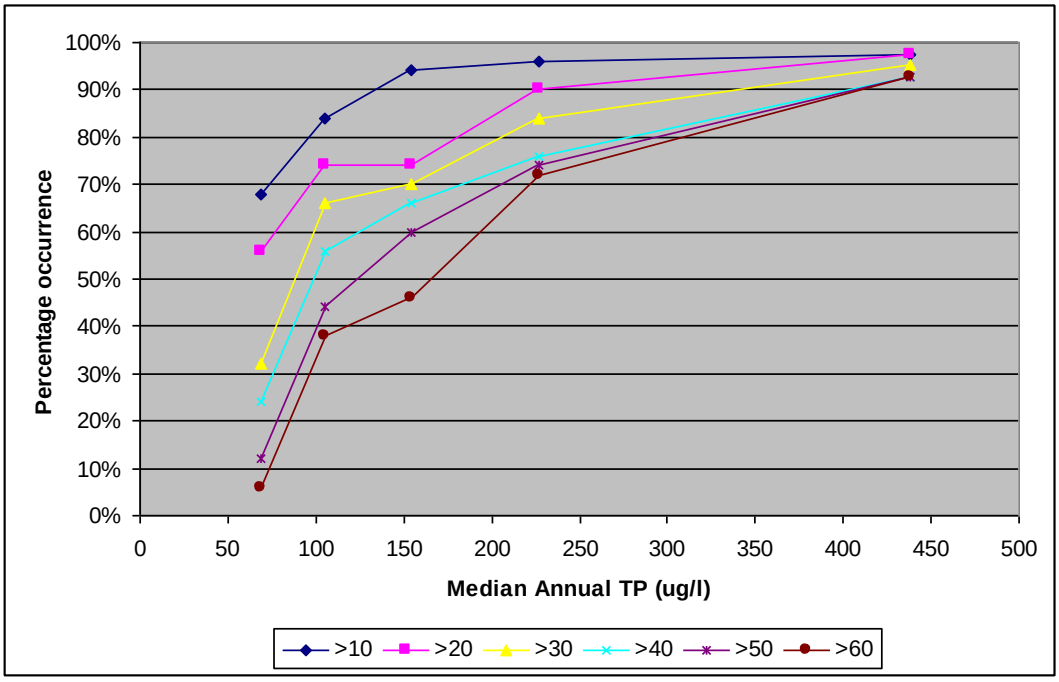

Figure 2-4(L2) Percent occurrence of chlorophyll-a concentrations in excess of specified levels in Bon Accord Dam.

Based on this analysis, the following chlorophyll-a-based risk categories can be provisionally defined for South African reservoirs as follows:

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	

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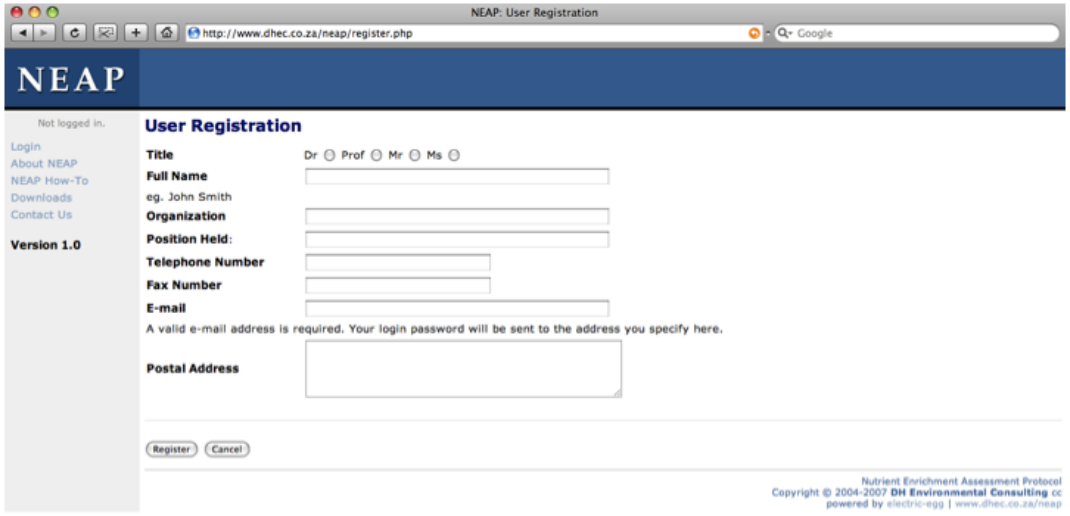
NUTRIENT LOADING ASSESSMENT

Various options are available for undertaking basic assessments of the nutrient loading status and associated trophic state in a lake or reservoir. These range from simple application of Vollenweider-type relationships [2], to software packages that integrate nutrient loading and reservoir hydromorphology. An example of the latter is the NEAP V1.0 internet-based package developed for South Africa

(http://www.dhec.co.za/neap/login.php).

WHAT IS NEAP?

NEAP is an internet-based phosphorus based nutrient loading tool for lakes and/or reservoirs which, depending on the level of information entered, allows the user to select one or more outputs that describe, for example, the P-loading generated by the catchment, the trophic condition of the lake, and the lake's likely response to a change in phosphorus loading. NEAP is based on a range of existing phosphorus load-response relationships. By using available information, NEAP V1.0 has been calibrated for use under South African conditions, and in particular for use in reservoirs. It is a relatively simple process to adjust or re-calibrate the model for use in any particular country or region, or indeed for a particular waterbody. In many cases site-specificity overrides regional or national genericity – requiring that a site-specific calibration be used for an individual reservoir.



THE NEAP DEVELOPMENT PHILOSOPHY

NEAP has been purposefully designed as a simple, phosphorus-based, eutrophication screening tool. As such, it provides a means, which is not data intensive, of determining the degree of nutrient enrichment, or trophic status, of the water body. Once calibrated, it allows the user to determine the manner in which the annual mean concentration of phosphorus is likely to change in response to an increase or decrease in the loading of this element. Such determinations can be made with NEAP at a high (70%) level of confidence.

In most cases, the calibration of dynamic models is severely limited by the availability and/or quality of data. Increasing model complexity also often renders the model lake-specific. The purpose of a screening tool, such as NEAP, is to provide management-related answers without having to resort to an extended period of data collection. The underlying philosophy of NEAP has been to provide a fast and simple to use approximation of the level of eutrophication in a particular reservoir, and to inform options for management. Should more detailed examinations be required, more complex models can be employed as the data become available.

It is intended that subsequent releases of NEAP will incorporate a level of functionality that will support the integration of biogeochemical processes (fate and loss relationships), as well as refinements such as the inclusion of aquaculture impacts. Importantly, later versions will be able to include support for assessing 'virtual' nutrient load reductions relating to management approaches targeting 'top-down' foodweb manipulation.

WHAT IS NEAP'S LEVEL OF RESOLUTION?

NEAP is a First Level tool, with its central value in its simplicity. NEAP is an annual time-step (dT = 1 year) model, i.e. it requires the minimum level of data for all parameters. Notwithstanding this, the model is robust and allows for relatively rapid screening and classification of individual systems, as well as providing indications of how each assessed waterbody will respond to a change in phosphorus loading.

Once NEAP has been used to classify and rank systems, more sophisticated predictive tools, requiring monthly, weekly or daily data for a wide range of parameters may be employed. Decisions to rehabilitate a lake or reservoir should not be made on the basis of NEAP alone, nor should higher level predictive modelling necessarily have to follow the use of NEAP. For this reason, a risk assessment component has been integrated into NEAP, providing an indication of the confidence with which the final output is made.

It should be noted that estimates of catchment nutrient loading can contain errors as high as 50% - therefore accuracy requires a comprehensive assessment process.

INTRODUCTION TO THE MODEL BASE OF NEAP

NEAP is a single layer, single variable (total phosphorus) empirical model that incorporates simple allowances for aspects that are essentially features of multi-layer models, for example the very important need to include sediment loading sub-models.

Several single layer, single variable models have been developed to study the behaviour of phosphorus in different reservoirs. Internationally, the Vollenweider General Lake Model relationship provides the best generic starting point for modelling phosphorus in lakes [2]. Previously, work conducted on a limited number of South and southern-African reservoirs showed that the OECD-type models [3] provided the closest relationship between predicted and observed conditions [14]. This study, which examined 12 models, confirmed that the OECD relationship for phosphorus loading provided a generic fit for South African conditions. However, a predominant characteristic of South African impoundments and shallow lake/vlei environments is a high rate of water exchange (low hydraulic retention times). A more detailed comparison of these models on specific reservoirs indicated that the use of the Walker Reservoir Model [15], a relationship derived for systems with high flushing rates, was more appropriate. Both models have been incorporated and NEAP makes the appropriate selection based on the lake flushing rate determined from the hydrological information that is entered.

NEAP is an annual, single time-step model, i.e. it produces outputs based on annual total or mean values for each parameter.

Models used in NEAP, compared with the Vollenweider General Lake Model:

1. *Vollenweider General Lake Model*
   $P = L_p / q_s (1 + T_w^{0.5})$
2. *OECD (Combined Data Set)*
   $P = 1.55([P\}_j / (1+ \sqrt{T_w}))^{0.82}$
3. *Walker Reservoir Model*
   $P = L*T_w (1-R) / z$
   $R = 1 + [1-(1 + 4Nr)^{0.5}] / 2Nr$
   $Nr = (K_2*L*T_w^2)/z$
   $K_2 = 0.17q_s / (q_s + 13.3)$
   Where: P = average in-lake total phosphorus (mg L^{-1})
   $[P]_j$ = annual mean inflow of phosphorus (mg m^{-3})
   L_p = annual total phosphorus areal loading (mg m^{-2} y^{-1})
   q_s = annual areal water loading rate (m y^{-1})

T_w = hydraulic retention time, years
z = mean depth, m

FEATURES OF NEAP

NEAP V1.0 is a modular, web-based tool incorporating the following components:

- A user login and registration module;
- An "About NEAP" section that describes what NEAP can be used for;
- A "How-to" section that provides a step-by-step explanation, supported by worked examples, of how NEAP can be used, and which allows the user to download a checklist of requirements that can be completed, and the correct units established, prior to entering data into NEAP;
- Six calculation modules that allow the user to determine one or more of the following:
- An estimation of the total phosphorus load back-calculated from the observed in-lake condition;
- A phosphorus-loading module that allows for the aggregation of phosphorus loads from multiple sources, and which outputs a predicted in-lake mean annual phosphorus concentration. This module includes allowance for internal loads from sediments to be added;
- A chlorophyll-a prediction module – generating an annual mean and peak concentration for chlorophyll-a based on the calculated in-lake phosphorus concentration;
- A trophic state prediction module, with output in two formats;
- A load-reduction module that outputs the change in condition in response to a selected reduction in phosphorus loading;
- A risk assessment, based on the concentration at which problematical levels of bloom development (expressed as chlorophyll-a) are likely to be encountered.
- A user feedback section that allows the user to post queries to the NEAP developers, or to request assistance or advice for a particular problem.

USER UNDERSTANDING OF EUTROPHICATION

It is extremely important that the NEAP user has a reasonable working understanding of what eutrophication is – i.e. that eutrophication is not simply a function of phosphorus loads and concentrations – and that a wide variety of biophysical and chemical factors can enhance or constrain the observed level of eutrophication in a particular waterbody. It is as important for the water resource manager to be able to determine whether or not a particular resource is eutrophic as it is to determine the likelihood of it becoming so, or where it lies on a trend towards an impaired trophic state.

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ASSESSING THE POTENTIAL FOR TOXIN PRODUCTION

ASSUMPTIONS USED IN THE CYANOTOXIN PRODUCTION RISK ASSESSMENT MODEL

Table 2-1(L2) Assumptions and variables used in the simple cyanobacterial risk assessment model to derive growth and toxin production by cyanobacteria based upon phosphorus supply in reservoirs.

	Variables used in each Scenario Category		
Variable inputs and assumptions used for the risk assessment scenario model for calculation of cyanobacterial biomass, toxin and odour production	**Best Case**	**Most Likely Case**	**Worst Case**
Proportion of Total Phosphorus (TP) pool in the reservoir that is bioavailable	0.36	0.6	0.8
Proportion of the bioavailable P that is converted to Chl-a	0.5	0.8	1.0
Proportion of Chl-a that is either *Anabaena* or *Microcystis*	0.1	0.5	1.0
The Chl-a content of *Anabaena circinalis* (pg/cell) [8]	0.72	0.72	0.72
The Chl-a content of *Microcystis aeruginosa* (pg/cell) [8]	0.36	0.36	0.36
The production of saxitoxins by *Anabaena*	0.33	0.33	0.33
The ratio of microcystin to *Microcystis* Chl-a	0.04	0.12	0.4

The model calculates chlorophyll yield from available phosphorus concentration which can be modified depending upon the scenario selected. Chlorophyll-a is then translated to cell numbers of *Microcystis* or *Anabaena* using published cell chlorophyll quotas. Cellular content or 'cell quota' ranges for geosmin, saxitoxin and microcystin are applied to estimate the likely yield of the cyanobacterial metabolites under the chosen scenarios.

The assumptions and calculations used with the simple cyanobacterial risk assessment model and their justification are as follows:

1) Two general starting assumptions apply for this model:

   - that the climatic conditions are favourable for cyanobacterial growth and therefore the eventual population size is determined by the carrying capacity of the reservoir.
   - that all other conditions for optimum growth are met and the phosphorus concentration is the limiting factor that will determine the eventual algal and cyanobacterial biomass.

2) Phosphorus concentrations: The level of total phosphorus (TP) in the example here (i.e. 80 µg L^{-1} TP) was derived from the average spring/summer concentrations in an actual drinking water reservoir. For the scenario purposes the projected lower and upper levels were selected arbitrarily as half and double this concentration. If historical data is available for your reservoir then it is possible to select equivalent values for the model calculations.

3) Phosphorus availability: The proportion of total phosphorus (TP) that is bioavailable for uptake and utilisation by organisms will vary between water bodies and an empirical range is used here. The values selected here are: 0.36 for best case; 0.6 for most likely case; 0.8 for the worst case.

4) Incorporation of bioavailable P into algal biomass: The proportion of bioavailable P that is converted to chlorophyll-a is assumed to be in the range of 0.5 - 1 (i.e. 50-100%). The assumption is that some bioavailable P will be taken up by other organisms, but most bioavailable P is taken up by phytoplankton and directly translated into chlorophyll-a.

5) The proportion of chlorophyll-a that is attributable to either *Anabaena* or *Microcystis* depends upon the degree of dominance achieved by the cyanobacteria and a range of 0.1 – 1 (10%-100%) is used here. Major blooms of cyanobacteria can form practically monospecific populations and the 'worst case' scenario assumes that 100% of the chlorophyll-a is *Anabaena* or *Microcystis* accordingly. The 'most likely case' assumes a value of 50%. Reflecting the fact that minor blooms of cyanobacteria may account for less than half of the chlorophyll-a in the reservoir, the 'best case' assumes that 10% of chlorophyll-a is *Anabaena* or *Microcystis.*

6) The assumed chlorophyll-a content of *Anabaena circinalis* is 0.72 pg cell^{-1} and 0.36 pg cell^{-1} for *Microcystis aeruginosa.* These values are based on values published by Reynolds (1984). This is used to determine the number of cells mL^{-1} from the Chl-a concentration.

7) The ratio of microcystin to *Microcystis* chlorophyll-a is derived from the published data and depends upon the strain and environmental conditions. The 'worst case' scenario assumes a ratio of microcystin to *Microcystis* chlorophyll-a of 0.4, which is the maximum of the range published by Chorus and Bartram [10]. This is reduced to 0.12 for the 'most likely case' and 0.04 for the 'best case' scenario (the mean of the range published by Chorus and Bartram, 1999).

8) The production of saxitoxins by *Anabaena* can then be determined from the number of cells mL^{-1} using the estimated saxitoxin yield of 0.33 µg L^{-1} for *Anabaena* cell density of 5,000 cells mL^{-1} (Humpage & Falconer, unpublished). Cell quotas for toxin production will be variable within and between natural populations and over time and other cell quotas can be used where they are available.

The output from this simple model should be considered in the light of a number of factors that will modify and reduce the risk from toxins. For example:

- The cyanobacteria present may not necessarily produce toxins, even if they are known toxigenic species.
- Management strategies are available in the reservoirs to reduce the growth or impact of the cyanobacterial population (e.g. variable off take height, algicide use, destratification)
- A range of variables associated with local conditions including water chemistry and weather patterns may make the conditions unsuitable for cyanobacterial growth.

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POTENTIAL ALGAL GROWTH SCENARIOS FOR HUMBUG SCRUB RESERVOIR

INTRODUCTION

Although significant numbers of toxic cyanobacteria have not occurred historically, there are some records of two potentially toxic species (*Microcystis aeruginosa* and *Anabaena circinalis*) in Humbug Scrub Reservoir. Recently there has been an increase in the occurrence of small picoplanktonic cyanobacteria, such as *Aphanothece* in the reservoir. This may reflect improvements in taxonomic processes, or may be due to real increases in the diversity and numbers of these types of cyanobacteria. Both the potentially toxic and picoplanktonic cyanobacteria may present a challenge to the Humbug Scrub water treatment plant. The treatment plant is not able to completely remove algal metabolites such as odours and toxins if a bloom were to occur and may suffer from reduced filter performance if significant picoplanktonic cyanobacteria are present.

PROCESS

To address the future risk to water quality and potential challenges to the treatment plant, historical and current water quality data, including physical, chemical and cyanobacterial data were assessed against the current Australian Drinking Water Guidelines (2004)16 and the New South Wales (NSW) Department of Land and Water Conservation Algal Contingency Plan (2000) [17].

A frequency analysis of excursions above guidelines and industry-recognised alert levels (see Chapter 6 for examples of alert levels) was carried out to determine their potential impact on water quality, in particular, the growth of potentially toxic cyanobacteria. This data was used to develop three predicted scenarios. These scenarios can be defined as "likely", "reasonable worst case" and "worst case". They indicate potential outcomes for the growth of the problematic cyanobacterial species *Microcystis aeruginosa* (potentially hepatotoxic) and *Anabaena circinalis* (potentially toxic and odorous) given different defined combinations of conditions. The picoplanktonic cyanobacterium *Aphanothece* has also been included as a representative of this class of cyanobacteria which are small in size but are occurring more frequently in the reservoir.

The scenarios were developed for two sites in the reservoir. The two sites were chosen to represent an ‘open water’ offtake site and an upstream, shallow ‘arm’ site. The sites were considered separately to determine whether the shallow arm sites would have the potential to support higher concentrations of cyanobacteria, toxins or odours which may be transported to the open water offtake sites.

The initial conditions for the scenarios employ historical and current water quality data (ie nutrient concentrations, temperature profiles and cyanobacteria numbers) from Humbug Scrub Reservoir and are built upon projections for meteorological conditions that favour algal growth, combined with scenarios for increased nutrient concentrations.

The projections are arbitrary calculations based upon:
Favourable meteorological conditions for growth

and

Either static or two circumstances leading to increased concentrations of either available and/or total phosphorus

Favourable meteorological conditions are defined as air temperatures greater than 30°C with low wind speeds persisting for a period of weeks. These conditions are generally the result of a stable high-pressure system. These meteorological conditions result in a stable water column with elevated temperature, low turbulence and the development of thermal stratification. Stable air temperatures have been used in the scenarios in this case as these conditions are most likely to give rise to thermal stratification. Many studies have shown that thermal stratification is one of the main factors in supporting cyanobacterial blooms. Small fluctuations in air temperature would not generally allow for complete 'overturn' of the reservoir, especially if stratification was persistent and therefore temperature fluctuations have not been considered in the following scenarios.

DERIVATION OF SCENARIOS

In order for a substantial blue-green algal (cyanobacterial) population to develop, the appropriate physical and climatic conditions must occur. This is a combination of an extended period of low wind, above average air temperatures and adequate solar radiation input. If these conditions persist for long enough, usually for more than 10 to 14 days, the risk of a problematic cyanobacterial bloom is high. The magnitude of the risk will then be determined by the period of time that the conditions persist, the carrying capacity of the reservoir and the types and effectiveness of management operations implemented. The carrying capacity of the reservoir is the total algal biomass that the physico-chemical conditions in the reservoir will support. The factors that are considered to be limiting to the growth of phytoplankton, and therefore cyanobacteria, are the availability of light, phosphorus (P) and nitrogen (N). As turbidity levels are relatively low in Humbug Scrub, it is unlikely that light will be limiting. Nutrient monitoring in Humbug Scrub reservoir shows that the soluble N: soluble P ratio is greater than the Redfield ratio (7:1 by mass). Given this, it is likely that phosphorus will be the limiting nutrient in the reservoir and this assumption underlies the calculations to determine the cyanobacteria carrying capacity of the reservoir.

Theoretical calculations to determine the final algal population biomass in the reservoir based on nutrient levels employ variables that are based upon selected published empirical variables. The variables and assumptions required are:

- That certain proportions of bio-available P will be converted into chlorophyll-a, and,
- Assuming that conditions are then appropriate for cyanobacterial growth, a proportion of the chlorophyll-a can be attributed to certain problem species of cyanobacteria.

Published values for chlorophyll-a per cell of *Anabaena circinalis* and *Microcystis aeruginosa* used in this assessment were 0.72 and 0.36 pg cell^{-1} respectively [8]. Therefore for a given chlorophyll-a concentration, the maximum cell concentration for either species can be determined. Concentrations of taste and odour compounds and toxins per cell or per unit chlorophyll-are also published. Bowmer *et al.* [18] report a geosmin:chlorophyll-a ratio of between 59 and 360 ng μg^{-1} at 70 and 17 μmol m^2 s^{-1} photosynthetically active radiation (PAR). Phytoplankton may experience light intensities, fluctuating at the scale of minutes to hours, from 0 to 1800 μmol m^2 s^{-1} PAR. Therefore these light intensities represent a small part of the range experienced within an illuminated water column, and are not strictly applicable

to Humbug Scrub Reservoir. However, the relationship between geosmin and chlorophyll is stronger than geosmin and cell dry weight and is therefore more suitable. Chorus and Bartram [10] report a mean microcystin:chlorophyll-a ratio of 0.12 μg μg^{-1}. These relationships can be used to estimate the maximum geosmin concentration or microcystin concentration given a certain chlorophyll yield in the reservoir.

RESULTS

HUMBUG SCRUB RESERVOIR –SITE A

CALCULATIONS

The calculations for these scenarios can be found in Table 2-2(L2) - Table 2-4(L2).

MOST LIKELY CASE

The likely scenario is derived based upon variations to drivers to the current reservoir conditions. This would be the case if the reservoir has reached a state of equilibrium so that there are no significant changes to source water quality over an extended period.

The calculations indicate that, based upon the current phosphorus concentrations at site A, theoretical populations of either *Anabaena circinalis* or *Microcystis aeruginosa* may occur at cell densities of approximately 1,820 and 3,640 cells mL^{-1}, respectively. These cell concentrations would result in a medium alert level situation and geosmin (odour) and microcystin (toxin) concentrations of approximately 13 ng L^{-1} and 0.16 μg L^{-1}, respectively. While the microcystin concentration is below the Australian Drinking Water Guideline for microcystin, the geosmin concentration is sufficient to potentially cause customer complaints.

The picoplanktonic cyanobacterium *Aphanothece* could theoretically reach a cell concentration of approximately 100,000 cells mL^{-1} under this scenario. Using a biovolume conversion this would result in an Alert Level 1 status using the draft national protocol for monitoring cyanobacteria in Australian surface freshwaters, Alert Levels Framework. If cell concentrations are used a high algal alert level situation would occur using the NSW DLWC Algal Contingency Plan in the reservoir.

REASONABLE WORST CASE

The reasonable worst case scenario considers the situation where there is an increase in the proportion of the current total phosphorus concentration that is bio-available. This may occur due to the distribution of different "species" or types of phosphorus. This may be caused by changes in microbial activity associated with altered physico-chemical environment, which in turn affects the mobilisation of phosphorus in the reservoir.

The calculations for this scenario predict that under these conditions, populations of approximately 6,000 and 12,000 cells mL^{-1} of *Anabaena circinalis* or *Microcystis aeruginosa* may occur, respectively. These cell concentrations would result in a medium alert level situation and geosmin and microcystin concentrations

of approximately 43 ng L^{-1} and 0.52 μg L^{-1}, respectively. This geosmin concentration would cause widespread complaints if not removed by the treatment process, however the microcystin concentration remains below the drinking water guideline for microcystin (1.3 μg L^{-1}).

WORST CASE

This scenario considers the situation where the internal and external loads to the reservoir become almost entirely available, the problem species became completely dominant and the odour compound or toxin were produced at their maximum reported rates. For this scenario to develop the absolute total phosphorus concentration would be likely to increase to, or above, 100 μg L^{-1}. In this 'worst-case scenario' high geosmin and microcystin concentrations would occur if there was a bloom of the appropriate species. This extreme worst case is very unlikely to occur in the short term, and would require significant decline in the source water quality over a period of time.

HUMBUG SCRUB RESERVOIR –SITE B

CALCULATIONS

The calculations for these scenarios can be found in Table 2-5(L2) - Table 2-7(L2).

MOST LIKELY CASE

The calculations indicate that, based upon the current phosphorus concentrations at site B, theoretical populations of either *Anabaena circinalis* or *Microcystis aeruginosa* may occur at cell densities of approximately 2,100 and 4,100 cells mL^{-1}, respectively. These cell concentrations would result in a medium alert level situation and geosmin (odour) and microcystin (toxin) concentrations of approximately 15 ng L^{-1} and 0.18 μg L^{-1}, respectively. While the microcystin concentration is below the Australian Drinking Water Guideline for microcystin, the geosmin concentration is sufficient to potentially cause customer complaints.

REASONABLE WORST CASE

The calculations for this scenario predict that under these conditions, populations of approximately 13,000 and 25,000 cells mL^{-1} of *Anabaena circinalis* or *Microcystis aeruginosa* may occur, respectively. These cell concentrations would result in a medium alert level situation for *Anabaena circinalis* and a high alert level status for *Microcystis aeruginosa* and geosmin and microcystin concentrations of approximately 91 ng L^{-1} and 1.1 μg L^{-1}, respectively. This geosmin concentration would cause widespread complaints if not removed by the treatment process and the microcystin concentration is approximating the drinking water guideline for microcystin (1.34 μg L^{-1}), and would require health risk assessment and appropriate water treatment for toxin removal.

WORST CASE

The worst case scenario for Site B is similar to site A

CONCLUSIONS

These scenarios indicate that Humbug Scrub Reservoir could develop taste and odour problems associated with cyanobacterial growth without any deterioration in source water quality, provided the seed source for problem cyanobacteria were available and could reach their potential under favourable meteorological conditions, or there was an increase in internal nutrient load - i.e. for the "likely" case. It is not possible to estimate or speculate upon the introduction or occurrence of the problematic cyanobacterial species in the reservoir, however these species have been recorded in the past. It must also be added that the exact nature of the physico-chemical environment that favours one type of algae or cyanobacteria over another is not entirely understood. These issues relate to subtle factors such as, for example, trace element chemistry, and microbial or grazing interactions.

The "likely" scenario which can be considered quite possible given the current reservoir conditions, could result in a medium alert level/Alert Level 1 situation. The reasonable worst case scenario can be considered to be an infrequent event (1 in 5-10 years), however as it could result in an high alert level status (Alert Level 2) there would be considerable risk associated with this scenario.

It is apparent that under certain circumstances, in the analysis of the scenarios for each site in the reservoirs, that the upstream, arm sites are more likely to support a significant cyanobacterial bloom, which could lead to significant taste, odour or toxin production. An example of this is evident in the reasonable worst case scenario for Humbug Scrub Reservoir. In this situation the predicted levels of both *Microcystis aeruginosa* and *Anabaena circinalis* are approximately twice as high at site B compared to the levels at site A. The corresponding geosmin and microcystin levels are also two-fold higher at the upstream sites.

The algal growth scenarios also show that the reservoir can support high concentrations of picoplanktonic cyanobacteria such as *Aphanothece*. The likely scenario shows that blooms could develop which contain numbers of around 100,000 cells mL^{-1} to 200,000 cells mL^{-1} of *Aphanothece*. This corresponds to around the Alert Level 1 category of the Alert Levels Framework for freshwater algae in drinking water (see Chapter 6).

RISK REDUCTION

To assess risk reduction it is necessary to first identify the factors that affect the consequence and frequency ratings of the hazard.

CONSEQUENCE

The factors that may influence the consequence of an event include,

- Whether the cyanobacterium that occurs is toxic and/or odorous
- Is the monitoring frequency sufficient to detect the cyanobacteria to allow a management response?
- What management strategies and options are available when an alert level event occurs?

In consideration of the factors which influence the consequence of an event, a number of points should be documented.

- There is no possibility for control over the toxicity of taste and odour compounds produced.
- We would expect to exceed the detection or low alert level/Alert Level 1 threshold if the monitoring program in place is adequate. This means that during normal circumstances, even when monitoring is adequate, a low alert level status may persist in the reservoir.
- The 'in reservoir' management strategies that may be applied are destratification, variable offtake height and algicide use. These management options will vary in their feasibility and effectiveness for Humbug Scrub Reservoir. For the use of algicides there are the operational, legislative (permits or registration) and environmental issues associated with their use. These have not been addressed in the past, although they are not insurmountable as an option.

FREQUENCY

The factors that may affect the frequency rating include,

- Weather patterns associated with the El Niño Southern Oscillation
- In reservoir management strategies such as destratification and algicide application.

In consideration of the factors which influence the frequency of an event, a number of points should be documented.

While the weather patterns associated with the El Nino cannot be controlled, it may be predictable and accounted for in the management of monitoring programs.

Table 2-2(L2) Calculation Table for "Likely" Scenario in Humbug Scrub Reservoir, site A

Calculations												
Scenario Assumptions:	The climatic conditions are favourable for cyanobacterial growth	The proportion of bio-available TP is:	The proportion of bio-available P converted to Chl-a is:	The proportion of Chl-a that is *Anabaena*:	The proportion of Chl-a that is *Microcystis*:	The Chl-a content of *Anabaena circinalis* is: (pg/cell)	The Chl-a content per cell of *Microcystis aeruginosa* is: (pg/cell)	The ratio of geosmin to *Anabaena* Chl-a is:	The proportion of extra-cellular geosmin is:	The ratio of microcystin to *Microcystis* Chl-a	The proportion of Chl-a that is *Aphanothece:*	The Chl-a content of *Aphanothece* is: (pg/cell)
Value:	Yes	**0.364**	**0.8**	**0.5**	**0.5**	0.72	0.36	100	0.1	0.12	**0.41**	0.01
Comments:	Population size is then determined by carrying capacity of the reservoir	Some total P unavailable due to binding to particles etc	Some bioavailable P will be taken up by other organisms	Will depend upon the degree of dominance achieved	Will depend upon the degree of dominance achieved	Published value (Reynolds 1984)	Published value (Reynolds 1984)	Mean of published range (Bowmer *et al.* 1992)	Mean of published range (Bowmer *et al.* 1992)	Mean of published range (Chorus and Bartram (1999)	Will depend upon the degree of dominance achieved	Value found in Laboratory Cultures (Hobson Pers Com)
Justification:	Given Stable conditions phosphorus concentration is likely to determine biomass in freshwaters	This value is calculated from Humbug Scrub historical data.	Most bio-available P is taken up by phytoplankton	Minor blooms of cyanobacteria may account for less than half the Chl-a	Minor blooms of cyanobacteria may account for less than half the Chl-a			Depends upon the strain and environmental conditions	Depends upon the strain and environmental conditions	Depends upon the strain and environmental conditions	Mean of calculated proportion of chlorophyll-attributable to small cyano's in Humbug Scrub	
	Concentration in Reservoir											
	TP	FRP	Chl-a	Chl-a	Chl-a	*Anabaena*	*Microcystis*	geosmin	geosmin	microcystin	Chl-a	*Aphanothece*
	ug/L	ug/L	ug/L	ug/L	ug/L	cells/mL	cells/mL	ng/L	ng/L	ug/L	ug/L	cells/mL
	50	18.20	14.56	7.28	7.28	10,111	20,222	728.00	72.80	0.87	5.97	596,960
	20	7.28	5.82	2.91	2.91	4,044	8,089	291.20	29.12	0.35	2.39	238,784
Current Level	9	3.28	2.62	1.31	1.31	1,820	3,640	131.04	13.10	0.16	1.07	107,453
	5	1.82	1.46	0.73	0.73	1,011	2,022	72.80	7.28	0.09	0.60	59,696
	2	0.73	0.58	0.29	0.29	404	809	29.12	2.91	0.03	0.24	23,878

Table 2-3(L2) Calculation Table for "Reasonable Worst Case" Scenario in Humbug Scrub Reservoir, site A

Calculations												
Scenario Assumptions:	The climatic conditions are favourable for cyanobacterial growth	The proportion of bio-available TP is:	The proportion of bio-available P converted to Chl-a is:	The proportion of Chl-a that is *Anabaena*:	The proportion of Chl-a that is *Microcystis*:	The Chl-a content of *Anabaena circinalis* is: (pg/cell)	The Chl-a content per cell of *Microcystis aeruginosa* is: (pg/cell)	The ratio of geosmin to *Anabaena* Chl-a is:	The proportion of extra-cellular geosmin is:	The ratio of microcystin to *Microcystis* Chl-a	The proportion of Chl-a that is *Aphanothece:*	The Chl-a content of *Aphanothece* is: (pg/cell)
Value:	Yes	**0.6**	**1**	**0.8**	**0.8**	0.72	0.36	100	0.1	0.12	**1**	0.01
Comments:	Population size is then determined by carrying capacity of the reservoir	Some total P unavailable due to binding to particles etc	Some bioavailable P will be taken up by other organisms	Will depend upon the degree of dominance achieved	Will depend upon the degree of dominance achieved	Published value (Reynolds 1984)	Published value (Reynolds 1984)	Maximum of published range (Bowmer *et al.* 1992)	Maximum of published range (Bowmer *et al.* 1992)	Maximum of published range (Chorus and Bartram (1999)	Will depend upon the degree of dominance achieved	Published value (Reynolds 1984)
Justification:	Given Stable conditions phosphorus concentration is likely to determine biomass in freshwaters	Higher proportions of TP are bioavailable in more eutrophic conditions	Most bio-available P is taken up by phytoplankton	Major blooms of cyanobacteria can form practically monospecific dominance	Major blooms of cyanobacteria can form practically monospecific dominance			Depends upon the strain and environmental conditions	Depends upon the strain and environmental conditions	Depends upon the strain and environmental conditions	Maximum of calculated proportion of chlorophyll-attributable to small cyano's in Humbug Scrub	
	Concentration in Reservoir											
	TP	FRP	Chl-a	Chl-a	Chl-a	*Anabaena*	*Microcystis*	geosmin	geosmin	microcystin	Chl-a	*Aphanothece*
	ug/L	ug/L	ug/L	ug/L	ug/L	cells/mL	cells/mL	ng/L	ng/L	ug/L	ug/L	cells/mL
Increase	50	30.00	30.00	24.00	24.00	33,333	66,667	2400.00	240.00	2.88	30.00	3,000,000
	20	12.00	12.00	9.60	9.60	13,333	26,667	960.00	96.00	1.15	12.00	1,200,000
Current Level	9	5.40	5.40	4.32	4.32	6,000	12,000	432.00	43.20	0.52	5.40	540,000
	5	3.00	3.00	2.40	2.40	3,333	6,667	240.00	24.00	0.29	3.00	300,000
Decrease	2	1.20	1.20	0.96	0.96	1,333	2,667	96.00	9.60	0.12	1.20	120,000

Table 2-4(L2) Calculation Table for "Worst Case" Scenario in Humbug Scrub Reservoir, site A

Calculations												
Scenario Assumptions:	The climatic conditions are favourable for cyanobacterial growth	The proportion of bio-available TP is:	The proportion of bio-available P converted to Chl-a is:	The proportion of Chl-a that is *Anabaena*:	The proportion of Chl-a that is *Microcystis*:	The Chl-a content of *Anabaena circinalis* is: (pg/cell)	The Chl-a content per cell of *Microcystis aeruginosa* is: (pg/cell)	The ratio of geosmin to *Anabaena* Chl-a is:	The proportion of extra-cellular geosmin is:	The ratio of microcystin to *Microcystis* Chl-a	The proportion of Chl-a that is *Aphanothece:*	The Chl-a content of *Aphanothece* is: (pg/cell)
Value:	Yes	**0.8**	**1**	**1**	**1**	0.72	0.36	360	0.4	1	**0.8**	0.01
Comments:	Population size is then determined by carrying capacity of the reservoir	Some total P unavailable due to binding to particles etc	Some bioavailable P will be taken up by other organisms	Will depend upon the degree of dominance achieved	Will depend upon the degree of dominance achieved	Published value (Reynolds 1984)	Published value (Reynolds 1984)	Maximum of published range (Bowmer *et al.* 1992)	Maximum of published range (Bowmer *et al.* 1992)	Maximum of published range (Chorus and Bartram (1999)	Will depend upon the degree of dominance achieved	Published value (Reynolds 1984)
Justification:	Given Stable conditions phosphorus concentration is likely to determine biomass in freshwaters	Higher proportions of TP are bioavailable in more eutrophic conditions	Most bio-available P is taken up by phytoplankton	Major blooms of cyanobacteria can form practically monospecific dominance	Major blooms of cyanobacteria can form practically monospecific dominance			Depends upon the strain and environmental conditions	Depends upon the strain and environmental conditions	Depends upon the strain and environmental conditions	Minor blooms of cyanobacteria may account for less than half the Chl-a	
	Concentration in Reservoir											
	TP	FRP	Chl-a	Chl-a	Chl-a	*Anabaena*	*Microcystis*	geosmin	geosmin	microcystin	Chl-a	*Aphanothece*
	ug/L	ug/L	ug/L	ug/L	ug/L	cells/mL	cells/mL	ng/L	ng/L	ug/L	ug/L	cells/mL
Increase	50	40.00	40.00	40.00	40.00	55,556	111,111	14400.00	5760.00	40.00	32.00	3,200,000
	20	16.00	16.00	16.00	16.00	22,222	44,444	5760.00	2304.00	16.00	12.80	1,280,000
Current Level	9	7.20	7.20	7.20	7.20	10,000	20,000	2592.00	1036.80	7.20	5.76	576,000
	5	4.00	4.00	4.00	4.00	5,556	11,111	1440.00	576.00	4.00	3.20	320,000
Decrease	2	1.60	1.60	1.60	1.60	2,222	4,444	576.00	230.40	1.60	1.28	128,000

Table 2-5(L2) Calculation Table for "Likely" Scenario in Humbug Scrub Reservoir, site B

Calculations												
Scenario Assumptions:	The climatic conditions are favourable for cyanobacterial growth	The proportion of bio-available TP is:	The proportion of bio-available P converted to Chl-a is:	The proportion of Chl-a that is *Anabaena*:	The proportion of Chl-a that is *Microcystis*:	The Chl-a content of *Anabaena circinalis* is: (pg/cell)	The Chl-a content per cell of *Microcystis aeruginosa* is: (pg/cell)	The ratio of geosmin to *Anabaena* Chl-a is:	The proportion of extra-cellular geosmin is:	The ratio of microcystin to *Microcystis* Chl-a	The proportion of Chl-a that is *Aphanothece:*	The Chl-a content of Aphanothece is: (pg/cell)
Value:	Yes	**0.13**	**0.8**	**0.5**	**0.5**	0.72	0.36	100	0.1	0.12	**0.41**	0.01
Comments:	Population size is then determined by carrying capacity of the reservoir	Some total P unavailable due to binding to particles etc	Some bioavailable P will be taken up by other organisms	Will depend upon the degree of dominance achieved	Will depend upon the degree of dominance achieved	Published value (Reynolds 1984)	Published value (Reynolds 1984)	Mean of published range (Bowmer *et al.* 1992)	Mean of published range (Bowmer *et al.* 1992)	Mean of published range (Chorus and Bartram (1999)	Will depend upon the degree of dominance achieved	Value found in Laboratory Cultures (Hobson Pers Com)
Justification:	Given Stable conditions phosphorus concentration is likely to determine biomass in freshwaters	This value is calculated from Humbug Scrub historical data.	Most bio-available P is taken up by phytoplankton	Minor blooms of cyanobacteria may account for less than half the Chl-a	Minor blooms of cyanobacteria may account for less than half the Chl-a			Depends upon the strain and environmental conditions	Depends upon the strain and environmental conditions	Depends upon the strain and environmental conditions	Mean of calculated proportion of chlorophyll-attributable to small cyano's in Humbug Scrub	
	Concentration in Reservoir											
	TP	FRP	Chl-a	Chl-a	Chl-a	*Anabaena*	*Microcystis*	geosmin	geosmin	microcystin	Chl-a	*Aphanothece*
	ug/L	ug/L	ug/L	ug/L	ug/L	cells/mL	cells/mL	ng/L	ng/L	ug/L	ug/L	cells/mL
	100	13.00	10.40	5.20	5.20	7,222	14,444	520.00	52.00	0.62	4.26	426,400
	50	6.50	5.20	2.60	2.60	3,611	7,222	260.00	26.00	0.31	2.13	213,200
Current Level	28.6	3.72	2.97	1.49	1.49	2,066	4,131	148.72	14.87	0.18	1.22	121,950
	15	1.95	1.56	0.78	0.78	1,083	2,167	78.00	7.80	0.09	0.64	63,960
	10	1.30	1.04	0.52	0.52	722	1,444	52.00	5.20	0.06	0.43	42,640

Table 2-6(L2) Calculation Table for "Reasonable Worst Case" Scenario in Humbug Scrub Reservoir, site B

Calculations												
Scenario Assumptions:	The climatic conditions are favourable for cyanobacterial growth	The proportion of bio-available TP is:	The proportion of bio-available P converted to Chl-a is:	The proportion of Chl-a that is *Anabaena*:	The proportion of Chl-a that is *Microcystis*:	The Chl-a content of *Anabaena circinalis* is: (pg/cell)	The Chl-a content per cell of *Microcystis aeruginosa* is: (pg/cell)	The ratio of geosmin to *Anabaena* Chl-a is:	The proportion of extra-cellular geosmin is:	The ratio of microcystin to *Microcystis* Chl-a	The proportion of Chl-a that is *Aphanothece:*	The Chl-a content of *Aphanothece* is: (pg/cell)
Value:	Yes	**0.4**	**1**	**0.8**	**0.8**	0.72	0.36	100	0.1	0.12	**1**	0.01
Comments:	Population size is then determined by carrying capacity of the reservoir	Some total P unavailable due to binding to particles etc	Some bioavailable P will be taken up by other organisms	Will depend upon the degree of dominance achieved	Will depend upon the degree of dominance achieved	Published value (Reynolds 1984)	Published value (Reynolds 1984)	Maximum of published range (Bowmer *et al.* 1992)	Maximum of published range (Bowmer *et al.* 1992)	Maximum of published range (Chorus and Bartram (1999)	Will depend upon the degree of dominance achieved	Published value (Reynolds 1984)
Justification:	Given Stable conditions phosphorus concentration is likely to determine biomass in freshwaters	Higher proportions of TP are bioavailable in more eutrophic conditions	Most bio-available P is taken up by phytoplankton	Major blooms of cyanobacteria can form practically monospecific dominance	Major blooms of cyanobacteria can form practically monospecific dominance			Depends upon the strain and environmental conditions	Depends upon the strain and environmental conditions	Depends upon the strain and environmental conditions	Maximum of calculated proportion of chlorophyll-attributable to small cyano's in Humbug Scrub	
	Concentration in Reservoir											
	TP	FRP	Chl-a	Chl-a	Chl-a	*Anabaena*	*Microcystis*	geosmin	geosmin	microcystin	Chl-a	*Aphanothece*
	ug/L	ug/L	ug/L	ug/L	ug/L	cells/mL	cells/mL	ng/L	ng/L	ug/L	ug/L	cells/mL
Increase	100	40.00	40.00	32.00	32.00	44,444	88,889	3200.00	320.00	3.84	40.00	4,000,000
	50	20.00	20.00	16.00	16.00	22,222	44,444	1600.00	160.00	1.92	20.00	2,000,000
Current Level	28.6	11.44	11.44	9.15	9.15	12,711	25,422	915.20	91.52	1.10	11.44	1,144,000
	15	6.00	6.00	4.80	4.80	6,667	13,333	480.00	48.00	0.58	6.00	600,000
Decrease	10	4.00	4.00	3.20	3.20	4,444	8,889	320.00	32.00	0.38	4.00	400,000

Table 2-7(L2) Calculation Table for "Worst Case" Scenario in Humbug Scrub Reservoir, site B

Calculations												
Scenario Assumptions:	The climatic conditions are favourable for cyanobacterial growth	The proportion of bio-available TP is:	The proportion of bio-available P converted to Chl-a is:	The proportion of Chl-a that is *Anabaena*:	The proportion of Chl-a that is *Microcystis*:	The Chl-a content of *Anabaena circinalis* is: (pg/cell)	The Chl-a content per cell of *Microcystis aeruginosa* is: (pg/cell)	The ratio of geosmin to *Anabaena* Chl-a is:	The proportion of extra-cellular geosmin is:	The ratio of microcystin to *Microcystis* Chl-a	The proportion of Chl-a that is *Aphanothece:*	The Chl-a content of *Aphanothece* is: (pg/cell)
Value:	Yes	**0.7**	**1**	**1**	**1**	0.72	0.36	360	0.4	1	**0.8**	0.01
Comments:	Population size is then determined by carrying capacity of the reservoir	Some total P unavailable due to binding to particles etc	Some bioavailable P will be taken up by other organisms	Will depend upon the degree of dominance achieved	Will depend upon the degree of dominance achieved	Published value (Reynolds 1984)	Published value (Reynolds 1984)	Maximum of published range (Bowmer *et al.* 1992)	Maximum of published range (Bowmer *et al.* 1992)	Maximum of published range (Chorus and Bartram (1999)	Will depend upon the degree of dominance achieved	Published value (Reynolds 1984)
Justification:	Given Stable conditions phosphorus concentration is likely to determine biomass in freshwaters	Higher proportions of TP are bioavailable in more eutrophic conditions	Most bio-available P is taken up by phytoplankton	Major blooms of cyanobacteria can form practically monospecific dominance	Major blooms of cyanobacteria can form practically monospecific dominance			Depends upon the strain and environmental conditions	Depends upon the strain and environmental conditions	Depends upon the strain and environmental conditions	Minor blooms of cyanobacteria may account for less than half the Chl-a	
	Concentration in Reservoir											
	TP	FRP	Chl-a	Chl-a	Chl-a	*Anabaena*	*Microcystis*	geosmin	geosmin	microcystin	Chl-a	*Aphanothece*
	ug/L	ug/L	ug/L	ug/L	ug/L	cells/mL	cells/mL	ng/L	ng/L	ug/L	ug/L	cells/mL
Increase	100	70.00	70.00	70.00	70.00	97,222	194,444	25200.00	10080.00	70.00	56.00	5,600,000
	50	35.00	35.00	35.00	35.00	48,611	97,222	12600.00	5040.00	35.00	28.00	2,800,000
Current Level	28.6	20.02	20.02	20.02	20.02	27,806	55,611	7207.20	2882.88	20.02	16.02	1,601,600
	15	10.50	10.50	10.50	10.50	14,583	29,167	3780.00	1512.00	10.50	8.40	840,000
Decrease	10	7.00	7.00	7.00	7.00	9,722	19,444	2520.00	1008.00	7.00	5.60	560,000

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

BACKGROUND

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

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

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

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

VISUAL INSPECTION

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

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

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

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

Figure 3-1 Cyanobacteria blooms and scums

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

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

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

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

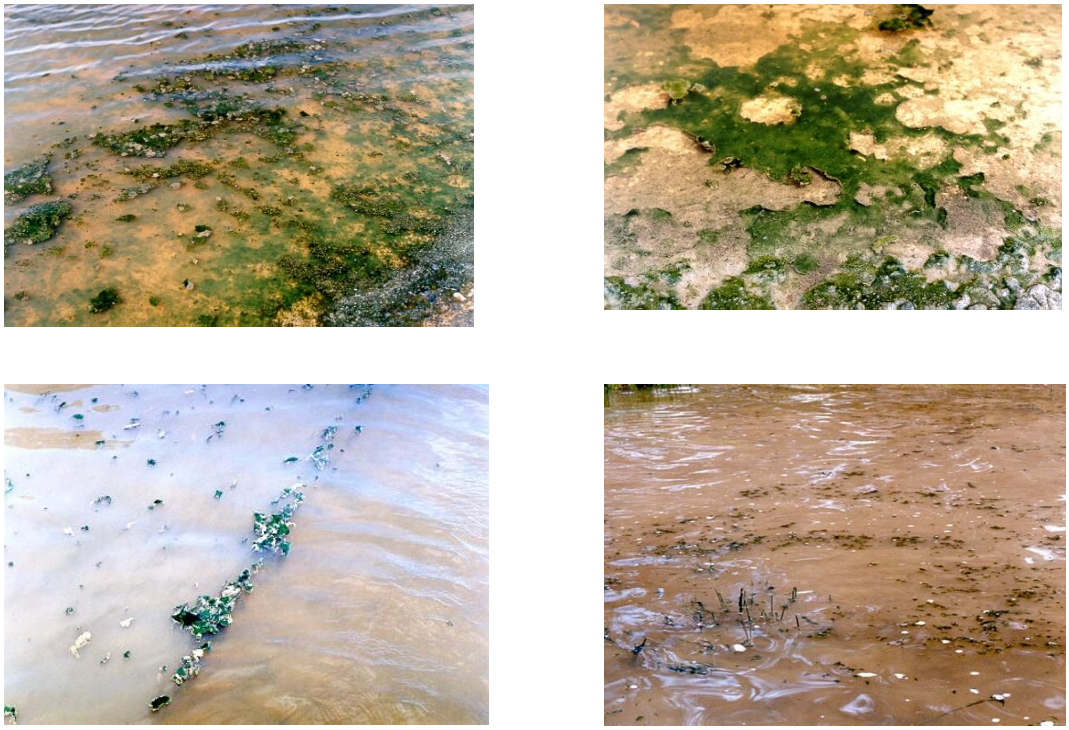

Figure 3-3 Benthic cyanobacteria attached to sediments and rock surfaces, and floating on the surface after breaking free from the substrate

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

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

SAMPLING PROGRAM DESIGN

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

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

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

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

ACCESS FOR SAMPLE COLLECTION

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

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

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

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

SAMPLE COLLECTION METHODS

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

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

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

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

SAMPLES FOR BENTHIC CYANOBACTERIAL SURVEYS

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

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

WATER SAMPLES FOR CYANOBACTERIAL IDENTIFICATION AND COUNTING

RESERVOIR/RIVER SAMPLING BY BOAT

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

Go to Level 2 for more information on open water sampling

Integrated water column samples

Discrete depth samples

SURFACE GRAB SAMPLES FROM SHORELINE

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

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

SAMPLES FOR TOXIN ANALYSIS

QUALITATIVE

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

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

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

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

QUANTITATIVE

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

SAMPLING FREQUENCY

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

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

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

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

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

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

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

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

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

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

SAMPLING REPLICATION

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

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

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

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

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

TRANSPORT AND STORAGE OF SAMPLES

SAMPLES FOR CYANOBACTERIAL IDENTIFICATION AND ENUMERATION

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

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

SAMPLES FOR TOXIN ANALYSIS

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

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

ANALYSIS FOR CYANOBACTERIA AND THEIR TOXINS

CYANOBACTERIA

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

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

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

DIRECT CELL COUNTING AND IDENTIFICATION

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

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

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

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

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

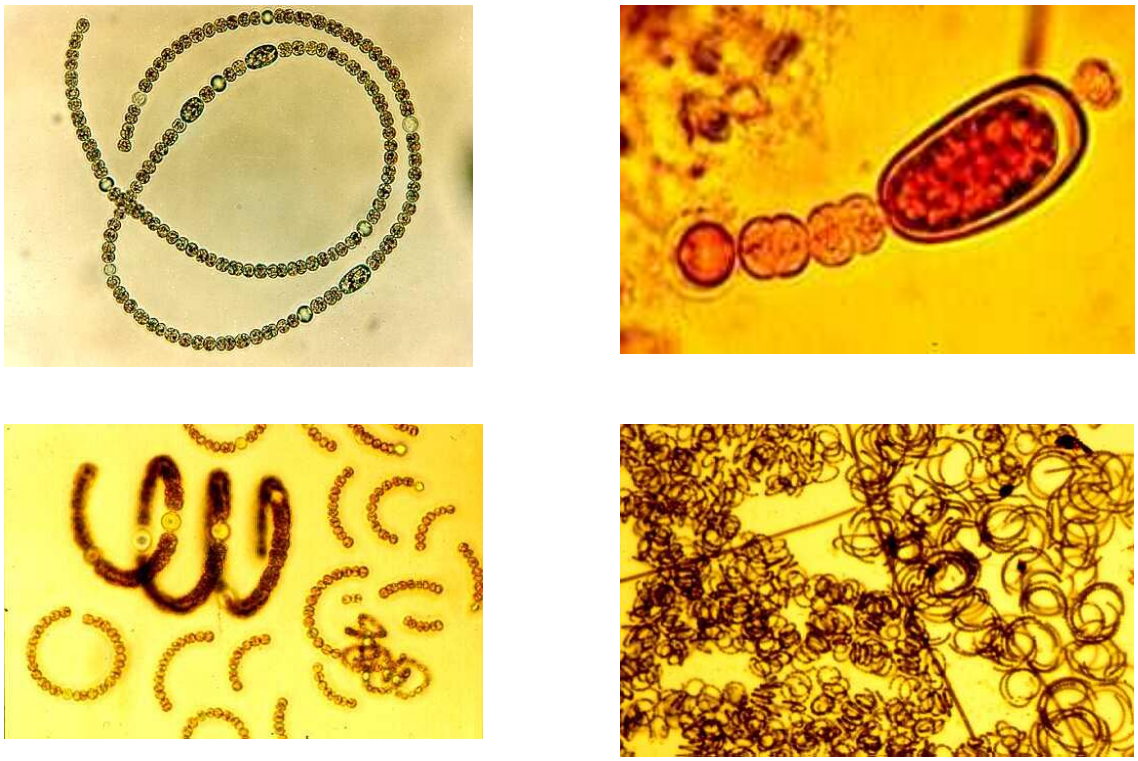

Figure 3-6 Different strains of the same cyanobacterium, *Anabaena circinalis*, several of which are toxic. This figure illustrates the difficulties inherent in microscopic identification for the determination of toxicity.

PRECISION OF CELL COUNTING

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

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

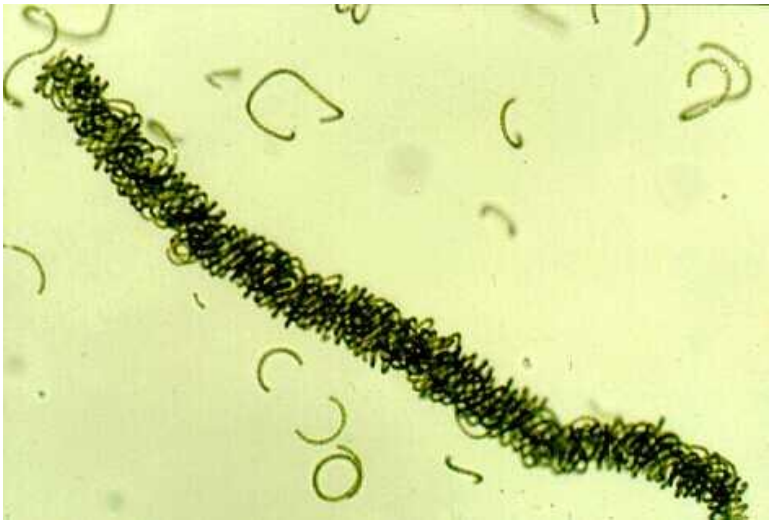

Figure 3-7 Uncertainty of enumeration of cyanobacteria is largely attributable to the clumped distribution of cells in colonies and filaments

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

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

Level 3 detailed analytical techniques:

cell counting procedure using the sedimentation technique,

cell counting procedure using the filtration technique

calculation of biovolume

MEASUREMENT OF PIGMENT CONCENTRATIONS

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

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

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

Level 3 detailed analytical techniques:

spectrophotometric technique for the determination of chlorophyll-a

CYANOTOXINS

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

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

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

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

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

ELISA

Protein phosphatase inhibition assays (PPIA)

Instrumental analysis

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

For more information on sample concentration follow this link

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

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

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

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

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

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

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

MEASUREMENT OF PARAMETERS INFLUENCING THE GROWTH OF CYANOBACTERIA

TEMPERATURE

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

Level 3 detailed analytical techniques:

determination of temperature in the field

PHOSPHORUS

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

Level 3 detailed analytical techniques:

flow injection analysis and photometric detection of ortho-phosphate

SECCHI DEPTH

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

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

Level 3 detailed analytical techniques:

for a procedure on Secchi depth measurements, click here

PH AND DISSOLVED OXYGEN

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

Level 3 detailed analytical techniques:

determination of pH in the field

determination of dissolved oxygen in the field

TURBIDITY

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

Level 3 detailed analytical techniques:

determination of turbidity

PARTICLES

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

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

VISUAL INSPECTION

RECORDING SHEET FOR A VISUAL INSPECTION

Sampling Point:……..

Date: …… Time: ……..

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

……

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

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

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

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

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

Algae/plant sample collected: No ☐ Yes ☐

Comments ……

……..

Sampler's name: ……

Received by: …… Date ……..

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SAMPLING FOR CYANOBACTERIAL IDENTIFICATION AND COUNTING

INTEGRATED WATER COLUMN SAMPLES

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

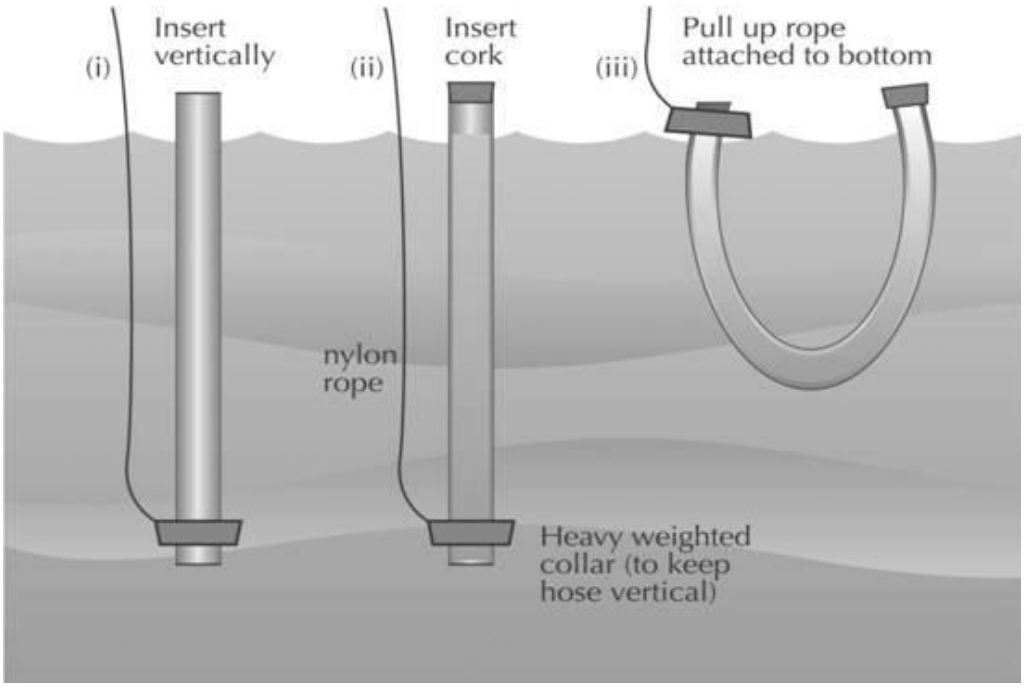

Figure 3-1 (L2) Using a hosepipe sampler to collect an integrated water column sample.

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DISCRETE DEPTH SAMPLES

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

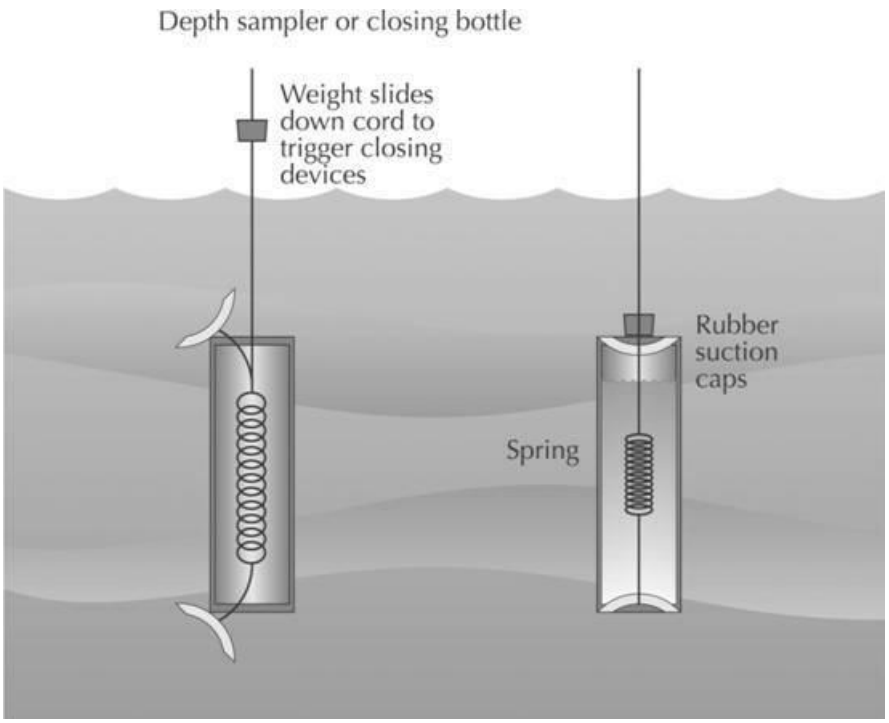

Figure 3-2(L2) Using a depth sampler or closing bottle to collect a grab sample at a discrete depth

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WORKED EXAMPLE ILLUSTRATING THE IMPACT OF SAMPLE REPLICATION

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

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

The estimated sample mean **m** is:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

SITE DESCRIPTION

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

ROUTINE SAMPLING PROGRAM

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

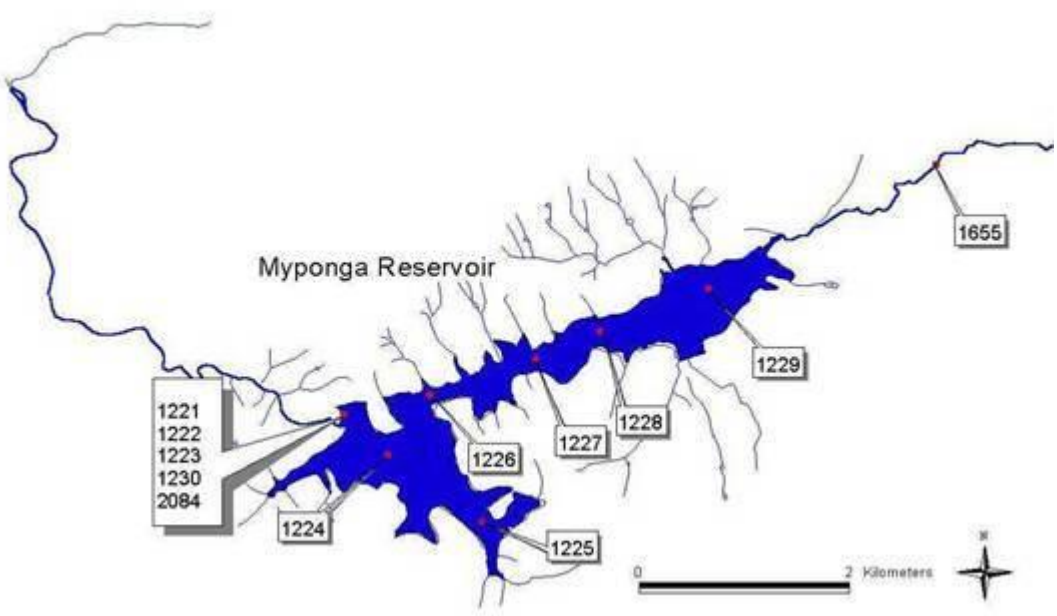

Figure 3-3(L2) Sampling Locations in Myponga Reservoir

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PRECISION OF CELL COUNTING

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

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

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

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

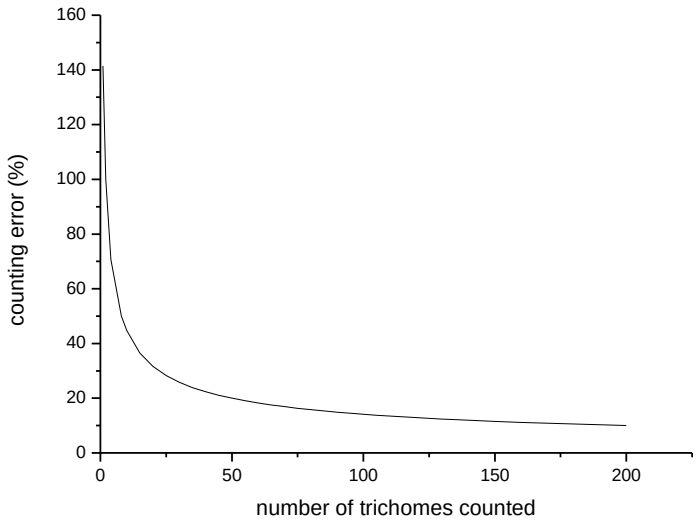

Figure 3-4(L2) Variation of counting error as a function of number of tricomes, or colonies, counted

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

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

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

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ANALYSIS FOR CYANOBACTERIA AND THEIR TOXINS

ELISA

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

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

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PROTEIN PHOSPHATASE INHIBITION ASSAYS

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

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INSTRUMENTAL ANALYSIS

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

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

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

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

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SAMPLE CONCENTRATION

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

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

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MEASUREMENT OF TOTAL, INTRACELLULAR AND EXTRACELLULAR CYANOTOXINS

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

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

ANALYSIS FOR CYANOBACTERIA AND THEIR TOXINS

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

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

CYANOBACTERIA IDENTIFICATION AND ENUMERATION BY MEANS OF A SEDIMENTATION METHOD.

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

PRINCIPLE OF THE METHOD

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

APPARATUS, MATERIALS AND REAGENTS

INSTRUMENTS AND EQUIPMENT

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

GLASSWARE

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

OTHER MATERIALS

- Lens cleaning tissue
- Lens cleaning liquid

REAGENTS

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

Figure 3-1(L3) Inverted light microscope

PROCEDURE

SAMPLE PREPARATION

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

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

IDENTIFICATION AND ENUMERATION

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

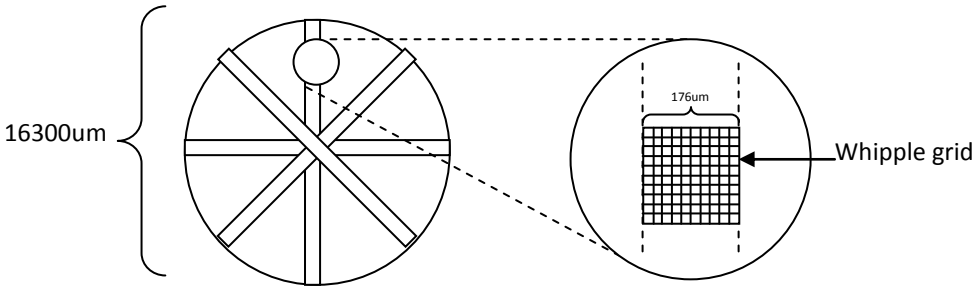

Figure 3-2(L3) Line diagram showing the orientation of lanes and the Whipple grid.

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

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- Huber-Pestalozzi, G. 1962a. Das Phytoplankton des Süsswassers: Systematik und Biologie. Tl. 1. Algemeiner Teil. Blaualgen. Bakterien, Pilze. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.
- Huber-Pestalozzi, G. 1962b. Das Phytoplankton des Süsswassers: Systematik und Biologie. Tl. 2. Diatomeen. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.
- Huber-Pestalozzi, G. 1962c. Das Phytoplankton des Süsswassers: Systematik und Biologie. Tl. 2. Hlf. 1. Chrysophyceen, Farblose Flagellaten, Heterokonten. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.

SAFETY PRECAUTIONS

HAZARD WARNING

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

SAFETY INSTRUCTIONS WHEN WORKING WITH FORMALDEHYDE (MERCK, 2004)

- Formaldehyde is toxic by inhalation, in contact with skin and if swallowed it could lead to serious irreversible effects. It could also cause burns, lead to sensitivity during skin contact and there is evidence suggesting carcinogenicity.
- Formaldehyde should always be stored at 15°C - 25°C in a tightly closed container in a well ventilated place.
- When handling this substance, personal protective equipment, such as latex gloves, a laboratory coat and safety glasses, should be used.
- Formaldehyde is heavier than air and should always be used in a suitable extraction cabinet, that is, one with a down flow extraction system.
- Never inhale the substance and avoid any generation of vapours of this substance. The inhalation of fresh air is best after inhalation of formaldehyde.

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

SAFETY INSTRUCTIONS WHEN WORKING WITH ETHANOL (MERCK, 2006)

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

CALCULATIONS AND EXPRESSION OF RESULTS

CALCULATION OF THE PHYTOPLANKTON BIOMASS AS CELLS/MℓL

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

- Calculate the area of the circular sedimentation chamber floor:

Sedimentation chamber floor area	=	πr^2
	=	π x $(8150\mu m)^2$
	=	208 672 438μm^2

- Calculate the area of one rectangular lane:

Lane area =	diameter of sedimentation chamber x width of Whipple grid	
=	16 300µm x 176µm	
=	2 868 800μm^2	

- Calculate the conversion factor:

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

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

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

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

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

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

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

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

Calculating the percentage composition of a taxon

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

REPORTING PHYTOPLANKTON RESULTS

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

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PHYTOPLANKTON IDENTIFICATION AND ENUMERATION BY MEANS OF THE FILTRATION METHOD

BACKGROUND

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

PRINCIPLE OF THE METHOD

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

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

APPARATUS, MATERIALS AND REAGENTS

INSTRUMENTS AND EQUIPMENT

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

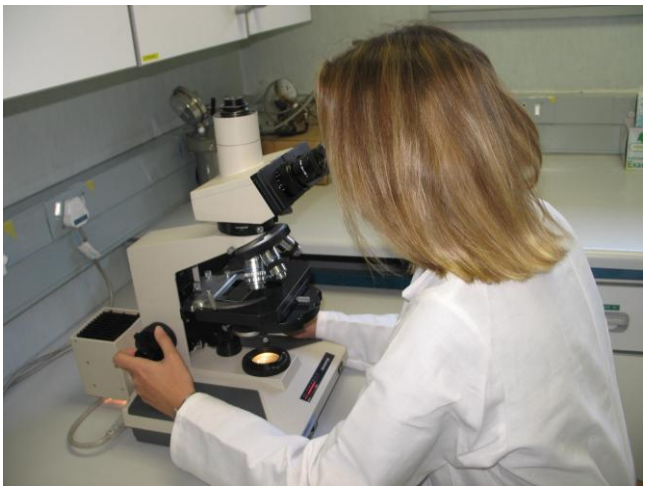

Figure 3-3(L3) Compound light microscope

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

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

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

GLASSWARE

25mL, 50mL and 2000mL measuring cylinders.

OTHER MATERIALS

0.45µm filters of appropriate quality.

REAGENTS

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

PROCEDURE

SAMPLE PREPARATION

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

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

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

IDENTIFICATION AND ENUMERATION

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

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

SAFETY PRECAUTIONS

HAZARD WARNING

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

CALCULATION AND EXPRESSION OF RESULTS

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

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

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

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

The results are expressed as counts per mL.

Sources of error may arise from the following:

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

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BIOVOLUME CALCULATION OF PHYTOPLANKTON AND CYANOBACTERIA [25]

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

The method for determining cyanobacterial cell biovolume is:

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

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

This process should be repeated individually for each cyanobacterial species.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

BACKGROUND

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

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

Certain interferences with the method have been identified:

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

APPARATUS, MATERIALS AND REAGENTS

INSTRUMENTS AND EQUIPMENT

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

GLASSWARE

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

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

OTHER MATERIALS

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

REAGENTS

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

PROCEDURE

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

SAFETY PRECAUTIONS

HAZARD WARNING

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

CLOTHING

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

SAFETY INSTRUCTIONS WHEN WORKING WITH ETHANOL

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

SAFETY INSTRUCTIONS WHEN WORKING WITH ACID

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

CALCULATION AND EXPRESSION OF RESULTS

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

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

Where:

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

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

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

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

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FLOW INJECTION ANALYSIS AND PHOTOMETRIC DETECTION OF ORTHO PHOSPHATE.

BACKGROUND

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

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

PRINCIPLE OF THE METHOD

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

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

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

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

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

APPARATUS, MATERIAL AND REAGENTS

APPARATUS

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

- Reaction unit or manifold
- Colorimetric detector
- Data system

MATERIAL:

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

REAGENTS:

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

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

PROCEDURE

PREPARING OF SOLUTIONS:

AMMONIUM MOLYBDATE SOLUTION

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

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

POTASSIUM ANTIMONY TARTRATE SOLUTION

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

MOLYBDATE COLOUR REAGENT

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

ASCORBIC ACID REDUCING SOLUTION

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

SODIUM HYDROXIDE - TITRIPLEX RINSE

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

CALIBRATION OF STOCK SOLUTIONS:

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

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

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

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

CALIBRATION STANDARDS

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

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

SAMPLE PREPARATION:

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

OPERATING INSTRUCTIONS OF ANALYZER / DATA SYSTEM:

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

CALCULATION AND EXPRESSION OF RESULTS:

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

Return to level 1

SECCHI DEPTH MEASUREMENTS

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

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

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

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

PROCEDURE

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

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

Return to level 1

DETERMINING TEMPERATURE IN THE FIELD

BACKGROUND

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

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

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

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

APPARATUS, MATERIAL AND REAGENTS

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

APPARATUS

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

REAGENTS

- 0.01M KCl
- Deionised water

PROCEDURE

TEMPERATURE VERIFICATION (QUALITY CONTROL) IN THE LABORATORY

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

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

TEMPERATURE READING IN THE FIELD

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

Return to level 1

DETERMINING PH IN THE FIELD

BACKGROUND

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

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

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

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

PRINCIPLE OF THE METHOD

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

APPARATUS, MATERIAL AND REAGENTS

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

APPARATUS

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

- Teflon sample cups (250mL)

REAGENTS

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

PROCEDURE

PH CALIBRATION:

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

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

PH READING IN THE FIELD

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

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

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

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DETERMINATION OF DISSOLVED OXYGEN IN THE FIELD

BACKGROUND

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

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

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

Erratic results may be due to:

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

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

PRINCIPLE OF THE METHOD

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

APPARATUS, MATERIAL AND REAGENTS

APPARATUS

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

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

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

REAGENTS

- Deionised water
- 0.01M KCL
- 0.4M Na_2SO_3

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

PROCEDURE

CALIBRATION

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

DISSOLVED OXYGEN READING IN THE FIELD

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

Return to level 1

METHOD FOR THE DETERMINATION OF TURBIDITY

BACKGROUND

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

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

PRINCIPLE OF THE METHOD

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

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

APPARATUS, MATERIAL AND REAGENT

APPARATUS:

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

MATERIALS:

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

REAGENTS:

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

PROCEDURE

CALIBRATION OF INSTRUMENT

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

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

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

MEASUREMENT PROCEDURE:

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

Return to level 1

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CHAPTER 4 TABLE OF CONTENTS

CHAPTER 4 MANAGEMENT AND CONTROL IN SOURCE WATERS (LEVEL 1)

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 damage or destroy the cyanobacteria. Management strategies have recently been comprehensively summarised and reviewed by Cooke et al. [1].

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 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. [2, 3, 4].

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 4-1(a).

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.

Level 2 link to more detail about aerators

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 4-1(b).

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.

Links to Myponga Reservoir case studies

Effect of mixing on stratification and the phytoplankton community

Effect of mixing on nutrient release and algal biomass

Using mathematical models to predict cyanobacterial growth

Simulation of various management strategies

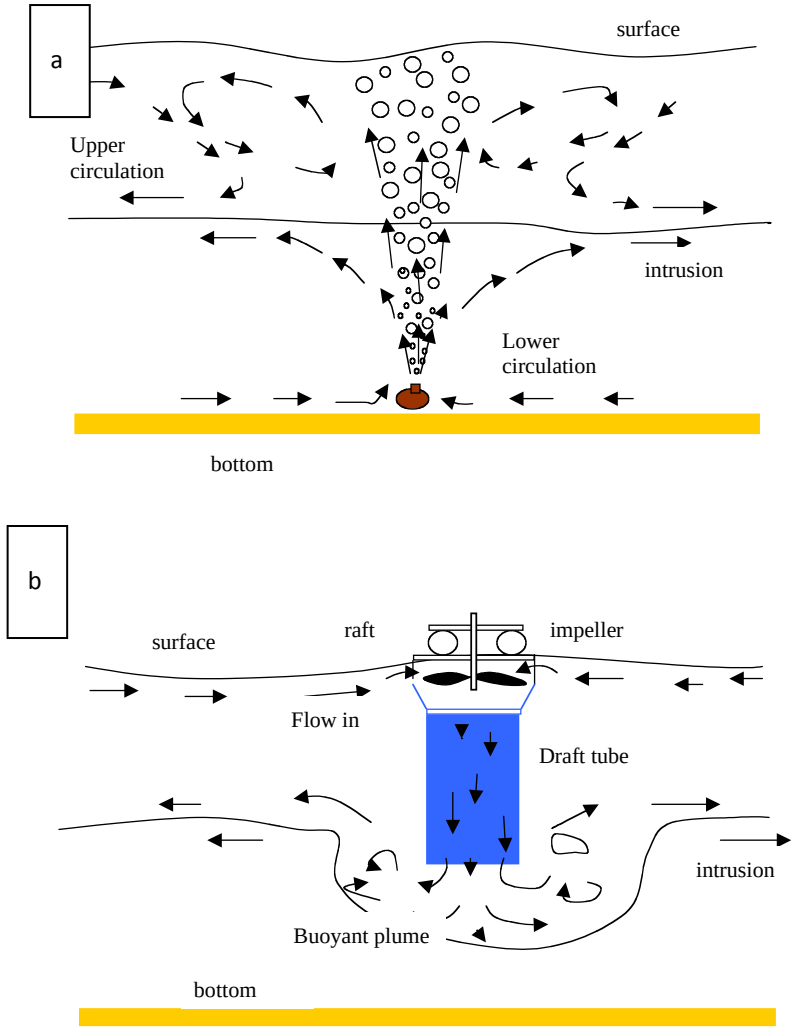

Figure 4-1 Flow and circulation fields created by a bubble plume aerator a) and a surface-mounted mechanical mixer b) in reservoirs

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 [5]. It is therefore important to apply the appropriate mixing processes for a particular water body. Schladow [6] 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.

A summary of some factors influencing the application of destratification can be found here

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 [7] reported the development of criteria for flow manipulation that may result in destratification sufficient to disrupt cyanobacterial growth. 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 [8] 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 [9], 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 [10]. 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 [5].

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 [11]. 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.

Link to phosphorus precipitation case study

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 [12]. 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)

Chemicals that have been utilised as algicides are 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 [13])

Compound	Formulation	References
Copper sulphate	$CuSO_4.5H_2O$	14, 15,16,17
Copper II alkanolamine	Cu alkanolamine.$3H_2O^{++}$	18
Copper-ethylenediamine complex	$[Cu(H_2NCH_2CH_2NH_2)_2(H_2O)_2]^{++}SO_4$	18
Copper-triethanolamine complex	Cu $N(CH_2CH_2OH)_3.H_2O$	18
Copper citrate	$Cu_3[(COOCH_2)_2C(OH)COO]_2$	19,20
Potassium permanganate	$KMnO_4$	21 22
Chlorine	Cl_2	21
Lime	$Ca(OH)_2$	23
Barley straw		24, 25

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 [26]. 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 [27, 28]. 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 [16].

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)).

a) 

b) 

c) 

d) 

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

Recommendations for copper sulphate dosing techniques, including dose rate and application

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 [16]. 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.* [18]. 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. [19]. It is available either as a commercial preparation [29] or by simultaneously dosing copper sulphate and citric acid [19]. 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 [30].

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 [22]. Fitzgerald [22] 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 [15]. 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} [15].

Hydrogen peroxide: Hydrogen peroxide has been shown to selectively damage cyanobacteria over other planktonic species such as green algae [31]. 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 [32]. A range of microorganisms have been shown to very effectively degrade several of the major cyanotoxins, including microcystins and cylindrospermopsin [33, 34]. However, the time taken for total toxin degradation varies widely from 3-4 days to weeks or months depending upon the circumstances [35]. 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 [33, 36]. 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 [34]. However, in water bodies where the cylindrospermopsin is found regularly degradation has been shown to occur relatively rapidly [37].

Saxitoxins have not been shown to be degraded by bacteria so, if a toxic bloom of *Anabaena circinalis* is dosed, it may be necessary to have water treatment strategies for dissolved toxin removal [38]. In addition, although saxitoxin appears to be non-biodegradable, it can undergo biotransformations involving conversion from less toxic forms to more toxic variants [39].

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 [5]

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 [5]. 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 culturing large numbers of microorganisms, and the ability of the cyanobacteria to become immune to infection [5].

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 [5].

Click here for more detailed information on the manipulation of the foodweb to improve water quality

Click here to read a case study of biomanipulation

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 [24, 25, 40, 41]. 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 [25]. While reservoir trials with barley straw appeared to confirm these laboratory observations [41, 42], other trials resulted in no observable effect [43, 44].

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 [45].

Although some water authorities have applied this method due to the low cost and appeal as a natural treatment, Chorus and Mur [5] 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 [46] as well as causing sedimentation due to disruption of the gas vesicles [47] depending on the energy and length of time of application. The observed effects are also dependent on the species of cyanobacteria [48]. 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 [49]. 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 4 MANAGEMENT AND CONTROL IN SOURCE WATERS (LEVEL 2)

PHYSICAL CONTROLS

POSITIONING OF AERATORS IN A RESERVOIR

Destratification devices are usually placed near the offtake or dam wall in a deep area of the reservoir. It is possible to simulate reservoir destratification using a hydrodynamic-ecological numerical model to determine whether the destratifier will maintain cyanobacterial growth below 2000 cells mL^{-1} (for geosmin producing *Anabaena circinalis*) and dissolved oxygen (DO) at greater than 4mg L^{-1}. The one-dimensional hydrodynamic ecological model, DYRESM-CAEDYM, is ideal for this type of modelling. DYRESM-CAEDYM was developed by the Centre for Water Research and is available as free-ware from www.cwr.uwa.edu.au. Combinations of the various management options (e.g. no artificial intervention, aerator operating, surface mixers etc) can be simulated to determine which operational strategies would give the desired result of low cell numbers and increased DO. Informed operational strategies can then be implemented according to the results of the simulation.

Return to level 1

MIXING CASE STUDY – MYPONGA RESERVOIR

THE PHYTOPLANKTON COMMUNITY

This case study was derived from [50 and 58].

Current management at Myponga Reservoir in South Australia includes both artificial destratification and chemical algicides to control cyanobacteria. Although there are two different destratifying systems in Myponga Reservoir, there is still strong persistent stratification in the surface layer at particular times when high nocturnal temperatures and low wind speed inhibit cooling (Figure 4-1 (L2)). However, modelling studies have shown that the destratifiers have significantly reduced the period over which *Anabaena* can grow. The phytoplankton community in Myponga Reservoir is dominated by green algae and diatoms, which rely on turbulence to remain entrained, and the conditions when cyanobacteria grow have been narrowed to short periods each year (Figure 4-2(L2)).

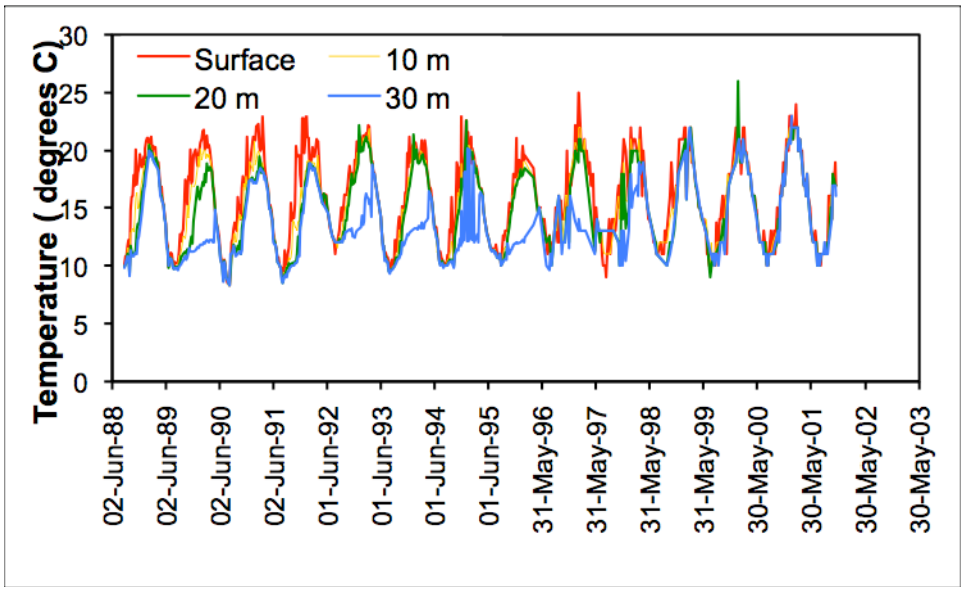

Figure 4-1(L2) Temperature measured weekly at the surface, 10 m, 20 m and 30m depth adjacent to the off-take point at Myponga Reservoir. The aerator was installed in 1994.

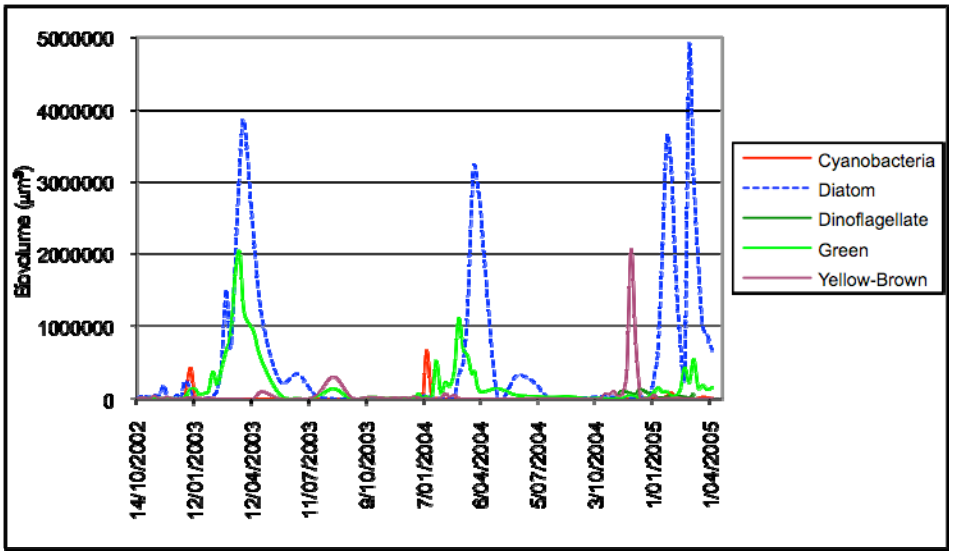

Figure 4-2(L2) Relative abundance of the different phytoplankton groups in Myponga Reservoir

Return to level 1

ARTIFICIAL DESTRATIFICATION TO CONTROL THE NUTRIENT LOAD

Seasonal temperature stratification was evident at Myponga Reservoir during summer from 1984 until 1994. Since installation of the aerator in 1994, close to isothermal conditions have been maintained at the sampling site (Figure 4-1(L2)). However, surface layer heating is evident at other sites in the reservoir outside of the immediate bubble plume, which is consistent with other reservoirs where bubble plume aerators are operating [56,52]. Dissolved oxygen concentrations were

below 4 mg L^{-1} for extended periods during 1992/93 and 1993/94, which provided conditions suitable for contaminant resolubilisation. Since aerator operation in 1994 the dissolved oxygen concentration at 30 m has been maintained above 4 mg L^{-1}.

Prior to 1994 the concentration of filterable reactive phosphorus (FRP) at 30 m depth was consistently higher than the surface concentrations during summer and autumn (Figure 4-3(L2)). This coincides with the periods of extreme temperature stratification and low dissolved oxygen in the hypolimnion. Filterable reactive phosphorus at 30 m depth reached a maximum concentration of 0.259 mg L^{-1} in April 1986. The vertical gradient in FRP concentration has decreased since deployment of the bubble plume aerator and the large flux events have been eliminated.

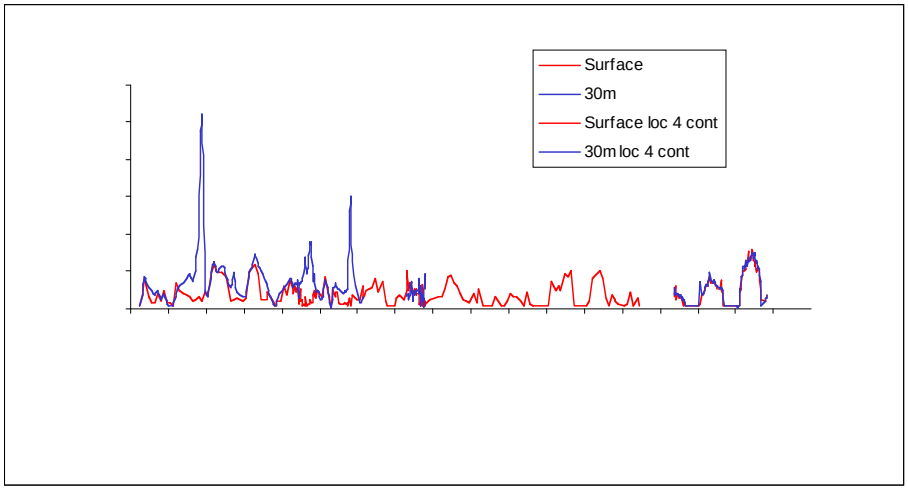

Figure 4-3(L2) Filterable reactive phosphorus at the surface and 30 m at Location 1 near the dam wall and from Location 4 from October 1998. Aerator installation decreased the internal nutrient load and high concentrations in the hypolimnion were not observed following aerator deployment.

RELATING NUTRIENTS TO ALGAL BIOMASS

In Myponga Reservoir the nutrient loading from the catchment occurs predominantly during winter and early spring. The nutrient pool is not utilised immediately as phytoplankton growth is limited by cool water temperatures and grazing pressure. As water temperature increases the phytoplankton grow rapidly and chlorophyll-a concentration increases with an associated decrease in FRP (Figure 4-4(L2)). FRP concentrations decrease to below the detection limit (0.005 mgL^{-1}) and the chlorophyll decreases some time later and the seasonal cycle is repeated.

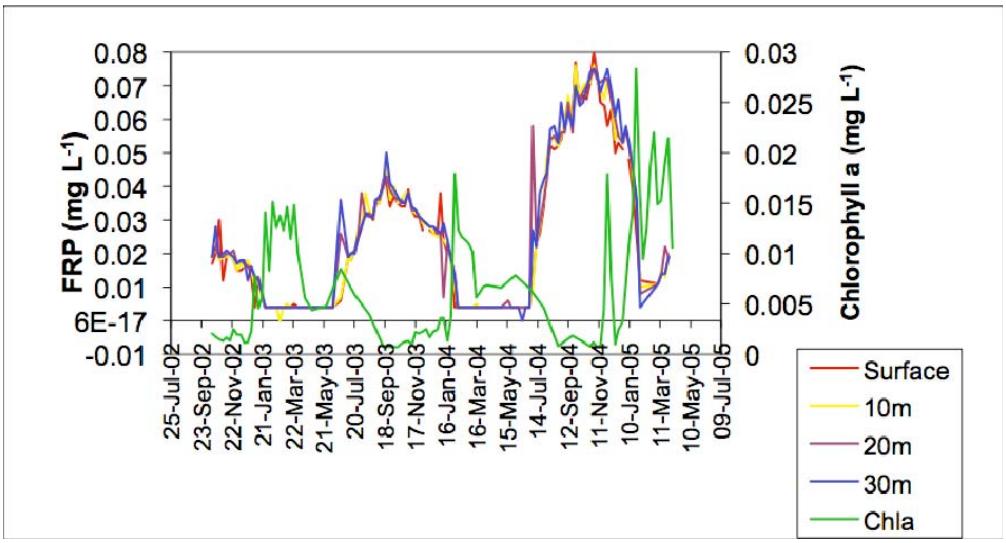

Figure 4-4(L2) Filterable reactive phosphorus at four depths and chlorophyll concentration integrated over the top 5m.

With the internal nutrient load largely controlled in Myponga Reservoir by the aeration system, the catchment is the dominant source of nutrients. In Myponga Reservoir two tributaries contribute the majority of the nutrient load, but loading is both seasonally and inter-annually variable. High inflow to the reservoir results in high total phosphorus (TP) loads and reservoir concentrations. A high maximum TP concentration in Myponga Reservoir results in a high chlorophyll-a concentration. Figure 4-5(L2) shows the relationship between the maximum annual TP concentration and the maximum chlorophyll-a found in the following growth period in the years between 1985 and 2000. Two outlier years, 1988 and 1993, are excluded from the regression in the figure. 1988 was an unusual year in that rains were early, and consequently there was a 6 month interval between the TP and Chl-a maximum. In 1993, hypolimnetic anoxia caused by thermal stratification, released higher than usual FRP concentrations from the sediments, sustaining high algal biomass and resulted in a high maximum chlorophyll-a concentration. The operation of the bubble plume aeration system since 1994 has most likely prevented this situation from recurring [53].

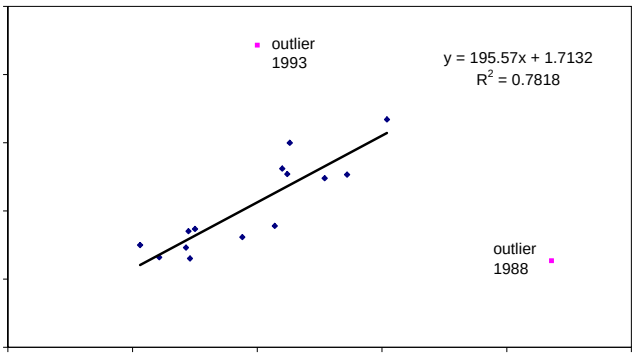

Figure 4-5(L2) Relationship between maximum total phosphorus and maximum chlorophyll *a* in the following growing period

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MODELLING ALGAL GROWTH

Because weather and limnological conditions are never constant it is difficult to determine whether destratification has an impact on cyanobacterial growth without very extensive historical data sets. An alternative approach is the use of numerical models to simulate the hydrodynamics and cyanobacterial growth. DYRESM-CAEDYM is a coupled hydrodynamic, water quality and algal growth model available as free-ware from the Centre for Water Research, The University of Western Australia (http://www.cwr.uwa.edu.au/). The modelling approach has been used in these studies to evaluate destratification in Myponga Reservoir. Meteorological variables measured at the stations on the reservoir were used for model inputs. Algal growth was simulated using equations describing nutrient and light-limited growth of *Anabaena circinalis* and floating velocity.

The DYRESM-CAEDYM simulation of the phytoplankton community was undertaken for the period September 2000 to March 2001. The observed and simulated total Chl-a concentrations are shown in Figure 4-6(L2). The simulated biomass captures the timing of the summer peak in the field data, but did not simulate the unseasonal peak that occurred in December 2000. This peak was attributed to the excessive growth of *Chroomonas* sp., a species which was not included in the model. The simulated growth of *Anabaena circinalis* from September-2000 to March-2001 produced a reasonable match with the observed field data (Figure 4-7(L2)), although the simulated growth started earlier in the season than observed in the field.

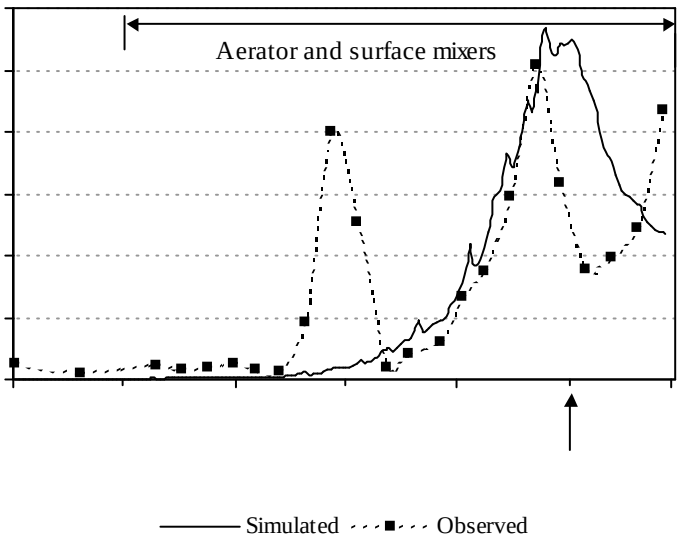

Figure 4-6(L2) Observed and simulated total Chl-a concentration (µg Chl-a L^{-1}), with simulated $CuSO_4$ dosing on 31-January-2000, and surface mixers and aerator operating between 1-October-2000 and 28-February-2001.

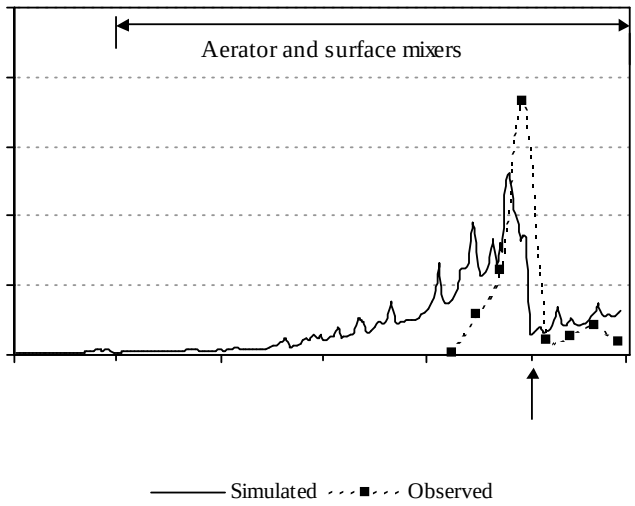

Figure 4-7(L2) Observed and simulated *Anabaena circinalis* concentration (µg Chl-a L^{-1}) from 1-September-2000 to 1-March-2001.

The simulation of the 3 types of phytoplankton that were representative of the assemblage in Myponga Reservoir from September 1999 to March 2001 produced reasonable results considering the limitations of the model. The observed phytoplankton community consisted of more than the three species simulated in this model. Other species will dominate with changes in nutrients, light and temperature as highlighted by the excessive growth of *Chroomonas*. An improvement to the CAEDYM model would be to increase the number of species simulated, although this would require intensive calibration. A trial and error approach as used in this study would be insufficient.

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SIMULATION OF VARIOUS MANAGEMENT STRATEGIES

The CAEDYM model output compared with observed field data gave a reasonable representation of phytoplankton biomass (as total Chl-a) for three species in Myponga Reservoir. The comparison between observed and simulated for *Scenedesmus* showed a strong correlation whereas a moderate correlation was observed with *Anabaena circinalis*. The next step involved using the model to determine the individual and combined impact of the surface mixers and the aerator for destratification and control of cyanobacteria. The following strategies were investigated for their ability to maintain DO greater than 4 mg L^{-1} and to limit *Anabaena circinalis* below 2,000 cells mL^{-1}.

1. No artificial intervention
2. Aerator and surface mixers with no $CuSO_4$ dosing
3. Aerator only
4. Surface mixers at measured flow rate (3.5 $m^3 s^{-1}$)
5. Surface mixers at design flow rate (5 $m^3 s^{-1}$)
6. Surface mixers at increased flow rate (8 $m^3 s^{-1}$)
7. Intermittent operation
8. Equivalent aerator energy input using surface mixers

Detailed results of this modelling can be found in [58].

The effectiveness of the various operational strategies used to limit the growth of *Anabaena circinalis* and maintain DO concentration in the water column is summarised in Table 4-1(L2). The simulation employed for validation, including the surface mixer, bubble plume aerator and $CuSO_4$ dosing algorithms, produced similar results to the observed field data. If no artificial mixing or $CuSO_4$ dosing were employed, excessive growth of *Anabaena circinalis* would occur and permanent stratification would lead to the presence of anoxic conditions. The use of the aerator without $CuSO_4$ dosing adequately maintained well-mixed conditions and DO throughout the water column. However, the growth of *Anabaena circinalis* could exceed 1,000 cells mL^{-1} (for a total of 16 days) but would not reach the threshold of 2,000 cells mL^{-1}.

When the aerator is coupled with the surface mixers (at 3.5 $m^3 s^{-1}$), the growth of *Anabaena circinalis* was further reduced with the peak concentration falling from ~ 1,400 cells mL^{-1} to ~ 1,000 cells mL^{-1}. The operation of the surface mixers (3.5 $m^3 s^{-1}$) alone would not be able to destratify the water column and maintain DO at acceptable levels, and importantly the growth of *Anabaena circinalis* would exceed 2,000 cells mL^{-1}. Increasing the flow rates of the surface mixers improves their destratification ability and reduces the growth of *Anabaena circinalis*. With a surface mixer flow-rate of 8 $m^3 s^{-1}$, optimal results were achieved maintaining DO above 4 mg L^{-1} and limiting the maximum concentration of *Anabaena circinalis* to ~ 1,000 cells mL^{-1}.

Using intermittent mixing, the growth of cyanobacteria was restricted to a maximum concentration of ~ 700 cells mL^{-1} and well-mixed conditions were maintained. The use of $CuSO_4$ dosing would not be required under this strategy and operational costs would be lower due to the reduced use of the aerator and surface mixers. The use of 25 surface mixers, using the same energy as the existing aerator, adequately destratified Myponga Reservoir and almost completely inhibited the growth of *Anabaena circinalis*.

Table 4-1(L2) Results from existing and simulated water quality management strategies.

Artificial mixing operation	Maximum cyanophyte concentration (cells.mL^{-1})	Days above 1000 cells.mL^{-1}	Minimum DO (mgL^{-1})	Simulated phytoplankton assembly composition		
				Chlorophytes	Cyanophytes	Diatoms
Existing - Field	1625	1	~5.00	96.30%	0.50%	3.20%
Existing - Sim	278	0	4.70	96.60%	0.70%	2.70%
Strategy 1	4444	196	1.00	91.30%	6.80%	1.90%
Strategy 2	1069	3	4.70	94.10%	2.90%	3.00%
Strategy 3	1389	16	4.70	92.90%	4.00%	3.10%
Strategy 4	2361	133	1.20	93.90%	4.70%	1.40%
Strategy 5	1556	21	4.70	95.30%	3.40%	1.30%
Strategy 6	1014	1	4.70	96.40%	2.40%	1.20%
Strategy 7	667	0	4.70	97.10%	1.70%	1.20%
Strategy 8	153	196	4.70	98.30%	0.60%	1.10%

The addition of the surface mixer and $CuSO_4$ dosing algorithms to DYRESM-CAEDYM enabled the phytoplankton succession and DO concentration to be adequately simulated and validated against observed field data for the period 1 September 1999 to 1 September 2000. This enabled various management strategies to be investigated. Modelling showed that the potential for growth of *Anabaena circinalis* would occur during periods of thermal stratification and with the presence of a

shallow surface mixed layer. This coincided with oxygen depletion in the hypolimnion and adequate levels of nutrients (FRP> 0.01 mg L^{-1} and NO_x > 0.1 mg L^{-1}).

The actual mixing program with an aerator at Myponga Reservoir adequately maintains DO throughout the water column, and coupled with $CuSO_4$ dosing, limits the growth of *Anabaena circinalis* to a maximum concentration of ~ 1,600 cells mL^{-1} or 1.17 µg Chl-a L^{-1} (0.5% of the total biomass as Chl-a). The simulation of the existing aerator, surface mixers and $CuSO_4$ dosing produced similar results, affirming the need for intervention to maintain manageable levels of cyanobacteria and DO concentrations. The simulation showed that when the surface mixers and aerator are used without $CuSO_4$ dosing (strategy 2) the *Anabaena circinalis* would not exceed concentrations that would be of concern for water supply. The sole use of the surface mixers was found to be adequate at maintaining water quality if the flow rate could be increased to 8 m^3s^{-1}. However, at their current flow rate (3.5 $m^3 s^{-1}$) they are unable to fully destratify Myponga Reservoir and limit the growth of *Anabaena circinalis* to below 2,000 cells mL^{-1}.

The use of intermittent artificial mixing would reduce operational costs as the aerator and surface mixers would run at 50% less than the current operational schedule. Using this technique, destratified conditions are maintained, DO concentrations are kept high and the growth of *Anabaena circinalis* is minimal and importantly, the use of $CuSO_4$ dosing is not necessary. Under the current operating conditions, the simulation demonstrated that the use of $CuSO_4$ dosing is not necessary, as *Anabaena circinalis* concentrations did not exceed 2,000 cells mL^{-1}. As demonstrated with DYRESM-CAEDYM, the current nutrient concentrations, light climate, meteorological forcing and artificial mixing operations at Myponga Reservoir do not favour the excessive growth of *Anabaena circinalis*. However, even at these concentrations taste and odours can be problematic and require additional treatment.

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FACTORS INFLUENCING DESTRATIFICATION

The theoretical requirements for mixing to cause destratification and reduce cyanobacterial growth and biomass can be explained as follows. The reduction of cyanobacterial biomass is dependent upon the relationship between the depth to which the water column is mixed (Z_{mix}) and the depth of the penetration of light or photosynthetically active radiation (PAR, 400-700nm) into the water column. Light penetration is often described as the euphotic depth (Z_{eu}) which is the depth to which 1% of the subsurface irradiance penetrates [51]. The ratio between these depths can be used to evaluate the potential for light availability to limit the growth of phytoplankton which are circulating within the surface mixed layer. For example Z_{mix}:Z_{eu} ratios of 2.5 [3] or 3 [52] are regarded as ratios that will not support cyanobacterial growth. This means that the surface layer must mix to much deeper than light penetrates. Therefore, both the mixing and the clarity of the water column determine the Z_{mix}:Z_{eu} ratio. It follows that if a water body is inherently turbid or coloured it is theoretically more suitable to use mixing as a control technique than in clear water because the euphotic depth is shallower.

Artificial destratification has achieved good results in reducing iron and manganese problems for water treatment plants [53, 54], however the results in relation to the control of nuisance algae and cyanobacteria have been more variable [55]. This is most likely due to the complex interaction of the effects of destratification upon the availability of nutrients and light which are both required for the growth of photosynthetic organisms such as algae and cyanobacteria.

Destratification systems operating in deep reservoirs (mean depth >15m) have generally been more successful in changing the composition of the phytoplankton community [56, 3], while studies in shallower water bodies show less impact [57,52]. Even in deep reservoirs destratifiers may not be able to prevent the development of a stratified surface layer, outside of the immediate influence of the plume or mixer, which means that there is still a habitat for buoyant cyanobacteria to exploit [56].

It is likely that in situations where artificial destratification has failed to reduce cyanobacterial growth, neither nutrients nor light were limited sufficiently to impact on growth. Either there was a large enough external load to continue to supply adequate nutrients, and therefore limiting the internal load was inconsequential, or the artificial mixing was not adequate to light-limit the cyanobacteria.

A detailed description and comparison of the use of aerators and mechanical mixers to control cyanobacteria is provided in [58].

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CHEMICAL CONTROLS

PHOSPHORUS PRECIPITATION CASE STUDY

Taken directly from the USEPA website:

http://www.epa.gov/owow/lakes/kezar.html

Watershed Protection: Clean Lakes Case Study

Phosphorus Inactivation and Wetland Manipulation

Improve Kezar Lake, NH

EPA 841-F-95-002

Office of Water (4503F)

Kezar Lake, located in central New Hampshire, has had a long history of water quality problems. Following a major fish kill and persistent algae blooms beginning in the early 1960s, a Diagnostic/Feasibility Study (Phase I of the Clean Lakes Program) was initiated in 1980 under section 314 of the Clean Water Act. The study established that the lake's problems were from internal loading of phosphorus, and outlined a management strategy to restore the lake. Lake sediments, contaminated by years of effluent discharge from a nearby wastewater treatment facility, were the source of this internal loading.

A Restoration/Protection Project (Phase II of the Clean Lakes Program) commenced in 1984 to implement the recommended management strategy for Kezar Lake. Two main approaches were employed to reduce phosphorus concentrations in the lake. First, aluminum salts were injected into the hypolimnion to inactivate sediment phosphorus. The injections were performed using a modified barge system that was an efficient and cost-effective means of aluminum salts application. Second, upstream riparian wetlands were manipulated by elevating water level and planting new species to encourage phosphorus removal by sedimentation and vegetative uptake.

From 1984 to 1994, comprehensive water quality monitoring programs (including part of the Phase II project, a state-assisted volunteer program, and an EPA Phase III Post-Restoration Monitoring Project) were conducted to assess the effects of the restoration activities. Results from these efforts have generally indicated that water quality has improved following aluminum salts injection, although some parameters did worsen during 1988 and 1993. Furthermore, recreational use of Kezar Lake has increased substantially since restoration.

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RECOMMENDATIONS FOR COPPER SULPHATE DOSING

When determining the dose rate it is recommended to obtain the current pH, alkalinity and dissolved organic carbon (DOC) of the water to be dosed as these parameters will influence the action of the copper sulphate in the water as already mentioned. The conditions that will significantly reduce the toxicity of copper sulphate treatment are alkaline pH i.e. >7.5-8.0; high alkalinity i.e. > 40 mg L^{-1} as $CaCO_3$; and moderate to high DOC i.e. > 4 mg L^{-1}. Guidelines for copper sulphate treatment are given by Cooke at al. [1] .

To accurately determine the required dose rate it is useful to do a range-finding bioassay test with the target organism in the reservoir water to be treated. This is like a water treatment 'jar' test where cyanobacterial cells are treated with a range of concentrations of copper sulphate ($CuSO_4.5H_2O$) - for example 6-8 concentrations in the range from 0.01 to 0.5 mg Cu L^{-1}, and maintained at room temperature for either 24 or 48 hours. Subsamples are removed and either stained with cell activity stains and assessed by fluorescence microscopy and/or counted by conventional cell counts. This allows the calculation of the MLD_{100} or "Minimum Lethal Dose to 100% of cells" at the time end point you require – either 24 or 48 hours.

From this data the amount of copper required for the dosing can be calculated for the volume to be treated. In some cases for treating buoyant cyanobacteria it may only be necessary to dose a zone of the top 5m, which is approximately equivalent to the surface mixed layer in the reservoir. The majority of cells will be located in this layer if conditions are calm and stable and especially if the reservoir is stratified. It follows that if treatment is done under these conditions there is a greater of chance of achieving the maximum contact of toxic copper with the target cyanobacterium as the copper dissolves and disperses at a high concentration throughout the surface layer. Also when stratification is present, it is recommended to dose early in the day, as buoyant cyanobacteria are more likely to be at the surface of the water column. It is therefore beneficial to turn off any mixing or aerating apparatus prior to dosing with copper sulphate.

If treatment is done on a regular basis it is recommended that a procedure be developed to track and guide the boat using GPS, to move in a systematic pattern to achieve optimum coverage of the reservoir surface with the chemical.

Once a waterbody has been dosed with copper sulphate it is important to monitor the water for copper residuals, to ensure that guidelines for drinking water are not likely to be exceeded. For species of cyanobacteria known to be toxic or taste and odour producers, it may also be necessary to monitor for toxins, tastes and odours.

Figure 4-8(L2) shows a flow diagram of actions recommended for copper sulphate dosing.

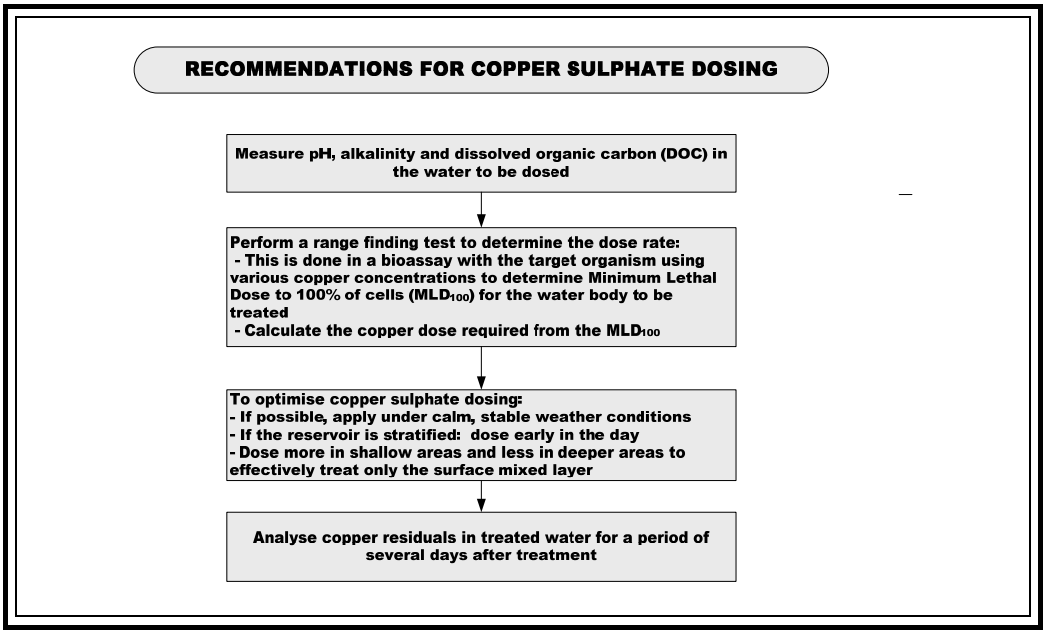

Figure 4-8(L2) Flow diagram for copper sulphate dosing: determining dose rates, application guidelines and follow-up monitoring

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BIOLOGICAL CONTROLS

IMPACTS OF MANIPULATION OF THE FOODWEB

Eutrophication problems that result in algal blooms, although commonly linked to a non-limiting supply of nutrients that support algal growth, may also occur as the result of other factors or a combination of factors. Various biophysical factors exacerbate the degree to which algal blooms occur or the frequency at which they occur. As the ambient concentration of phosphorus in reservoirs increases, so does the total biomass of fish. Research has shown that coarse fish, typically species of benthivorous and/or zooplanktivorous species – such as carp, barbell or canary kurper, tend to become dominant unless actively managed; this is also known as foodweb manipulation. Imbalanced fish populations results in an increased rate of availability of nutrients in the water column, via benthic disturbance and sediment resuspension - which also increases turbidity and decreases light availability- as well as via increased rates of excretion or recycling of nutrients into the water column. The same process also results in the uprooting of submerged macrophytes and hinders, or even precludes, the re-establishment of rooted macrophytes in disturbed sediments. The loss of macrophyte stability can force the system towards dominance by phytoplankton (see Figure 4-9(L2)).

In addition to impacts on the sediments and nutrient availability, high levels of zooplanktivore activity reduces the zooplankton within the reservoir foodweb, leading to destabilisation of the zooplankton-phytoplankton grazing dynamic. Current applied research in South Africa shows that these imbalances can be mitigated via a process of sustained and targeted foodweb management applied to the reservoir fishery [59].

These examples illustrate the value of knowing and understanding the key drivers that may be influencing the conditions in a particular waterbody. Figure 4-10(L2) broadly describes the major interactions occurring in a reservoir foodweb and, importantly, how an increase or decrease in any one or more may occur. This simple flowchart (Figure 4-10(L2)) allows the user to understand and/or determine the consequences of an action directed at one or more aspects of the water body's environment. The (+) or (-) signs on the directional arrows indicate the effect that the component has on the next. The effects are added in a multiplicative fashion – based on the mathematical relationships whereby a (+) multiplied by a (-) = (-) and a (-) x a (-) = (+).

For example: an increase in nutrients will cause algal levels to increase (+); an increase in numbers of benthivorous fish will increase the level of sediment resuspension (+), which in turn will increase nutrients and turbidity, and so on. Increased turbidity will have a negative (-) impact on vegetation, i.e. the increase will result in light reduction and blanketing, and reduce vegetation growth and coverage. So, an increase in turbidity has a negative (-) impact on vegetation, and in turn a negative (-) x (+) impact on the zooplankton which now have less vegetative habitat or cover available.

A second example: what would be the net impact on zooplankton of reducing nutrients: This would be (-) x (+) [effect of nutrients on algae] x (+) [effect of algae on turbidity] x (-) [effect of turbidity on vegetation] x (+) [effect of vegetation on zooplankton] = (-)x(+)x(+)x(-)x(+) = net positive effect on zooplankton. This would lead to more zooplankton which in turn would reduce (-) the levels of algae though grazing. Lastly, creating more aquatic vegetation habitat (the floating wetlands, littoral and riparian reedbeds, or protecting existing stands of pondweed) would support the development of a greater biomass of zooplankton able to graze on and reduce the algae, provided the fish grazing on zooplankton is in balance.

The flowchart includes options for assessing fishery, nutrient loading and bird management, in each case considering the management as reducing the impact caused by one or more. Waterfowl can have a profound and often unnoticed impact on nutrient loading (see chart), especially on smaller systems or on the shallow littoral environments in sheltered bays in large reservoirs.

By taking the time to assess as much information or knowledge about a particular reservoir or waterbody as possible, managers can make reasonable assessments of the likely drivers and knock-on effects using the flowchart. In many cases this will underpin a balanced management approach, as opposed to a single and often unsuccessful approach based on nutrients alone. Importantly, severe foodweb imbalances can produce impacts that have the appearance of nutrient-bolstered eutrophication.

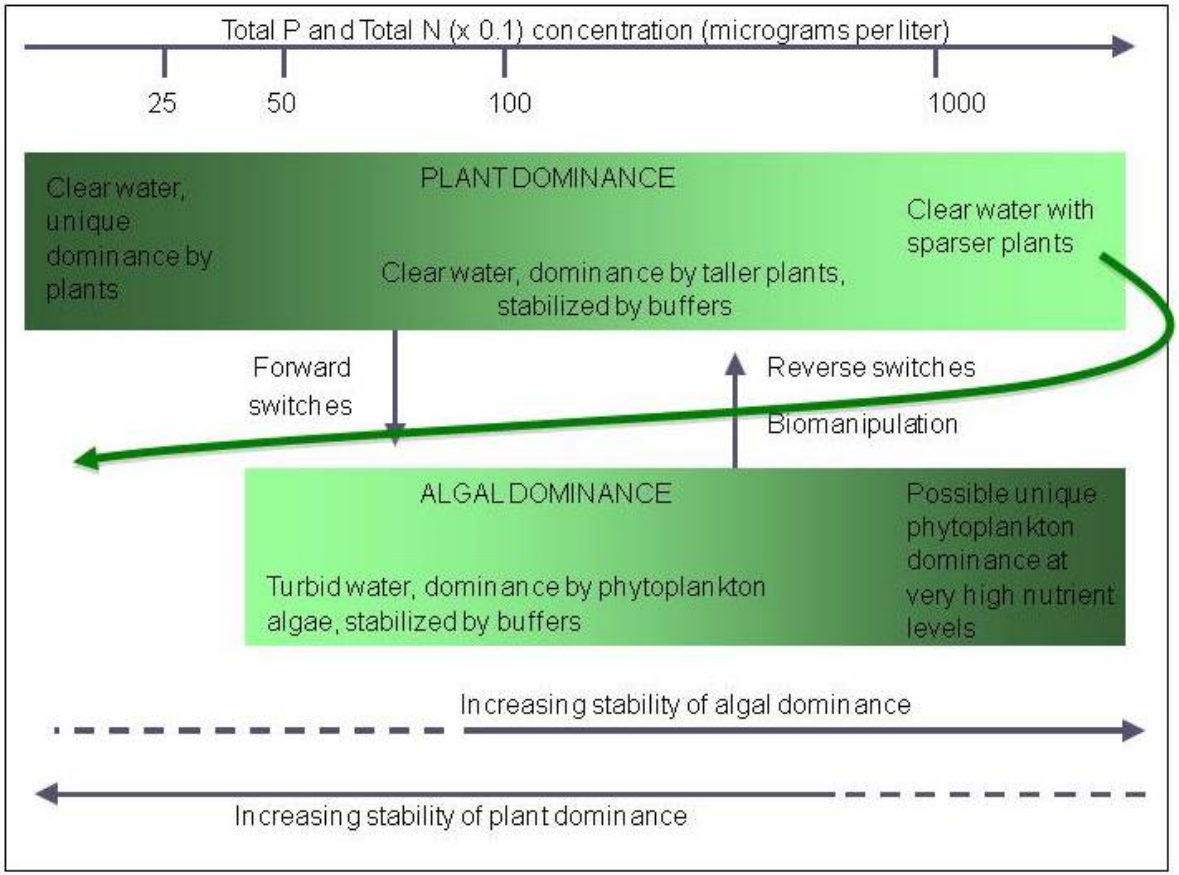

Figure 4-9(L2) Elements of the food web influencing cyanobacterial abundance

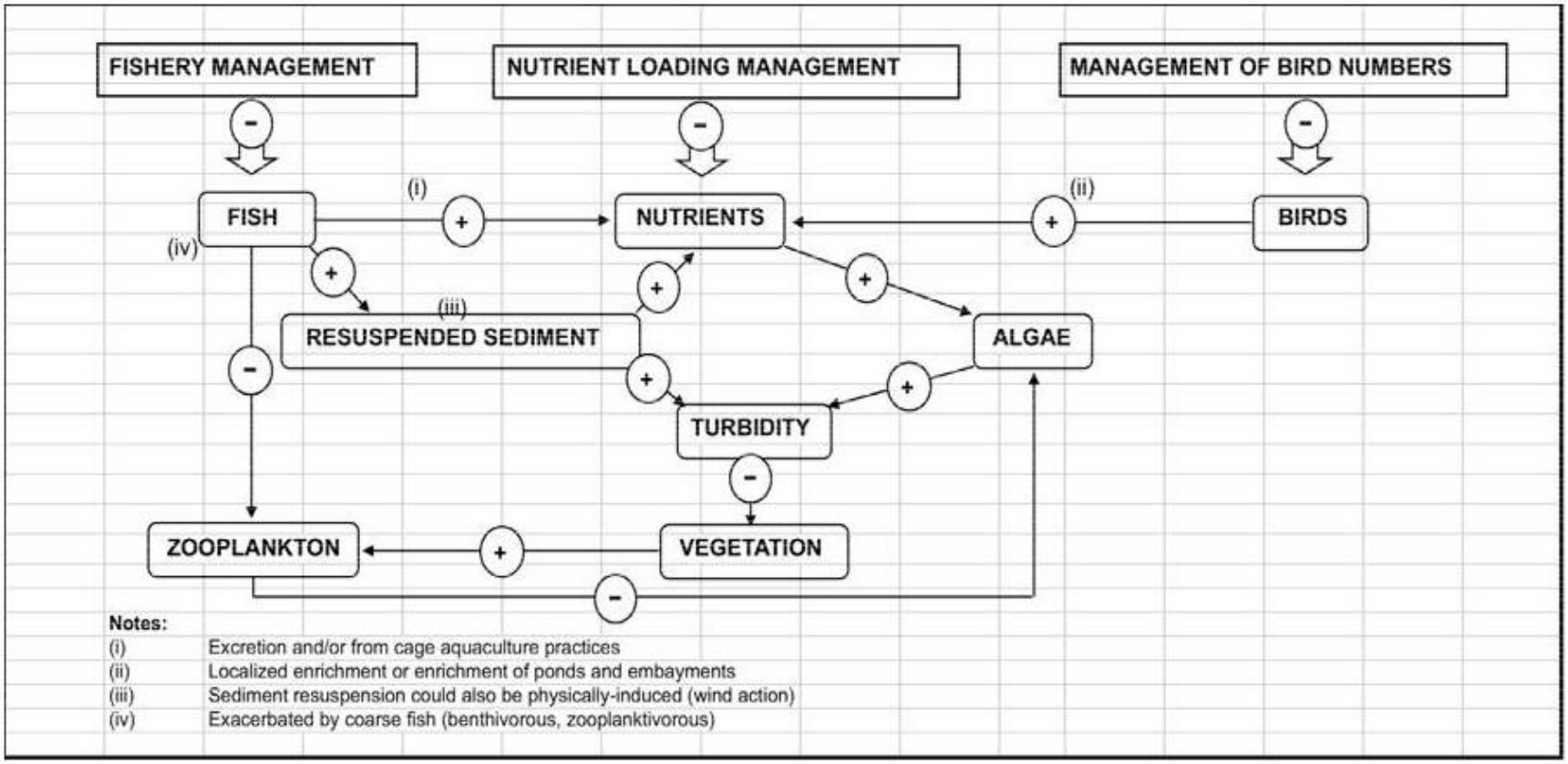

Figure 4-10(L2) Simple flowchart illustrating impacts of variation in various parameters on the aquatic environment

The flowchart is augmented by Figure 4-11(L2) – which broadly divides reservoirs into two types – the classically nutrient-driven case of eutrophication (Group 1) and where the situation is exacerbated by coarse fish dominance. This table broadly divides eutrophication problems into two areas (Group 1) the component that is associated with oversupply of nutrients originating from the catchment on a sustained basis and which is only effectively addressed at the catchment level; and (Group 2) that is associated with the long-term effects of poor impoundment management, with or without nutrient excesses, that has resulted in a gross disturbance in the foodweb - and in particular that associated with the change in the fish population from indigenous to rough fish. There are very few options for effective management available for large waterbodies falling within Group 1 other than short-term attention to the problem of algal blooms. An exception to this would be the case of a waterbody where the loading is primarily internal (external loading curtailed to manageable levels). In the latter case, and depending on the size of the waterbody, bottom sealing (physical or chemical) or dredging would now be a viable option - although perhaps expensive, the benefits would be both immediate and sustained.

Chemical in-lake controls might be effective in Group 1 waters where flushing rates are very low, especially during the summer in winter rainfall (Mediterranean) regions - where low availability of P during the summer, often geological in origin, could be reasonably offset by low level iron dosing during the latter months of the winter. Problems associated with Group 2 waters provide a genuine opportunity for effective in-lake control, even in the face of continuing nutrient loading from external sources. Obviously in such cases where external loading is not - or no longer - a problem, attention to foodweb restructuring offers significant potential for impoundment restoration/rehabilitation.

SYMPTOMS vs CAUSES ANALYSIS FOR IMPOUNDMENTS

COMMON SYMPTOMS		INDICATOR	PRIMARY CAUSES
GROUP 1			
N:P < 10-20	Increased phosphorus availability	Water chemistry	EXCESS NUTRIENT LOADING
Increased algal biomass	Phytoplanktonic or filamentous	Chlorophyll-a Water transparency	
Reduced algal diversity	Sustained dominance by few genera	Algal assemblage	
Cyanobacterial dominance	Colouration Blooms and/or scums	Visual Visual	
Increased frequency of algal blooms	In number per season or duration per event	Monitoring records	
Reduced water clarity	Organic origin (algal biomass)	Secchi depth	
Aquatic macrophyte dominance	Floating and/or rooted aquatic plants	Visual	
GROUP 2			
Increased inorganic turbidity	Sediment resuspension	Water transparency Turbidity measurements	COARSE FISH DOMINANCE
Increased P availability	Resuspension (fish, wind action) Excretion (fish, birds)	Water chemistry	
Reduced zooplankton dominance	Diversity, assemblage, body size	Monitoring	
Cyanobacterial dominance	Colouration Blooms Algal assemblage	Visual Visual Taxonomic	
Decrease in rooted macrophytes	esp. pondweeds	Spatial mapping	

Figure 4-11(L2) Symptoms and causes of various water quality issues in the water body

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CASE STUDY OF BIOMANIPULATION

In the late 1960s, Lake Veluwe, The Netherlands, displayed a transformation in its ecosystem from a macrophyte-dominated state when the total phosphorus levels exceeded 0.20 mg L^{-1}. The water of the Lake became turbid and remained so despite a significant reduction in the external nutrient load due to catchment management strategies. It was found that after these strategies were in place the Chl-a levels decreased, indicating a drop in levels of algal; however the light attenuation due to turbidity remained high due to the interaction of wind and benthivorous fish resuspending fine sediment particles. After several years macrophytes recolonised the shallower parts of the lake, resulting in localised clear water, while the deeper sections remained turbid. Once the causes were identified, a program to reduce the population of benthivorous fish commenced. This resulted in a recolonisation of the lake with zebra mussels, leading to further clarification of the water through filtration by the mussels. Finally this enabled the re-establishment of macrophyte species such as *Chara* and the clarification and rehabilitation of the entire lake [60].

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CHAPTER 5 TREATMENT OPTIONS (LEVEL 1)

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 [1]. 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, [2] 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. [3] has shown that potassium permanganate, applied at a concentration necessary to oxidise moderate levels of managanese, 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.

For more details follow this link.

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 [4]. 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 centimeters 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.

For more details, follow this link.

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 (turbidity, dissolved organic carbon removal etc) under the conditions of high numbers of cyanobacteria, optimum removal of cells, and therefore intracellular toxin, will be achieved [5]. 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.

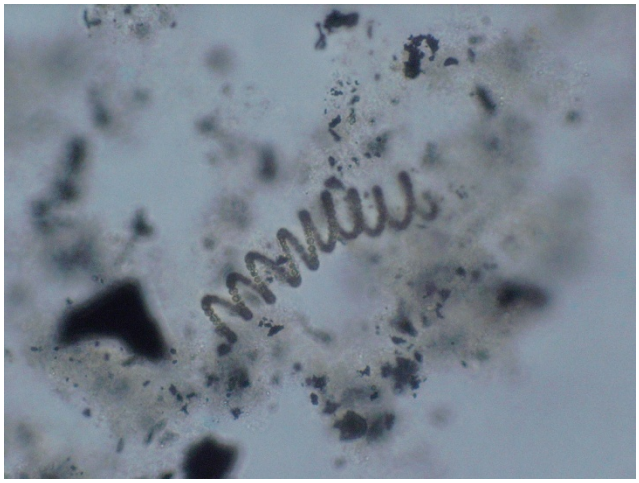

Figure 5-1 *Anabaena* filament encased in an alum floc. Dark areas are powdered activated carbon particles used to remove dissolved tastes and odours and cyanotoxins.

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 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

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 [6]. 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. As a result, if possible, all sludge and sludge supernatant should be isolated from the plant until the toxins have degraded sufficiently. Microcystins are readily biodegradable [7] so this process should take 1-4 weeks. Cylindrospermopsin appears to be slower to degrade [8] 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.

For more details, follow these links for

Coagulation and flocculation

Clarification

Rapid filtration

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 [9]. 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 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.

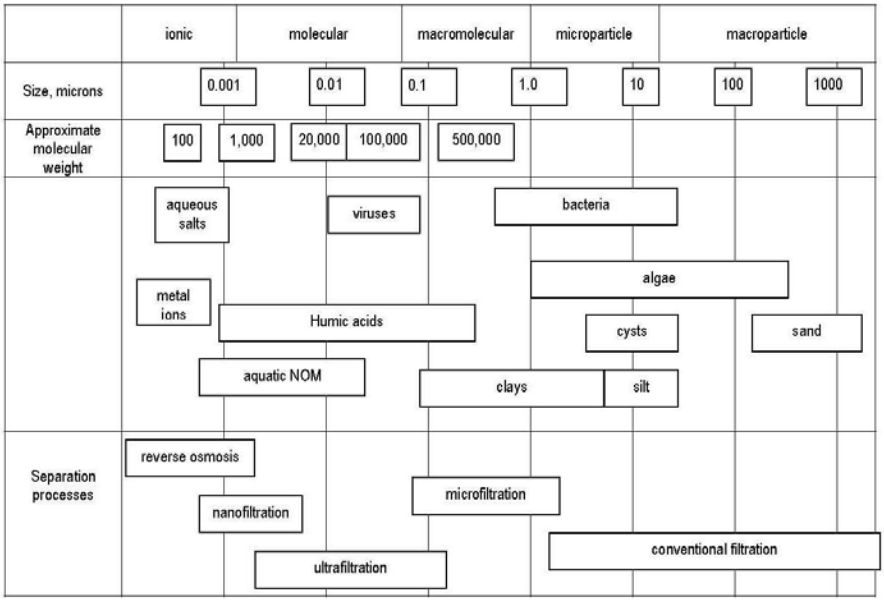

Figure 5-2 Efficiency of various filtration processes

For more details, follow these links:

Membrane modules

Permeate flow rate

Pre-treatments

Monitoring and control

Pressurised or submerged membranes

Dead-end or crossflow

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, possibly more, 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*** e.g. removal using activated carbon, membranes
- ***Chemical processes*** e.g. oxidation with chlorine, ozone and potassium permanganate
- ***Biological processes*** 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.

Follow these links for more information on activated carbon:

Manufacture

Characterisation

The adsorption process

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 floc, and should be avoided if possible. The 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.

For details of process design for PAC application click here

PAC TYPE AND DOSE REQUIREMENTS

Natural organic material plays a large role in controlling the removal of microcontaminants using activated carbon. The 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) [10] is 2000 times that of the target compound, the toxin. Clearly it 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, climatic conditions etc. As a result, only broad guidelines can be given and, as with the choice of activated carbon, it is suggested doses are determined on a site-specific basis.

Click here for a simple PAC comparative test

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.

Click here for a simple PAC dose requirement test

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. Simple testing procedures can be found by following the links in the previous section. 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 doses, 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 m-LR 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 m-LR 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 m-LR and mLA at 1 $\mu g\ L^{-1}$ 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 [11]. 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 3-4μg L^{-1}.

ANATOXIN-A.

The limited data that exists for anatoxin-a removal by PAC suggests that similar removals to that of m-LR can be expected [12].

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	m-LR	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 (e.g. $C/C_o>0.5$) is achieved. 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 $C/C_o >0.9$ [13]. 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.

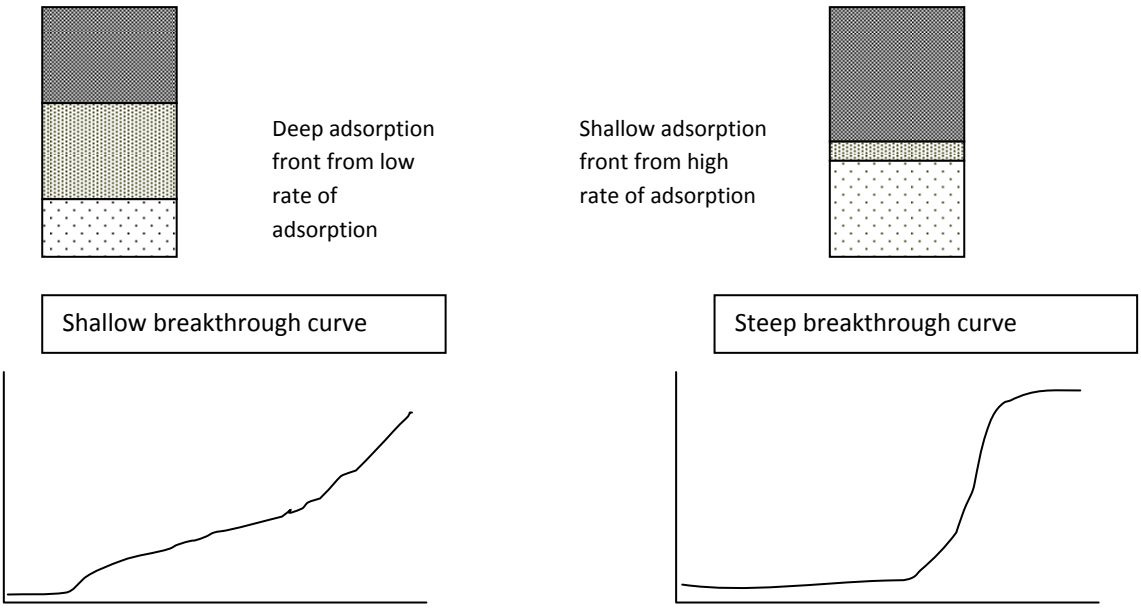

Figure 5-3 Effect of the adsorption front on the shape of the breakthrough curve

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

- the capacity of a particular carbon for the organic compound(s) in question
- the contact time between the water and the carbon
- the concentration of the organic compound in the feed, and the desired removal
- the 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 10C 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.

More information about monitoring and control of GAC processes, including determination of regeneration frequency, can be found here

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.

Click here for a simple GAC comparative test.

Click here for more general guidance on selection of GAC

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.

Click here for more information on EBCT

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:

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.

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 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 [14].

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.

Click here for a simple monitoring test for GAC

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 well removed by GAC, and good removals (up to 75% removal of toxicity) have been reported after 12 months of running laboratory scale GAC columns [15].

ANATOXIN-A.

Similar to PAC, the limited data that exists for anatoxin-a removal by GAC suggests that similar removals to that of m-LR can be expected [12].

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 5-1 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 5-1, microcystins should be rejected by RO membranes and nanofiltration 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.

More operational information about membranes can be found here

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. From the table 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 Cl_2

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 we use chlorine for the removal of algal toxins we should be aware that 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 into account this 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 [16].

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 [17]. Of the four most common microcystins, the ease of oxidation by chlorine is given by:

mYR>mRR>m-LR>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 considerations section, below.

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 [3].

CYLINDROSPERMOPSIN

The limited data available on the chlorination of cylindrospermopsin suggests it is more susceptible to chlorination than microcystins [18]. The conditions outlined above for the chlorination of microcystins are also applicable for cylindrospermopsin.

ANATOXIN-A

Anatoxin-a is not susceptible to chlorination [12].

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 [19].

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 [20]. 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 [21]. 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 [22]. There is a competitive effect with NOM, always at higher concentration than the toxins, as it can be expected that 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 [23]. 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 [23].

ANATOXIN-A.

Application of ozone as for microcystins will result in significant oxidation of anatoxin-a [24].

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 is not recommended as a major treatment barrier

For a description of the ozonation process, follow this link

POTASSIUM PERMANGANATE

Potassium permanganate has been shown to reduce the concentration of microcystins and anatoxin-a considerably [25] and may also be effective for the reduction of cylindrospermopsin [26]. If potassium permanganate application is practised to control manganese it should be maintained in the presence of these toxins. Unfortunately, the data currently available is not sufficient to allow recommendations for dose requirements or to allow us to consider potassium permanganate as an effective barrier.

For a description of the application of potassium permanganate and some laboratory results follow this link

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 [27, 28]. As with ozone, the presence of hydrogen peroxide promotes the formation of hydroxyl radicals, and increases the oxidising potential of the UV treatment.

For some laboratory results click here

HYDROGEN PEROXIDE

Hydrogen peroxide is 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

MORE INFORMATION ON OXIDATION

Reaction rates

Modelling of oxidant processes

BIOLOGICAL PROCESSES

Microcystin variants and cylindrospermopsin show great potential for significant biological removal, even at flow rates approaching those encountered in rapid sand filters [29]. 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.

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 be considered an effective barrier to cyanotoxins at present. 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 [4]. There is also good preliminary evidence that these processes will be effective for cylindrospermopsin removal.

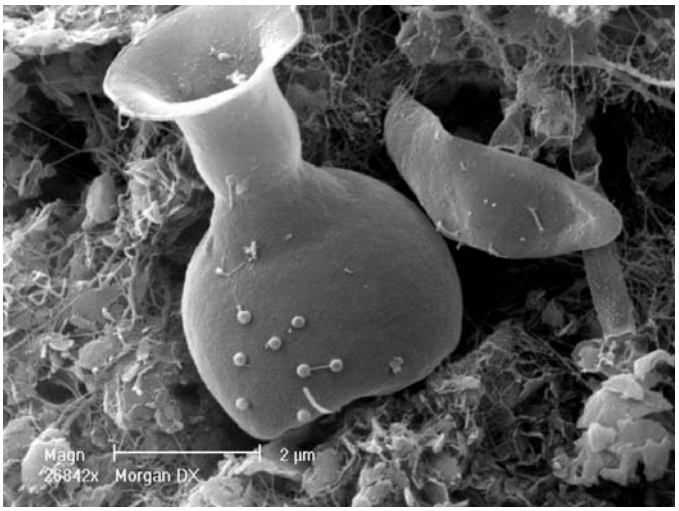

Figure 5-4 Scanning electron micrograph of biofilm on a sand particle from the rapid sand filter at Morgan Water Filtration plant, South Australia

For more information on riverbank and slow sand filtration, click here

CHAPTER 5 TREATMENT OPTIONS (LEVEL 2)

CYANOBACTERIA CELL REMOVAL

MICROSTRAINERS

GENERAL CONSIDERATIONS

The essential features of a microstrainer, illustrated in Figure 5-1(L2) are:

- the drum, generally between 1.5 to 3 m in diameter and up to 5 m long with a variable speed drive
- fabric of either stainless steel mesh or polyester cloth with apertures normally in the range 20 - 40 µm for microstraining or larger (e.g. 1 mm) for fine screening. The fabric is normally attached to small frames fixed to the drum, which can be removed individually without draining down
- wash water jet arrangement with a trough for collecting screenings
- a tank in which the microstrainer is housed (usually concrete) consisting of an inlet chamber with a weir for water to flow into the interior of the drum and an outer chamber containing the drum itself with an outlet weir

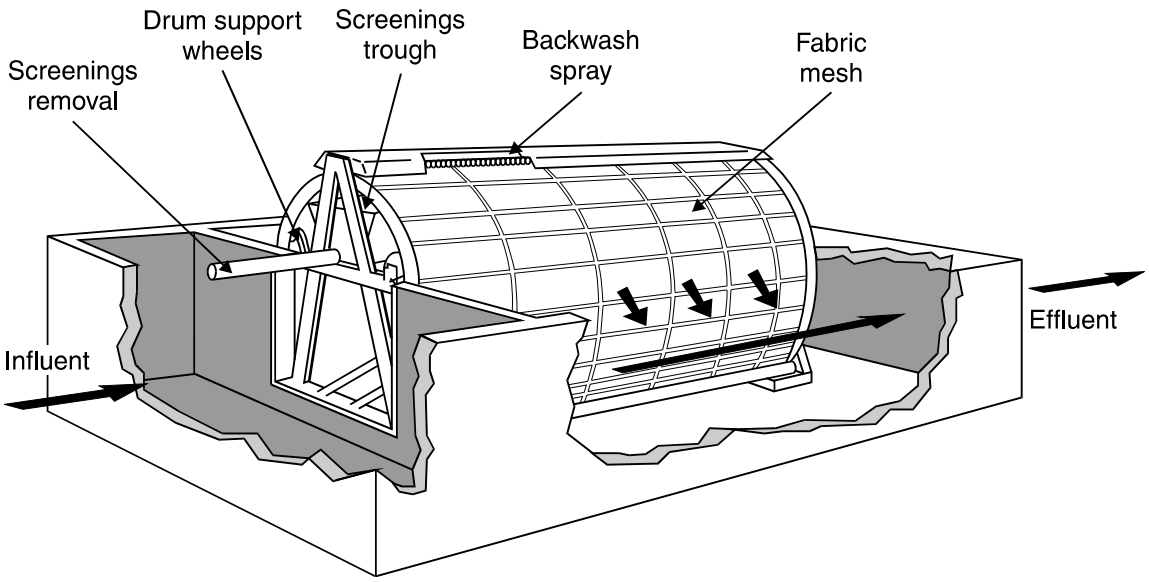

Figure 5-1(L2) Microstrainer

The main factors influencing performance are:

- speed of rotation. This will depend on solids loading. If solids loading increases, the fabric will block more quickly, and intensity of cleaning needs to be increased. This is achieved by increasing drum rotational speed. Drums usually operate up to a top speed of about 5 rpm;
- washing, which must be effective otherwise headloss across the fabric will be excessive. The maximum headloss is typically 0.3 m. The wash water demand is between 1 and 3% of the volume treated;
- sodium hypochlorite washing or ultraviolet irradiation to prevent blinding of the fabric by algae or a zoogloeal film. If build-up of calcium carbonate scale occurs, acid washing may also be necessary.

PROCESS MONITORING AND CONTROL

The only control variable is headloss which is controlled by varying the rotational speed of the drum. Headloss across the fabric is measured using a differential pressure cell or electrodes to determine water levels. The variable speed motor can be controlled automatically based on the signal from this cell.

Return to level 1

RIVERBANK, SLOW SAND AND BIOLOGICAL FILTRATION

PROCESS MONITORING AND CONTROL

The main parameters used for monitoring slow sand filters are flow rate, headloss and filtrate turbidity. Good operational practice for these parameters should also provide good performance for algal removal.

Filtration rate is controlled by means of a valve on the filter outlet. As the filter becomes blocked and headloss across the filter increases, the outlet valve must be progressively opened to allow the same filtration rate with a constant head above the sand. Valve adjustment can be manual on a daily basis (because headloss builds up slowly) or automatic, based on a signal from flow metering equipment. Headloss can be monitored using differential pressure cells, or measured manually using level indicating tubes. Data from headloss measurement can be used to predict when skimming of a bed will be necessary, and assist planning of works operation to minimise the number of filters out of use at any one time.

For river bank filtration sites monitoring of filtrate turbidity will yield information on the system's performance concerning particle removal. However, it needs to be taken into account that elevated turbidity and also increasing headloss may also result from processes in the groundwater body surrounding the well (physical or bio-chemical well-clogging). Monitoring of source water quality and determination of the nature of the particles encountered can help identify the cause.

PERFORMANCE OPTIMISATION FOR ALGAE REMOVAL

The following recommendations relate mainly to slow sand filter works with primary rapid gravity filtration. Works without primary filters may need a more conservative operating regime, for example in relation to maximum filtration rates and start-up conditions.

1) Slow sand filters typically contain a minimum of 300 mm depth of sand with an effective size of 0.3 mm (tolerance ±10%) and a uniformity coefficient of 1.7-2.3. All sources of new sand must be assessed for quality and grading before purchase. Sand removed during the cleaning process is usually washed on site to agreed quality (silt/particulate organic carbon) and grading specification. Only washed sand can be reused for resanding or rebuilding.

2) Filtration rate: slow sand filters may be operated within a band of 0.05-0.5 m h^{-1} (m^3 m^2 h^{-1}) downflow, although in practice the normal rate is narrower at 0.1-0.4 m h^{-1}. Pretreatment may be needed to achieve high filtration rates without excessive headloss build-up.

3) Where the level of sand in the filter has fallen to 300 mm, a decision needs to be made as to whether to top up the bed with clean media ("resand") or to replace the lower layers with clean media ("rebuild"). This decision is based on a number of factors, the main factor being the cleanliness of the sand in the bed. If the lower levels of sand accumulate a large mass of material, then the starting head loss may be high and the run

length short. Historically, dirty sand in the lower layers has not given rise to particle breakthrough although water quality can be adversely affected in terms of low dissolved oxygen and excessive growth of undesirable biological populations in the underdrains.

4) Following resanding or rebuilding, the bed is either run to waste or recycled, at a minimum flow rate of typically 0.025 m h^{-1}, until filtered water quality targets are met e.g. coliforms/*E. coli* below 100/10 per 100 ml.

5) During periods of increased cyanotoxin risk, consideration should be given to the possibility that the sand washwater may contain high concentrations of extracellular cyanotoxin because of cell lysis. Recycle should therefore be avoided if possible.

PERFORMANCE OPTIMISATION FOR TOXIN REMOVAL

The few parameters that can be optimised in bank filtration settings are the share of surface water compared to ambient groundwater (share of bank filtrate) and the minimum travel time of the bank filtrate in the subsurface. Both parameters depend on the distance from river to well and the pumping rate for a given hydro-geological setting. Simulation models (e.g. analytical/numerical GW models) can assist to determine the share of bank filtrate and the travel time for a given setting (e.g. BFS, MODFLOW, FEFLOW).

In order to assess the necessary travel time, it needs to be taken into account that under optimal conditions extra-cellular microcystin is usually well bio-degradable (half-lives may lie in the range of hours). However, in environments without an adapted microbial community, lag phases of up to one week may occur before degradation commences.

The following pre-requisites are postulated for sufficient removal of microcystin to < 1 µg L^{-1} by bank filtration at source waters with frequent cyanobacterial blooms (i.e. adapted microbial population):

- extra-cellular microcystin < 50 µg L^{-1},
- middle to fine grained sandy aquifer,
- aerobic conditions
- temperatures > 15 °C,
- residence times > 7 d (see figure 1)

For suboptimal conditions, residence times need to be much higher (> 70 d).

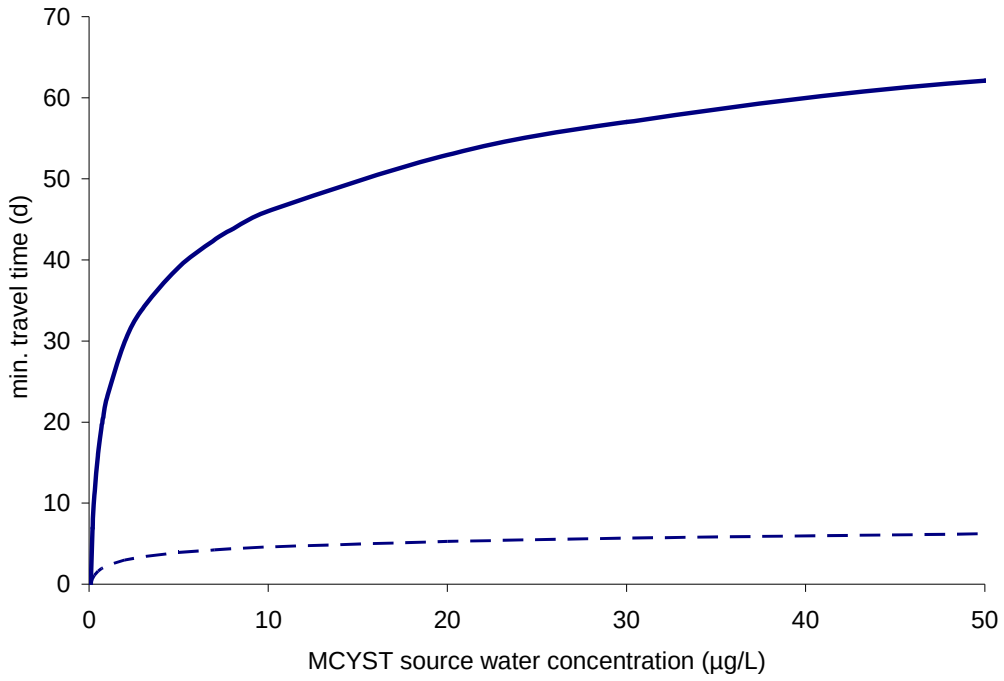

Figure 5-2(L2) Minimum subsurface travel time for sufficient removal of microcystin depending on source water concentration of extra-cellular microcystin for a) worst-case conditions (solid line), i.e. anoxic/anaerobic conditions, temperature < 15°C, and b) optimal conditions (dashed line).

Recent investigations have shown that for cylindrospermopsin biodegradation rates are similar to those determined for microcystin though their extra-cellular toxin share might be generally higher.

Return to level 1 (cyanobacteria removal)

Return to level 1 (cyanotoxin removal)

CONVENTIONAL TREATMENT

COAGULATION AND FLOCCULATION

PROCESS MONITORING AND CONTROL

Coagulation control is based on maintaining optimum doses and pH for effective algae removal as feed water quality varies. Automatic control requires flow proportional control of chemicals, with trimming to the optimum carried out by one of the following methods:

a) Feedback loop control from flocculated water characteristics. Proprietary systems (e.g. Streaming Current Detector) are available to control coagulant dose. Separate control of pH is usually necessary.

b) Feedback loop control based on product water quality from subsequent treatment processes, using signals from pH, turbidity, residual coagulant or colour monitoring instruments. This can require the successful operation of several instruments, depending on treatment requirements.

c) Feedforward control from feed water quality using empirical equations developed from historical data. Enough data are required to confidently relate required dose to quality which will limit its application for most sites. This method may also depend on the successful operation of several instruments, although UV absorbance is often used as the main on-line control parameter.

Methods (b) and (c) can be used as the basis for manual control, with operators regularly taking instrument readings and making appropriate adjustments to doses.

The success of any coagulation control strategy can be dependent on the hydraulic retention time in subsequent treatment. Long retention time systems can be more difficult to control by feedback from product water quality, but are less sensitive to short periods of non-optimum dosing.

PERFORMANCE OPTIMISATION FOR ALGAE REMOVAL

1) Jar tests should be carried out at suitable intervals, initially to identify relationships between coagulation conditions (dose and pH) and raw water quality, and subsequently to check that this relationship does not change with time. The required frequency for this will be site-specific, depending on the variability in raw water quality. It may also be valuable to use jar tests to compare alternative coagulants, to identify the most suitable for a particular site and conditions. Jar tests should be carried out on freshly collected samples, and at the same temperature as the raw water.
2) To maximise algal removal, jar tests need to be carried out on waters with high algal concentrations and appropriate algal counts carried out. However, this will not always be possible, and optimisation for removal of colour or UV absorbance at 254 nm (UV254) in filtered samples may give a working approximation of the requirements for good algae removal. This would need to be confirmed, however, at times of high algal concentrations.
3) Other important parameters in the jar test are total coagulant metal ion concentration and turbidity in the settled water, and soluble metal ion concentration in a filtered sample. Insoluble coagulant metal ion concentration is an indicator of settleability of the floc, and soluble metal ion concentration an indicator of the suitability of the chemical conditions.

Procedures are needed to ensure that operators maintain suitable dosing/pH conditions, identified from jar tests, with varying raw water quality. This can be particularly important for sites where sudden changes in raw water quality can occur, such as for direct river abstraction. Recommended ways of achieving this are outlined below.

- Provide graphs or tables, based on historical data, to relate dose/pH to raw water quality (rather than rely solely on operator experience in this).
- Initiate a program of jar tests initially to obtain data with which to check the validity of these graphs and tables, and then to ensure that the relationship between water quality and coagulation conditions does not change with time.
- Sampling of flocculated water (or coagulated water if this is not available) and measurement of appropriate parameters can provide a check that correct dosing conditions are being applied. Suitable procedures will need to be defined for this. For example it might be beneficial to provide a short period of stirring with a jar tester to establish a reproducible degree of flocculation. Appropriate parameters for measurement are insoluble coagulant metal ion and turbidity in settled samples (settleability of the floc), and colour, UV254 and soluble metal ion in filtered samples (coagulation chemistry). Target values for these will need to be identified on a site-by-site basis.

- The coagulant dose and pH can be checked by sampling and analysing the coagulated water. Polyelectrolyte doses should be checked by volumetric calibration.
- Measurement of turbidity/total coagulant metal ion concentration in clarified water, and colour/UV254 /coagulant metal ion concentration in works filtered water, would also give indications of the performance of coagulation. However, when raw water conditions are changing, there would be a time lag to take into account between coagulation and sampling. The performance of the solids-liquid separation, particularly for clarification, would also need to be taken into account.
- Sampling of clarified water for measurement of total metal ion coagulant and turbidity can give an indication of the success of the coagulation conditions in producing a readily separable floc. Turbidity above 2 NTU, Al above 0.5 mg L^{-1} or Fe above 1 mg L^{-1} would indicate scope for improvement in solids-liquid separation, which might be achieved by attention to coagulation conditions, and perhaps through the use of polyelectrolyte flocculant aid.

If filtered jar test samples appear to show better performance for colour and UV254 removal than that given on the plant for the same coagulation conditions, attention should be given to the initial chemical dosing and mixing conditions on the plant, as these may provide inadequate dispersion for the achievement of good coagulation chemistry. Similarly, if settled samples of flocculated water from the plant (or coagulated water if flocculated samples are not available) have markedly higher turbidity or total coagulant metal ion concentration than jar test samples, this could indicate a limitation in the plant coagulation or flocculation conditions to produce readily settleable floc. Attention should be given to mixing conditions on the plant, or the potential for polyelectrolyte to improve settleability.

Return to level 1

CLARIFICATION

GENERAL CONSIDERATIONS

Clarification processes involve either settling or flotation of the flocculated water. The objective of clarification is to reduce solids loadings to subsequent filters, thereby maximising run times and minimising the risk of breakthrough of particulates, including algae. This is achieved by operating clarification processes to prevent carry-over of solids, based on clarified water quality. The effectiveness of clarification is dependent upon achieving good chemical coagulation, and is influenced by hydraulic and solids loading rates. Ineffective desludging of clarifiers can also cause deterioration in clarified water quality because of carry-over of solids.

Well-operated clarification processes can therefore maximise removal of algal cells and associated cyanotoxin, but there is no evidence of any benefits for extracellular toxin. Biological activity in sludges held within clarifiers could potentially result in algal cell lysis and release of cyanotoxin. Effective sludge removal is therefore important to minimise risk from cyanotoxins.

Generally, clarification would be expected to remove at least 70-90% of the coagulant floc, and therefore give similar removal of algal cells provided these are effectively incorporated into floc by efficient coagulation. Some algal genera containing gas vacuoles (e.g. *Microcystis*) may be removed more effectively by flotation compared with settling.

The information provided in this manual relates to conventional clarification processes. There are many relatively new high-rate proprietary clarification processes available, and some of the principles discussed below would apply to these as well as the conventional processes.

PROCESS MONITORING AND CONTROL

Although good control of chemical coagulation is essential, floc blanket clarification can handle short periods of non-optimum dosing because of the long hydraulic retention times within the process. However, these long retention times can make good feedback control based on product water quality difficult to establish, as a result of long delay times between dose adjustment and effect on product quality.

Periodic removal of sludge from the floc blanket can be controlled based on blanket height by means of optical detector systems suspended in the tanks. Similar systems may allow control based on blanket solids concentration, particularly for use on the recirculation type systems. Desludging of concentrator cones can be controlled by weight of sludge accumulated in the cone.

Control of dissolved air flotation (DAF) is based largely on achieving and maintaining suitable chemical dosing conditions. Other operating variables e.g. air supply, scraper speed, etc. can be optimised once satisfactory chemical dosing has been achieved, and responses to changing raw water conditions can be made by manual adjustments. It may also be possible to implement automatic control by means of a feedback loop based on treated water quality. The latter can be more efficient for DAF than for processes that have a longer retention time.

PERFORMANCE OPTIMISATION FOR ALGAE REMOVAL

1) Ensure that suitable coagulation conditions are maintained not only to maintain good incorporation of particles into floc, but also to produce floc which is readily separable in clarification processes. Measurement of turbidity and coagulant metal ion concentrations in supernatant from settlement jar tests can be used to compare the relative performance of different coagulation conditions in terms of floc settleability. For DAF, the use of flotation jar tests, whilst not critical, would probably give more representative results.

2) Solids removal performance of individual clarifiers should be monitored using turbidity and insoluble coagulant metal ion concentration. Whilst target values need to be set for individual sites, turbidity above 2 NTU, insoluble Al above 0.5 mg L^{-1} or Fe above 1 mg L^{-1} would usually indicate scope for improvement in solids-liquid separation, which might be achieved by attention to coagulation conditions, and perhaps through the use of polyelectrolyte flocculant aid. However, if jar tests appear to give readily separable floc, the problem may lie in the plant hydraulic and mixing conditions.

3) This could be checked by taking samples of coagulated water directly from the plant, and carrying out settling or flotation tests with these samples. These should include periods of flocculation for the coagulated water. If the water quality (turbidity or total coagulant metal ion concentration) from these tests is better than that of the works clarified water, then attention should be given to works flocculation and solids-liquid separation, and the following should be investigated:

    - flocculation performance
    - hydraulic loadings and flow distribution in and between clarifier units
    - floc blanket depth and concentration
    - quantity of air supply to DAF (recycling ratio and saturator pressure)
    - relative performance of individual units

4) Assess whether any uneven flow distribution between units may be influencing the performance of individual units. Check also for any indications of uneven flows within units.

5) Take samples of floc blanket, if possible, and check the concentration as the settled volume after 30 minutes settlement. This should be around 15 - 20% of the original volume. Blanket depth should also be monitored

routinely to avoid any potential for overflow of the blanket into the clarified water launders. There may be scope to increase the frequency of sludge bleed to reduce variations in blanket depth.

6) DAF air supply should be estimated from suppliers' data (based on pressure and water temperature). With a packed saturator, a pressure of between 50 and 60 psi (350 and 450 kPa) and a recycle rate of 7% to 8% would correspond to a dose of between 8 and 10 g air per m^3 water treated. Lower doses than this may limit performance. Higher doses may be causing unnecessary turbulence.

7) Visual inspection of the DAF tanks should identify whether good dispersion of the bubbles across the width of the tank is being achieved, and that the "milky" appearance indicative of effective bubble generation is being achieved.

8) After start-up, avoid sudden changes in flow rate to either floc blanket clarification or DAF.

9) Any attempts to improve clarifier performance should be accompanied by appropriate monitoring of the filters, to identify the knock-on effects on filtered water quality and headloss build-up.

Return to level 1

RAPID FILTRATION

GENERAL CONSIDERATIONS

Generally, the aim is to operate the filters at the highest possible rate less than the maximum design loading, whilst maintaining acceptable filtered water quality for as long as possible between backwashing. However, for algal removal there is an additional consideration of accumulation of algal cells in filters, with the risk of cell lysis and the release of toxin into the filtered water.

The main performance criteria are filtrate quality, run length and headloss. The operational factors which influence performance are given below.

For general guidelines on filter operation the reader is directed to Logsdon et al. [30].

COAGULANT DOSING AND PH CONTROL

Coagulant dose and pH must be optimised for good performance. Dosing less than the predicted optimum reduces the "filterability" of the floc and hence reduces run length even though solids loading is reduced. Dosing more than the predicted optimum simply increases the solids loading onto the filters and consequently decreases run length. Metal ion coagulant can be partially substituted by a cationic polyelectrolyte coagulant, which, for direct filtration (without prior clarification) reduces solids loading to the filters and increases run length. Polyelectrolyte flocculant aids can also be dosed to increase run length.

FILTRATION RATE, FLOWRATE CHANGES AND START-UP

Filtration rates (volumetric flowrate per unit area of filter bed) are typically in the range 4 m per hour to 12 m per hour for conventional filtration applications. The effect of increasing filtration rate is to shorten filter run length. Higher filtration rates are possible if coarser media is used. To compensate for poorer filtration when using coarser media, deeper beds are used; typically the bed depth is 800 to 1200 times the particle diameter. The use of polyelectrolyte flocculant may also be beneficial when using coarser media.

Filter performance can be sensitive to changes in filtration rate, and accurate flow distribution between filters and banks of filters is important. Sudden changes in flowrate can lead to particle (and therefore algal) breakthrough. Filters can be less effective for solids removal, with higher turbidity and particle counts in the filtered water, for a short period after they are brought back into operation. A slow start-up, with gradually increasing filtration rate over the first hour or so, may be beneficial to minimise the effects of this. A “delayed” start, with the filter left to stand for a few minutes after backwashing, can also be beneficial in reducing the start-up peaks in turbidity and particle counts. This is known as filter “ripening”.

BACKWASHING

Backwashing should provide a minimum bed expansion of 10 to 20% to ensure fluidisation and adequate cleaning of the media. The wash rate needed is governed by media size, type and water temperature. Higher rates are needed at higher temperatures because of the lower viscosity of water. Dual-media and multi-media beds need slightly greater bed expansions than single media beds to maintain a good stratification of the media. Backwash flow should be confirmed with media suppliers, and the backwash performance should be checked (visual inspection, treated water quality and headloss on restart) to establish appropriate rates for specific plants.

Higher backwash rates may be needed at the start of the wash to overcome the initial resistance of the bed. To prevent this, air scour is usually used before backwash to create pathways for even distribution of backwash flow through the bed. Air scour rates are normally between 20 and 40 m per hour. A smaller bed expansion may be acceptable if air scour is used first. The durations of air scour and backwash vary, but typically would be less than 5 minutes for air scour and 10 minutes for backwash. It is important to achieve even distribution of backwash water over the whole area of the filter.

Some filters have simultaneous air scour and backwash, using each at about half the rate that they would be used individually. Simultaneous air scour and backwash produces aggressive cleaning but has a particularly disruptive effect on both the filter media and support material, and should not be used on filters without appropriate modifications to the underdrains, filter floor and washwater outlet weirs. Final backwash without air scour should then be used at a high enough rate to ensure re-segregation of dual or multi-media beds.

The efficiency of backwash influences the performance of the filter in terms of the subsequent run length and filtrate quality, particularly during the start-up period.

PROCESS MONITORING AND CONTROL

Efficient filtration should provide a very high degree of removal of algal cells from the water (>99%), and is achieved through:

- maintenance of suitable filtration rates
- monitoring of filtrate quality and headloss
- initiation of backwash at the appropriate time, and provision of suitable backwash conditions
- maintenance of satisfactory chemical dosing conditions
- prevention or minimisation of flow surges

Good flow distribution between filters is critical. Provision of permanently installed flow measurement devices on each filter can be expensive, but temporary methods are available for checking flow distributions.

Backwash is initiated either on time of operation or headloss. Turbidity can also be used, the backwash being initiated when turbidity reaches a particular value. Suitable monitors are therefore needed on individual filters.

PERFORMANCE OPTIMISATION FOR ALGAE REMOVAL

Operational practices that can minimise the risk of algal breakthrough are reviewed below. Some of these could be implemented only at times of greater algal toxin risk, although good operating practice could best be demonstrated through routine continuous use.

1) The risk of particle breakthrough into the filtered water is greatest during the early part of the filter run, because of the higher turbidity and particle counts which occur on filter start-up, and this period should be given particular attention for reducing the risk of algal breakthrough.
2) Check turbidity from individual filters to identify any poor performers. Measure flow distribution between individual filters, if possible, to see if this could be causing poor performance.
3) Check the suitability of backwash regimes with regard to flow rates and the achievement of good dispersion of air scour (visual inspection). Measure backwash and air flow rates. Routinely check quality of backwash water leaving the beds during the wash, and headloss on restart.
4) Monitor media depths to check for long term loss of media. Overflow of water from the filter during air scour should be avoided if possible, as this can lead to loss of media. Core samples of the media can give a useful indication of media quality and grading.
5) Backwashing of individual filters should be staggered over as long a period as possible to balance out the effects of startup quality and flow rate changes to remaining filters.
6) Attempts should be made to reduce the impact of filter start-up. Possible options to achieve this are:
    - run to waste or recycle of filtrate;
    - implementation of slow start or delayed start (delay between the end of the wash cycle and restart of filtration);
    - improved backwash conditions.

These are listed in probable order of effectiveness for most works. Combinations of these may be suitable for many sites.

1) Optimisation of the slow start conditions should be investigated based on particle counting rather than turbidity. Once optimised, performance could be monitored routinely using turbidity as an indicator.
2) Sudden flow rate changes to filters should be minimised. A maximum rate of 5% per minute has been suggested.
3) Most commonly flowrate change occurs to the remaining filters in a bank when one filter is taken out of service for backwashing. Slow shut-down of filters at the end of the run can minimise the impact of this.
4) If a filter is shut down before the normal end of the run, it should be backwashed, if possible, before restart, as this reduces the frequency and magnitude of peaks in particle counts/turbidity in the filtered water. The importance of backwashing before restart increases with the length of the run before the operation is stopped.
5) At times of high algal concentrations conditions in the filter may lead to algal cell lysis and significant release of toxin. At such times, backwashing should be carried out more regularly to remove accumulated algal biomass to minimise the risk from this. It may be possible to identify a maximum acceptable headloss,

and use this as a target to initiate backwash, rather than base backwash frequency on time or turbidity breakthrough. However, under summer conditions with warm water, it is likely that filter run times in excess of 24 hours would lead to biological breakdown of algae trapped within the filter.

Return to level 1

MEMBRANE FILTRATION

MEMBRANE MODULES

Membrane plants are designed to provide as large a surface area of membrane as possible for a given size of unit. The two types of membrane modules most commonly used to achieve this are the spirally-wound and hollow fibre configurations. Spirally-wound elements consist of two layers of membrane separated by sheets of porous fabric, wrapped around a central collection pipe for the treated water. Hollow fibre elements consist of bundles of fine diameter (50 - 100 µm) tubes of membrane packed into pressure vessels. In both of these configurations the water needs to pass through narrow constrictions, and pre-filtration is needed to prevent blockage by suspended material.

Return to level 1 (cyanobacteria removal)

Return to level 1 (cyanotoxin removal)

PERMEATE FLOW RATE

One of the most important aspects of membrane process design is the water flux, measured as permeate flow divided by the membrane area (l/m^2.h). Typical flux rates for each of the membrane types are shown in Table 5-1(L2).

Table 5-1(L2) Typical membrane flux rates

Membrane type	Typical flux rate (l/m^{2}h)
Microfiltration	50 - > 100
Ultrafiltration	40 - 80
Nanofiltration	25 – 35
Reverse Osmosis	20

Reduction in flux occurs through fouling of the membrane; this can result from inorganics (e.g. calcium carbonate), particulates, natural organics or biological growth. Particulate fouling can often be overcome by backwashing, whereas other types of fouling may require chemical treatment of the membrane.

Return to level 1 (cyanobacteria removal)

Return to level 1 (cyanotoxin removal)

PRETREATMENTS

Pretreatment is usually carried out to improve membrane performance; the most common method is pre-filtration which reduces the solids loading on the membranes, and reduces backwash requirements. Other pretreatments may be applied, which improve the overall treatment process, but may not improve membrane filtration. One example is the use of PAC applied upstream of a UF or MF membrane process, to remove disinfection by-product precursors; this could also be used for cyanotoxins. Pre-chlorination may be used for some types of membrane, for example to reduce biological fouling of the membrane, although this process should be used with caution in the presence of

cyanobacteria due to possible cell lysis and toxin release. Coagulation can be used prior to UF and MF to reduce DOC concentration and reduce fouling of the membrane. The coagulant used is often alum chlorohydrate, or ferric chloride.

Return to level 1 (cyanobacteria removal)

Return to level 1 (cyanotoxin removal)

PROCESS MONITORING AND CONTROL

Membrane flux decreases during operation, and frequent backwashing is carried out to overcome this. Backwashing is usually based on time of operation (e.g. hourly for UF and MF plants), although it may be initiated on loss of flux or increasing pressure drop. Long term loss of flux is overcome by chemically enhanced backwash (CEB) or chemical clean in place (CIP), using acid, alkali, hypochlorite or proprietary cleaning products, depending on the nature of the fouling.

The integrity of membranes can deteriorate over time, such that rejection decreases i.e. more of the material that the membrane should retain passes into the treated water. UF and MF plants have integrity test procedures to identify reductions in particulate removal that may not be apparent from other measurements. These tests usually involve application of air pressure, and small perforations of the membrane are apparent from a reduction in pressure and/or presence of bubbles in the permeate side of the membrane.

Return to level 1 (cyanobacteria removal)

Return to level 1 (cyanotoxin removal)

PRESSURISED OR SUBMERGED MEMBRANES

A major factor in choice of MF and UF membrane plant is whether to install a pressurised or a submerged membrane system. The former uses hollow fibre membrane modules in tubular pressure vessels, with feed water pumps driving the water though the membrane at typically 0.5 – 1 bar pressure differential. Submerged membranes use modules in tanks, with a vacuum applied to draw water through the membrane. Operating pressure differentials are lower for immersed systems, so more modules are needed, although this may be offset by a more compact packing arrangement for the modules compared with pressure systems. Operating costs for electricity are lower than for pressurised systems, because of the lower pressure differentials. Submerged membrane plants are less mechanically complex, particularly in relation to numbers of valves, and can therefore be easier to operate.

Return to level 1 (cyanobacteria removal)

Return to level 1 (cyanotoxin removal)

DEAD-END OR CROSSFLOW

Two alternative modes of operation can be used for pressurised systems: dead-end or crossflow. The latter involves recycling of water to provide a flushing action across the membrane surface, which can reduce fouling and cleaning frequencies, particularly for feed waters with higher suspended solids. Operating costs for pumping will be higher than for dead-end operation because of the recirculation flow.

Return to level 1 (cyanobacteria removal)

Return to level 1 (cyanotoxin removal)

CYANOBACTERIAL TOXIN REMOVAL

PHYSICAL PROCESSES

ACTIVATED CARBON

MANUFACTURE

Activated carbon is formed by the conversion of carbon from primary materials such as coal, wood, peat or coconut shells. The material is converted into a highly porous structure by heating in the presence of steam, air, or sometimes chemicals to temperatures in the range of 600-1000 ^{o}C. During this process the raw material is converted to layers of 6 membered carbon rings which are bound by physical forces into groups called microcrystallites. The spaces between these microcrystallites, the pores, provide the very large surface area for adsorption. Due to the nature of the starting materials there is always some inorganic material remaining on the surface (N, Fe, S, P, Na, Cl, Si), however, by far the most abundant elements present on the surface of activated carbon are carbon (approximately 80 to 98%) and oxygen (approximately 2 to 20%). The oxygen is present mainly as carbon-oxygen surface groups such as phenolic and carboxyl groups [31]. The internal structure of activated carbon, i.e. the sizes and numbers of the pores, as well of the chemistry of the surface, will depend on the starting material and the activation processes, and will affect the adsorption of target compounds such as algal toxins [32].

Figure 5-3(L2) shows some scanning electron micrographs of two types of activated carbon. The very different external structure of the carbons is also reflected in the internal porous structure and surface chemistry.

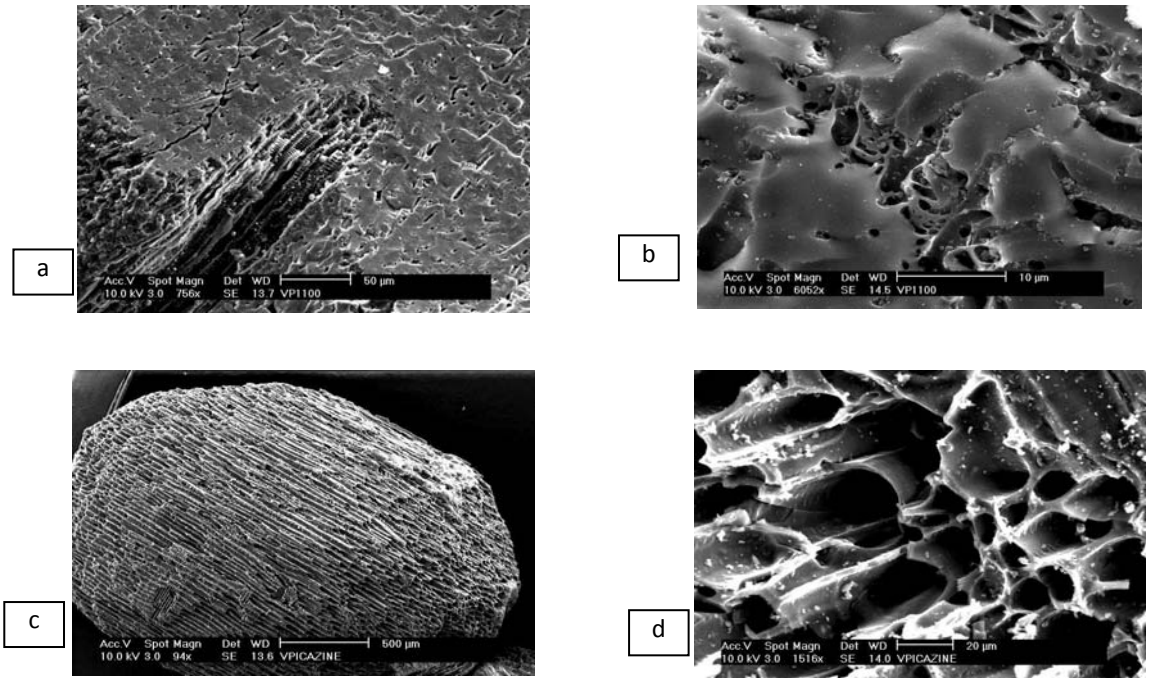

Figure 5-3(L2) Scanning electron micrographs of external activated carbon structure, a) and b) coconut-based activated carbon c) and d) wood-based, chemically activated carbon

Return to level 1

CHARACTERISATION OF ACTIVATED CARBONS

A number of tests are available for characterisation of activated carbons, and activated carbons are described in terms of these characteristics.

SURFACE AREA DETERMINATION AND PORE SIZE DISTRIBUTIONS

These parameters are usually determined using gas adsorption, most commonly nitrogen. The amount of nitrogen adsorbed is measured as a function of the relative pressure, and, based on the size of the N_2 molecule, and using one of a number of theoretical models, surface area and pore size can be calculated. The surface area can be a useful general guide for determining the overall area available for adsorption. For example, a carbon with a surface area of about 500 $m^2 g^{-1}$ would probably not be suitable for the removal of tastes and odours. However, a surface area of 1200 $m^2 g^{-1}$ (relatively high for an activated carbon) would not guarantee a high level of removal of these compounds, as the effectiveness of the adsorbent depends on the range of factors, mentioned above. The pore size distribution (PSD) will give a more reliable hint of whether the carbon will be suitable for a particular purpose, as the aim would be to have a carbon with a high volume of pores in the size range of the target molecule, as well as larger pores that will act as transport pores for the contaminant. The disadvantage of using PSDs is that the analysis is difficult, very low relative pressures of nitrogen are required, and the reproducibility between laboratories is not high.

The pores on activated carbon are categorised according to their size as follows-[33].

Primary micro pores	< 0.8 nanometre (nm)
Secondary micro pores	0.8 - nm - 2 nm
Mesopores	2 nm -50 nm
Macropores	> 50 nm

(One nanometre is one millionth of a millimetre.)

IODINE NUMBER

The iodine number is obtained from a series of adsorption experiments measuring the amount of iodine removed from solution by activated carbon. As iodine is a relatively small molecule it is assumed that the iodine number is an indication of the number of micropores, or the surface area. A value of 800 or higher suggests a high surface area, high “activity” carbon [34].

MOLASSES NUMBER

For this test a solution of backstrap molasses is prepared, and the activated carbon is added. The removal of colour in the solution is measured using UV spectroscopy. Molasses is the syrup remaining after processing sugar cane or sugar beet to obtain sugar. Backstrap molasses is the darkest of the by-products, and contains an unknown mixture of large organic molecules, some of which are highly coloured. It is assumed that the more colour adsorbed by the carbon, the more effective it will be for the adsorption of large organic compounds from water. In reality the number may reflect the volume of large pores, perhaps mesopores, in the carbon structure. A reasonable value for activated carbon is around 250.

TANNIN NUMBER

The tannin number is defined as the concentration of carbon, in mg L^{-1} required to reduce a standard tannin solution from a concentration of 20 to 2 mg L^{-1}. The standard Merck tannic acid recommended for use in this test has a molecular weight of approximately 1700 g mol^{-1}. The tannin number can give an indication of the adsorption capacity of the carbon for DOC, and the lower the tannin number the better the adsorption of tannin.

Essentially the four methods above give good general information, but give specific removal information only about the compound used in the test (e.g. iodine, tannin). Details of the three tests undertaken in aqueous solution are given in the American Water Works Association Standards list, published in 2002.

DENSITY

This parameter is often quoted by manufacturers. In general, a carbon with low density has a large volume of larger pores, such as macropores and mesopores, and relatively fewer micropores. It is also more likely to float, or be abraded during backwashing, which may be an issue for GAC.

ABRASION RESISTANCE

This number gives an indication of the "robustness" of an activated carbon particle. Of particular importance with GAC, where losses can be high through abrasion of particles during frequent backwashing.

PARTICLE SIZE

For GAC filtration, the particle size required will be determined by the physical requirements for effective filtration at the flow rates experienced in the plant, as well as the mode of backwashing utilised. The particle size of PAC is a major influence on the rate of removal of target compounds; the smaller the particle, the higher the rate of removal. As a result, shorter contact times and lower doses are required for smaller PAC particles. However, the advantages are somewhat overcome by the difficulties of removing and handling very small particles of black powder. A diameter of approximately 11 micron has been found to result in high rates of adsorption without major difficulties in removal and handling.

The interpretation of the data obtained using the tests listed above is not trivial, and any perceived relationship between the iodine number and, for example, the amount of cylindrospermopsin adsorbed in 30 mins, is tenuous at best. Although this information is useful, and many of these parameters can be supplied by the activated carbon manufacturer, it is very difficult to use them to help decide on a brand, or raw material for the removal of a particular compound (except, of course, if the target compound is iodine, tannin, or molasses). However, some generalisations can be made regarding the choice of activated carbon for the removal of algal toxins.

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THE ADSORPTION PROCESS

Removal of contaminants by activated carbon is a complex process. Figure 5-4(L2) is a schematic representation of the major processes occurring during adsorption, these are largely diffusion related. In order to be removed by activated carbon a molecule must diffuse:

-to the particle surface from the bulk liquid (1)

-through the liquid surface layer (2)

-through the pore structure of the carbon (3)

finally being removed from solution at the adsorption site (4) (see Figure 5-4(L2))

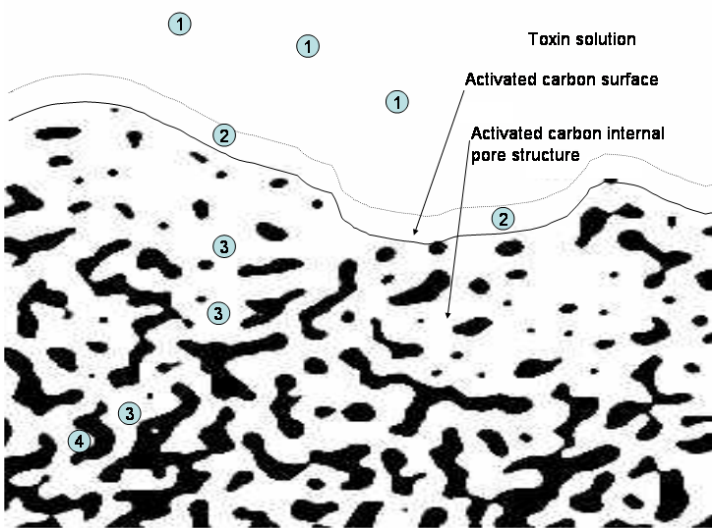

Figure 5-4(L2) Representation of diffusion into the activated carbon structure

Processes 1 and 2 depend on the physical parameters of the system, for example mixing conditions for PAC, flow rates for GAC. Processes 3 and 4 are dependent on the activated carbon pore size distribution and surface chemistry/hydrophobicity. In general, the most favourable energy for adsorption is provided by pores slightly larger than the adsorbing molecule, as there are more contact points for the compound to adhere, and it fits “snugly” into the pore. In water treatment another very important factor is how quickly the contaminant can reach a suitable adsorption site. This is strongly influenced by the access to the internal structure through the pores on the external surface, as well as the structure and size of the “transport pores”, those the contaminant must travel prior to reaching the adsorption site (i.e. step 3,Figure 5-4(L2)).

Physical adsorption is the primary means by which activated carbon works to remove contaminants from water. The highly porous structure provides a large surface area for contaminants (adsorbates) to collect. Physical adsorption occurs because all molecules exert attractive forces, especially molecules at the surface of a solid. The large internal surface area of carbon has many attractive forces which work to attract other molecules. One of the main forces is the attraction between the hydrophobic (“water fearing”) carbon surface and a hydrophobic molecule, or one with hydrophobic parts. The oxygen functional groups impart polarity and, if they dissociate, a charge to the surface, thus they allow adsorption through hydrogen bonding or electrostatic attraction [35].

Due to its very effective porous nature activated carbon adsorbs most compounds present in water to some extent. Although carbon has a very high surface area, invariably there are limited suitable adsorption sites available. A competition is set up between the different species for those adsorption sites, and adsorption of the compound of interest will usually be reduced [36]. The main competing species in surface water are those compounds formed by the breakdown of vegetable and animal matter in the environment, dissolved natural organic material (NOM). This mixture of compounds is collectively measured by dissolved organic carbon (DOC) analysis, or ultraviolet (UV) absorbance measurements.

The factors that influence the adsorption of contaminants, such as pore size distribution and surface characteristics, are dependent on the starting material and method of activation. Even small variations in the chemical composition of

the raw material and activation conditions can result in large differences in the finished product. A range of tests is available to characterise activated carbons with the aim of determining the most appropriate adsorbent for a particular contaminant. Broadly speaking these recommendations apply to both PAC and GAC. Both overall capacity and adsorption rates are also important for GAC filtration. As for PAC, a comparative test is recommended. Details of a useful comparative test for GAC are given below.

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POWDERED ACTIVATED CARBON

PAC PROCESS DESIGN

Flow conditions in the pipework carrying PAC dosed water, should be maintained at sufficient flow/velocity to avoid settlement of PAC and achieve good mixing. PAC particle size is typically in the range 40-80 μm and apparent (bulk) density in the range 360-740 kg/m^3 (assuming a voidage of 0.4, true density would be in the range 600 - 1233 kg per m^3). A particle of 80 μm diameter and density 1233 kg per m^3 would settle at a rate of about 3 cm min^{-1}. The particles should be maintained in suspension by maintaining turbulent flow conditions in the raw water main. Table 5-2(L2) shows minimum velocities and flows to maintain turbulent conditions in a range of pipe sizes.

Table 5-2(L2) Velocity and flow required to maintain turbulent conditions

Pipe diameter (mm)	Velocity required (m/s)	Flow required (m^3/h)
100	0.05	1.3
200	0.02	2.6
300	0.02	3.9
500	0.01	6.4
1000	0.005	12.9

Calculations assume a water temperature of 5 °C, smooth pipes and a Reynold's number of 3000.

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COMPARATIVE TEST FOR PAC

This test can be applied to determine the most cost-effective PAC for application in a water treatment plant.

1 Choose 3-6 good quality activated carbons with the general attributes required for the toxin of interest (see main text). The manufacturer will give general guidance regarding raw materials and average pore sizes.

2 Sample water from the position in the plant where the PAC will be applied. Spike the water with the concentration of toxin that might be expected at the application point. If this is unknown, 5 μg L^{-1} of toxin (for saxitoxins STX equivalents) is a value that will give representative results if converted to percent removals. Take a sample for toxin analysis.

3 Place 500 mL of spiked water into each of three jar testing vessels

4 Add 5, 10, and 30 mg L^{-1} of PAC* into the separate jar test vessels, with stirring.

5 Continue stirring for the average contact time expected after the point of application in the plant. This could be the middle of the range expected over the period of possible toxin contamination. Assume the effective contact time is only while the particles are in suspension in the plant. Disregard time during settling when determining contact time.

6 After the appropriate contact time, filter sample through membrane filter (0.45 μm), analyse samples for toxin concentration, or send to appropriate laboratory.

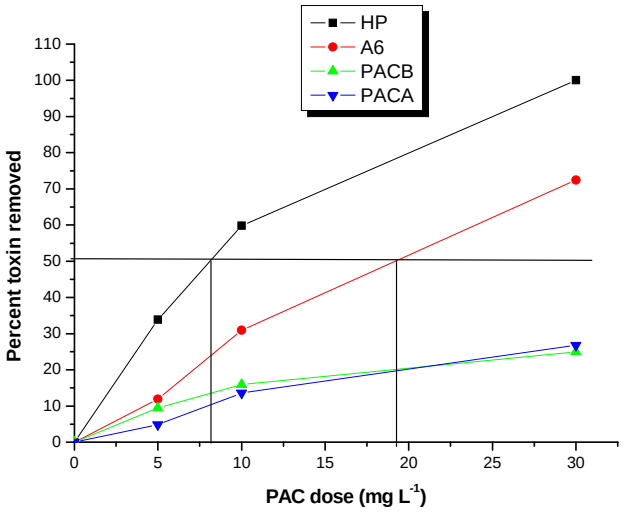
7 Undertake this test for each PAC.

8 Estimate the PAC dose required for 50% removal of the toxin. This can be determined approximately by interpolating a graph of percent removal vs carbon dose (see Figure 5-5(L2)).

9 Multiply the cost per kilogram of the carbon by the dose required, and a simple cost analysis of the carbons can be achieved.

* Prepare a slurry for each carbon by adding 50 mg to 50 cm^3 of milli-Q water or 1:1, one day before running the test

An example of this procedure for microcystins-LR for four carbons is shown in the graph and table in Figure 5-5(L2).



PAC	Dose required for 50% removal	Cost per kilogram	Cost per ML of water treated
1	8	4.20	**$34**
2	19	1.80	**$35**
3	>>30	3.80	**>>$ 114**
4	>>30	2.30	**>> $69**

Figure 5-5(L2) An example of a comparative test for PAC and cost analysis

In this example the most expensive carbon is the most cost effective for the removal of this contaminant.

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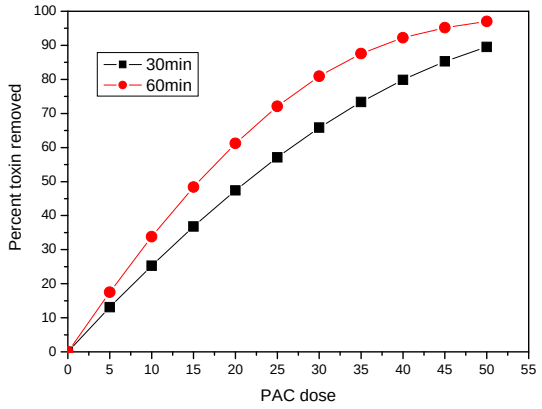
CONSTRUCTION OF PAC DOSE REQUIREMENT CURVES

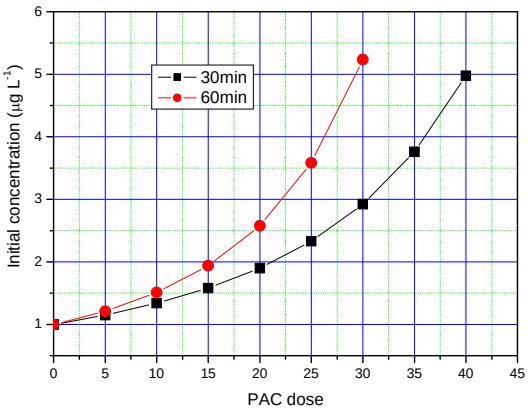
This method is simply an extension of the comparative test described in the section above. Once the most cost-effective activated carbon has been chosen a series of jar tests should be carried out over a larger range of doses, to obtain percent removals from 20 to 90%. These results can be applied to any concentration of toxin as the percent removal is independent of the initial concentration. At least 5 carbon doses should be used to obtain an accurate removal vs dose curve. This should be undertaken at two contact times if the plant could experience a variation in flow affecting the contact time for the PAC. An example is given in Figure 5-6(L2)a) below. To improve the ease of use of this graph, percent removal could be converted to initial concentration. If we assume a target concentration of 1 µg L^{-1} of toxin, the y axis data can be converted to initial concentration using the equation:

Initial concentration =100/(100-percent removal)
For example, 50% removal on the graph would apply to 100/(100-50)=100/50=2 µg L^{-1}
In other words, if the aim is to reduce the concentration of toxin from 2 µg L^{-1} to 1 µg L^{-1} the removal we need is 50%.

Figure 5-6(L2)b shows the same data as Figure 5-6(L2)a, with the percent removal axis converted to initial concentration. Both graphs are equally valid, although b) might be preferred for simplicity.



a



b

Figure 5-6(L2) Indicative PAC dose required for percent toxin removal (a) and to achieve a final concentration of 1 µg L^{-1} (b)

It is relatively easy to determine from Figure 5-6(L2)b that an inlet concentration of 2 µg L^{-1} will require a PAC dose of 15 mg L^{-1} with a contact time of 60 minutes, and 20 mg L^{-1} for a contact time of 30 minutes.

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GRANULAR ACTIVATED CARBON

PROCESS MONITORING AND CONTROL

When the contaminant driving the renewing of the GAC is present in the feedwater continuously, routine sampling of treated water from individual beds is the most common way of identifying breakthrough and the need for regeneration. Whilst this can provide a primarily reactive tool for ensuring that excessive breakthrough does not occur, and can monitor the suitability of an existing regeneration strategy, alone it does not allow any long term planning of regeneration schedules.

The most secure and practical schedule for regeneration (or the replacement of the GAC in the absence of regeneration facilities) involves “staggering”, with beds at different levels of exhaustion and regeneration of the longest running bed at fixed intervals. This offers the following advantages:

- the majority of the beds will have spare capacity to protect against shock loads
- GAC handling is spread over an extended period, rather than the whole works inventory needing to be regenerated over a short time
- each bed in turn can be operated to a predetermined breakthrough concentration above the concentration goal, because of dilution by water from the other beds, which increases the overall adsorption capacity of the system. The potential for this will depend upon the degree of security required, the sampling frequency and delay in obtaining the results, and the time taken to arrange regeneration and replace the GAC

The disadvantages of this mode of operation lie in the need to protect against breakthrough from the longest running bed, and to establish the stagger initially. This means either that new adsorbers have to be brought into service at intervals (probably not practical for most works) or some beds have to be regenerated early, thus losing some potential capacity during the first few years of operation.

For the simplest case, the stagger can be established by estimating the maximum bed life to a treatment target breakthrough concentration, and dividing this by the number of beds to identify the interval between regenerations. If breakthrough curves are available (e.g. from pilot plant trials), these can be used to refine the treatment target concentration from a single bed, using an iterative approach. From the breakthrough curve, the bed life to a treatment target is derived, and this bed life is divided equally by the number of adsorbers to identify the interval between regenerations. The concentration from each bed at this point, and therefore in the final water (i.e. mixed water from all beds), can be identified from the breakthrough curve. The concentration in the final water can be compared with the required quality standard. The treatment target can then be modified upwards or downwards, and the procedure repeated until the required margin of security is achieved (i.e. the minimum acceptable difference between quality standard leaving the works and the calculated mean from this approach). This is illustrated in Figure 5-7(L2). However, for seasonal contaminants such as toxins there will be a need to modify this approach to target higher risk periods, which is considered below.

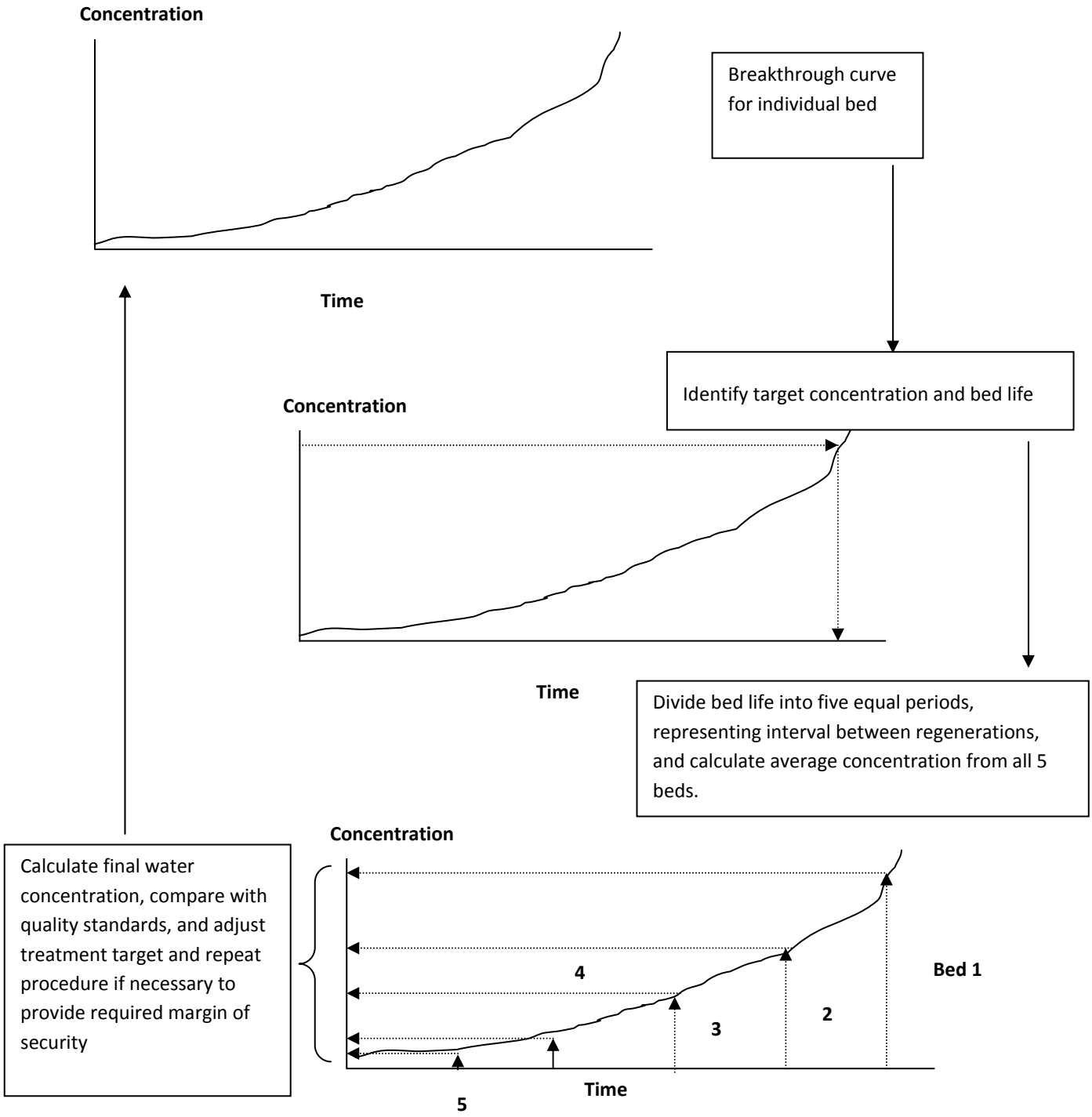

Figure 5-7(L2) Illustration of iterative approach to establishing regeneration schedule for plant with 5 adsorbers

REGENERATION SCHEDULE FOR SEASONAL CONTAMINANTS

Regeneration schedules should make allowance for any seasonal effects, for contaminants such as cyanotoxins. The stagger should be established to take this seasonality into account, to ensure that more beds have been recently

regenerated shortly before the cyanotoxin load is expected. Checks can also be made to evaluate whether shock loads can be dealt with, particularly by the longest running beds. A suggested operational approach for is as follows:

1) Take core samples at intervals, particularly before seasonal contamination might be expected, from the longest running bed and from the bed that has been in operation for the shortest time. Another bed could be included with intermediate run time between these two.

2) Take a representative sample from different depths within each bed (at least from the top half and bottom half).

3) Carry out adsorption isotherm tests on each sample, using appropriate water spiked with the relevant contaminants. Or use the *simplified GAC monitoring test*

Whilst this may not provide an accurate quantification of remaining capacity, it should allow an assessment to be made of the capability of the longer running beds to deal with a sudden increase in load. The most significant feature to identify would be the capacity of the lower part of the oldest bed compared with the upper parts of the bed, and with other beds.

Procedures should be in place to monitor the suitability of the regeneration schedule. As a minimum, this should involve routine sampling of treated water from individual adsorbers, with particular attention being given to the longest running beds. A more rigorous approach may be needed at some sites, particularly with widely varying concentrations of contaminants (e.g. due to algal growth), involving the estimation of loading and remaining capacity for specific contaminants.

The effectiveness of regeneration should be assessed and documented, using at least one procedure in each of the following categories:

- Adsorption properties before and after regeneration e.g. iodine number, methylene blue number.
- Chemical properties: e.g. ash content, leachables.
- Physical properties: e.g. apparent density, particle size range, attrition test.
- Loss of weight and volume on regeneration should also be obtained.

Consistency with relevant standards should be confirmed for regenerated GAC, and procedures should be in place to protect treated water quality on restart with freshly regenerated GAC. It may be necessary to operate a regenerated absorber to waste, until water quality (particularly pH) stabilises.

Monitoring of the feed water and flowrate can allow calculation of the approximate GAC loading at any point in time, and, based on historical data, an estimate of the remaining capacity. This may be adequate for situations where the raw water quality is relatively constant, and where data are available for estimating capacity.

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COMPARATIVE TEST FOR GAC

Testing to determine the most effective GAC in the laboratory is not as straightforward as PAC. Under normal conditions (i.e. 6-20 minute contact time) most virgin GAC will adsorb organic contaminants to below detection, perhaps for a prolonged period. It is therefore very difficult to compare several GACs. Long term pilot plant studies are recommended to determine the most effective GAC and the approximate time until breakthrough of the contaminant. However, these tests are difficult, expensive and time consuming. A simple alternative to determine the most

effective GAC is the short-bed adsorber test in combination with an equilibrium isotherm test. Equilibrium isotherms can be used to compare the capacities of the GACs for the contaminant, and short bed adsorber tests give an indication of the rates of adsorption. Two sets of experiments are required.

Equilibrium isotherms:

1 Sample water at the point in the treatment plant where the GAC will be situated, spike in toxin at a concentration of approximately 5 μg L^{-1}.

2 Place equal volumes of spiked water in each of 5 glass vessels. Volumes of 250-500 mL are preferred.

3 Add GAC, ground to < 45 μm, to 4 of the vessels at doses of 2, 6, 10 15 mg L^{-1}. The 6^{th} vessel will act as the control.

4 Mix vessels consistently to maintain activated carbon in suspension for 3 days.

5 Filter all samples and analyse for toxins.

6 Undertake his test for each carbon and plot percent removed vs carbon dose for each carbon (see Figure 5-8(L2)a).

Short bed column tests:

These tests are designed to force breakthrough of the contaminant for the comparison of different carbons

1 Pack GAC into small diameter column (1 cm) to a bed depth of 4 cm.

2 Pump toxin spiked test water through column at a flow rate equivalent to the filtration rate expected on the filters

3 Collect column outlet samples at regular intervals for a period of 2 hours

4 Analyse samples for toxins and plot percent toxin breakthrough vs time (Figure 5-8(L2)b)

A GAC that shows superior equilibrium capacity and removal in the short bed adsorber test could be expected to perform best at the full scale. In the example below, GACs 1 and 3 appear equivalent and the decision would depend on relative costs

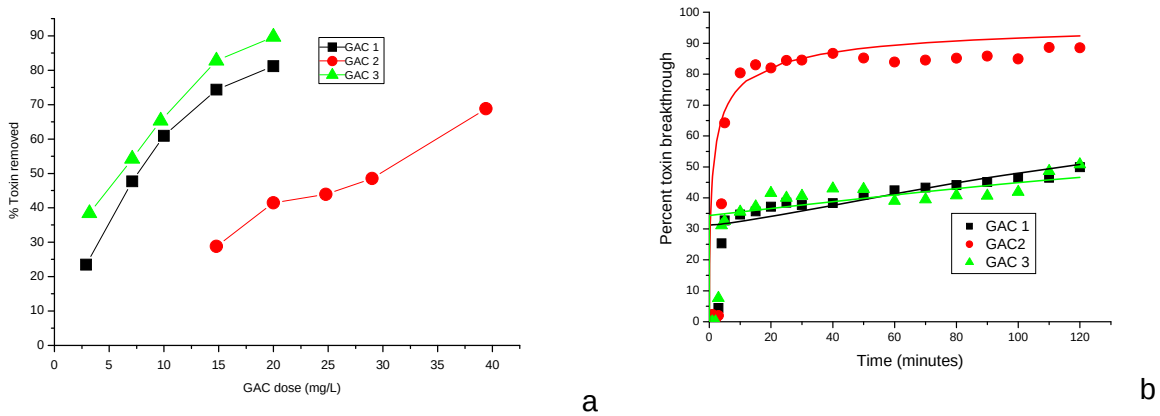

Figure 5-8(L2) Comparative test for GAC. Adsorption isotherms (a) and short bed column test (b)

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GENERAL GUIDANCE FOR GAC SELECTION

It is necessary to identify the most effective GAC for a particular situation, depending on the water type, application and contaminants to be removed. Reliable choice of the most cost effective GAC needs to be based at least on laboratory tests, and ideally on pilot plant trials. Table 5-3(L2) provides general guidance on GAC selection.

Table 5-3(L2) General guidance on selection of GAC

Application	Considerations for GAC selection
Removal of cyanotoxins from surface water, using post filter gravity adsorber	Smaller grain size (e.g. effective size 0.7 mm) and higher uniformity coefficient (e.g. 1.7 – 1.9). If headloss limitations, greater effective size (e.g. 0.9 mm) may be desirable to reduce backwash frequency. Higher overall capacity to deal with background organics (i.e. Iodine Number >1000 mg g^{-1}, Methylene Blue Number >240 mg g^{-1}).
Removal of cyanotoxins from surface water, using GAC as first stage filter media	Need GAC suitable for filtration applications, with larger grain size (e.g. effective size 1 mm) and lower uniformity coefficient (e.g. < 1.5). Higher overall capacity to deal with background organics (i.e. Iodine Number >1000 mg/g, Methylene Blue Number >240 mg g^{-1}). Lower values may be suitable for low TOC feed water (e.g. <2mg L^{-1}).

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EMPTY BED CONTACT TIME

The service life of the bed is dependent on the capacity of the carbon used and the empty bed contact time (EBCT):

$$\text{EBCT} = \frac{\text{Volume of GAC } (m^3)}{\text{Water flowrate } (m^3 / \text{minute})} \text{ (minutes)}$$

Or alternatively

$$\text{EBCT} = \frac{\text{depth of bed (m) x 60}}{\text{filtration rate (m / hour)}} \text{ (minutes)}$$

EBCTs are usually in the range 5 to 20 minutes, depending on the application. Doubling the EBCT will roughly double the service life, possibly giving some reduction in regeneration costs per unit volume treated (depending on the amount of carbon used), at the expense of higher capital cost.

By selecting suitable EBCT and GAC, a long service life (one year or more) can be obtained for many applications. For mixtures of organic compounds, the service life can be governed by the compound which breaks through first. GACs vary considerably in their capacity for specific organic compounds, which can have a considerable effect on service life. A guide to capacity can be obtained from batch equilibrium isotherm tests, but it is difficult to predict bed performance from such tests. Rapid column tests and mathematical models have been developed to help select the best GAC for a particular application, and provide a better prediction of bed life.

A parameter often used to compare GAC performance is the effective carbon dose (ECD) defined as:

$$ECD = \frac{\text{Weight of GAC in bed (g)}}{\text{Volume of water treated during service run } (m^3)}$$

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GAC MONITORING TEST

When GAC has been in use for 6 months or more it is worthwhile to begin to monitor for removal efficiency. For example, if a bloom of *Microcystis* were possible as the warmer months approach, a simple test for microcystin removal will give an indication of the GAC filter's ability to remove the toxin effectively.

Laboratory scale filter columns can be used for this test. A column diameter of 2.5 cm and a bed depth of 7-8 cm has been shown to be optimum. Larger pilot columns can also be used; in this case large volumes of water containing toxin will be required. This may prove an expensive exercise if the test is undertaken using commercial toxin standards.

The test can be conducted as follows

1. Take duplicate samples of 100 mL from the top of each GAC filter after backwash.
2. Place in glass column, 2.5 cm diameter, to a bed depth of 7-8 cm.
3. Pump water, sampled from the plant prior to the GAC filters and spiked with toxin, at a flow rate to achieve the same empty bed contact time as the full scale GAC filters.
4. After several hours take samples from the inlet to the column, and the outlet.
5. Repeat for other GAC samples
6. Analyse samples for toxin and calculate average percent removal.

Clearly this is not a definitive test to determine full scale removals as the samples will not necessarily be representative of the whole filter. However, it can be used to give an *indication* of how the GAC filters would perform. For example, if the small scale column showed an average of 50% removal of microcystin, and this is the level of removal that would be necessary in the plant, it would be wise to consider replacement of the GAC.

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CHEMICAL PROCESSES

OZONATION

PROCESS DESCRIPTION

The main features of an ozonation plant (Figure 5-9(L2)) are described below:

- Feed gas drying equipment: dry air or oxygen is needed to minimise power consumption by the ozoniser. The gas is dried by cooling to cause condensation, followed by adsorption of moisture.

- Ozone generator, usually of the tube type with concentric electrodes in glass tubes surrounded by cooling water.
- Contact tank in which the water is dosed with the ozone-enriched air by means of diffusers at the base. Water depth is typically 5 m deep, providing 10 to 20 minutes contact time in baffled tanks. Some designs allow for recycle and re-injection of this gas to increase the ozonation efficiency. However, these are not in common use.
- Ozone destructor to treat the off-gas from the contactor. The ozone is normally broken down catalytically using metal oxide catalysts, but some designs use an electric furnace heated to 300°C.

Care must be taken in selecting suitable materials for construction of the contactor and, more importantly, the pipework carrying ozonated air. Installation of ozonation plant in existing treatment works may involve considerable engineering complexity compared with the requirements for other chemicals or oxidants.

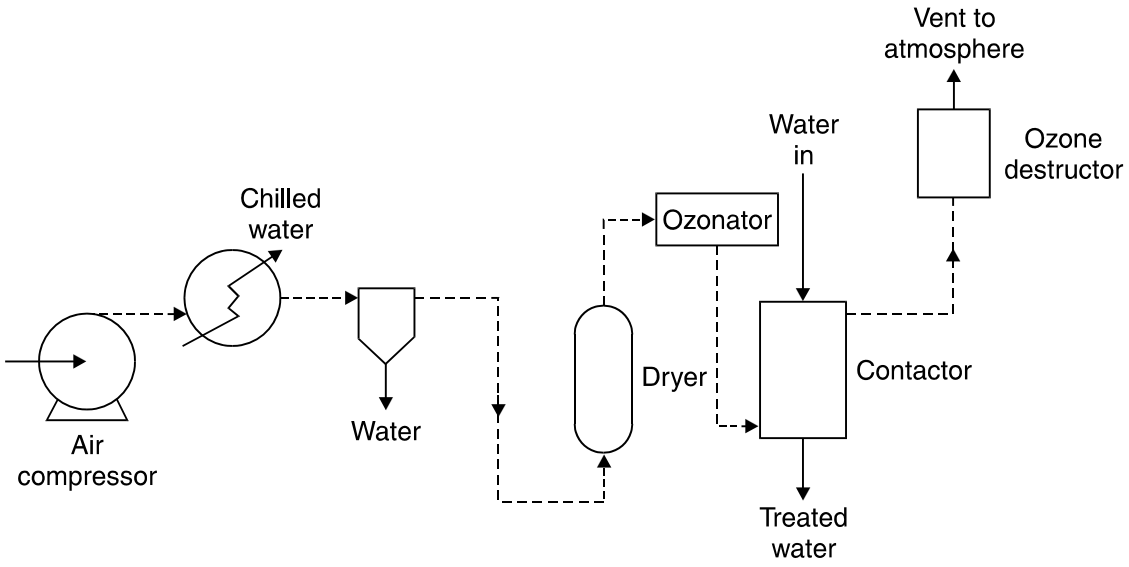

Figure 5-9(L2) Main features of an ozonation plant, ozone generation from air

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POTASSIUM PERMANGANATE

PROCESS DESCRIPTION

Potassium permanganate is normally supplied as a granular powder, and is dosed as a solution, typically at around 10 mg L^{-1}. It reacts with both organic and inorganic constituents in the water, but is most commonly used as a pre-treatment for oxidation of iron and manganese in raw water. The oxidant converts dissolved manganese into insoluble manganese oxides, which can be removed by filtration.

The dose rate is usually set manually, with automatic flow proportional control of pump speed. Although dedicated online monitors for potassium permanganate are not available, it would be possible to measure concentration by calibration of a spectrophotometer, measuring adsorption at 550 nm. Typically, the permanganate dose is adjusted to slightly less than the stoichiometric 'demand' for oxidation of manganese or iron, to avoid problems with pink coloration of the final water. The dose requirement will vary with pH and water temperature, and so requires some careful monitoring.

REMOVAL OF EXTRACELLULAR TOXINS

Doses of potassium permanganate in the range 2 to 10 mg L^{-1}, to a raw water with 2 h contact time, achieved a maximum of 48% removal of m-LR (initial concentration 4.6 µg L^{-1}). There was no residual oxidant at the end of the tests, which indicates that oxidant was probably consumed by competing natural organic matter, probably limiting removal of M-LR. A dose of 2 mg L^{-1} into treated water reduced the initial concentration of m-LR (4.0 µg L^{-1}) to below the limit of detection (0.9 µg L^{-1}). The tests were repeated for another water of different quality; 1 mg L^{-1}achieved 96% removal with raw water, and > 97% removal in treated water. Further tests with clarified and clarified/filtered water, under similar conditions, showed no difference between the two water types[5]. A dose of 0.7 mg L^{-1}achieved 76% removal of m-LR, and 1 mg L^{-1} achieved 88% removal, for initial concentrations of between 5 and 7.2 µg L^{-1}. Removal of anatoxin-a was also found to be effective, 0.5 mg L^{-1} achieving at least 85% reduction from an initial concentration of 4.3 µg L^{-1}, and 1 mg L^{-1} achieving greater than 93% removal. In another study[4], a dose of 1 mg L^{-1} potassium permanganate had no effect on intracellular cyanotoxin, whereas doses of 2 to 3 mg L^{-1} resulted in release of intracellular cyanotoxin and removal of extracellular cyanotoxin, such that the total concentration of M-LR was reduced from 1.4 µg L^{-1} to below the limit of detection (0.6 µg L^{-1}).

Example results of simulation modelling using potassium permanganate for microcystin-LR degradation are shown in Table 5-4(L2).

Table 5-4(L2) Results of simulation modelling of oxidation of microcystin LR using potassium permanganate

Oxidant	Temp (°C)	Concentration (mg L^{-1})	Time for 90% removal of Microcystin-LR
Potassium permanganate	10	1 2	35 minutes 18 minutes
	20	1 2	23 minutes 12 minutes

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UV/PEROXIDE

Pilot plant trials of UV irradiation for destruction of microcystin-LR and anatoxin-a, with and without hydrogen peroxide, were carried out by Anglian Water in the UK using ultrafiltration treated reservoir water spiked with 1µg/l microcystin LR and 0.2 µg/l anatoxin-a. UV doses of 1000 mJ/cm^2 gave around 50% removal of both cyanotoxins. Dosing of hydrogen peroxide at >7 mg L^{-1} had a beneficial effect for removal of anatoxin by UV, but not for microcystin.

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OXIDATION REACTION RATES

One objective the EU project "TOXIC" was to develop a database of cyanotoxin degradation by oxidation laboratory trials. A summary of the results for the oxidants used in water treatment is given in Table 5-5(L2) showing reaction rate constants ($M^{-1}s^{-1}$) and half-life of the cyanotoxins at 20°C and pH 7, based on an oxidant concentration of 1 mg L^{-1}.

Table 5-5(L2) Reaction rate constants for various oxidants and toxins. From [26]

Oxidant	Microcystin-LR	Anatoxin A	Cylindrospermopsin
Chlorine dioxide	1.1 (12 h)	"Low"	0.9 (>14 h)
Permanganate	348 (5 min)	"High"	0.3 (6 d)
Ozone	4×10^5 (0.1 s)	4×10^4 (1 s)	5×10^4 (0.7 s)
HOCl	90 (10 min)	<1 (>15 h)	1100 (48 s)
Monochloramine	0.012 (30 d)	< 1 (>14 h)	<1 (>14 h)

These results indicate the relative performance of the oxidants, and the simulation models, given below, can be used to predict performance in more detail.

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MODELLING OF OXIDATION PROCESSES

APPROACH

Treatment simulation models were developed as part of the EU "TOXIC" project, and the oxidation models have been used here to provide estimated performance information for chlorine, chlorine dioxide, chloramine, potassium permanganate and ozone for microcystin-LR degradation.

Reaction between microcystins and oxidants can be expressed by second order rate constants, and kinetic data have been derived for a range of appropriate oxidants. A modelling approach has been applied, using these rate constants, to develop simple relationships between oxidant concentration, contact time and cyanotoxin breakdown for all oxidants apart from ozone. Results are provided to allow the performance of the oxidants to be evaluated for design or operational purposes.

Interpretation of the modelling data needs to take into account the hydraulics of the contact system in relation to mixing and short-circuiting, and the actual oxidant concentrations which would occur in practice. Information given below on contact tank hydraulics can be used in conjunction with the process simulation model for oxidation available as an output from the TOXIC project.

The performance of a flow-through reactor, such as an oxidant contact tank, is influenced by the hydraulics. It is convenient to consider hydraulics in terms of two extreme types of reactor:

- Plug flow reactor (PFR), in which there is no axial mixing; and
- Continuous stirred tank reactor (CSTR), in which at any moment in time the concentration of any reactant at every point in the reactor and at the outlet is equal.

The CSTR therefore represents a perfectly stirred vessel. All things being equal, a reaction will proceed further in a PFR than in a CSTR, because in a CSTR the incoming reactants are being immediately diluted. Because there is no axial mixing in a PFR every element of fluid resides in the reactor for the same time, equal to the hydraulic residence time (HRT, reactor volume divided by the flow rate). Where there is axial mixing, the residence time of some fluid in the reactor in shorter than the HRT, and for some fluid is longer than the HRT. It is for these reasons that disinfection contact tanks are designed to promote plug flow.

A pipe reactor in which fully turbulent flow is maintained can approach PFR characteristics. The hydraulics of tanks will fall somewhere between the two extremes. A common approach to describing non-ideal hydraulics is to consider a tank as a series of n CSTRs of equal volume (V/n, where V is the tank volume). The effect of increasing n can be illustrated by considering what happens when an inert tracer is instantaneously injected into the inlet of the tank, and monitored at the outlet, Figure 5-10(L2).

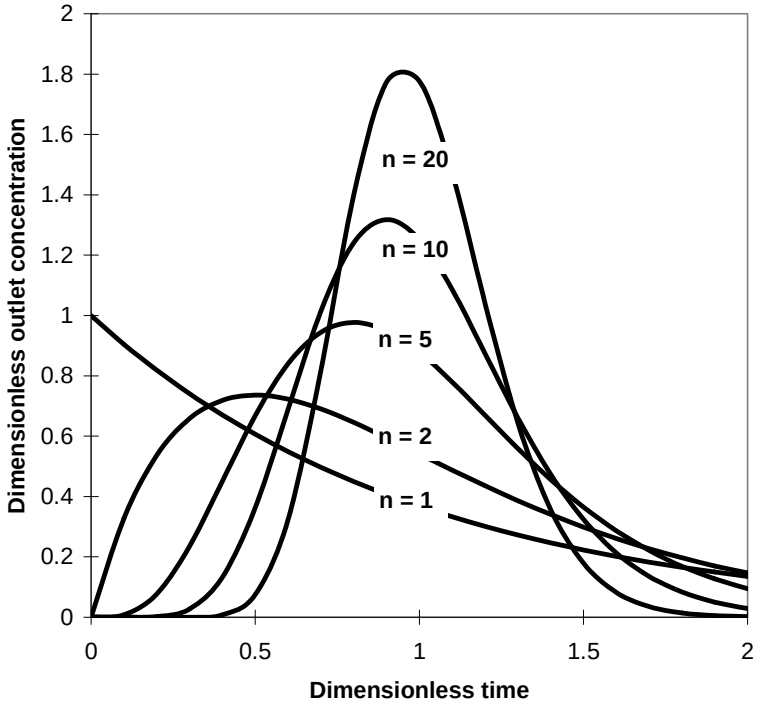


Figure 5-10(L2) Effect of number of CSTRs, n, on a tracer added instantaneously to the inlet of a tank. Note: dimensionless time = 1 is equivalent to the HRT of the tank

It can be seen in Figure 5-10(L2) that when n = 1, which represents the perfectly mixed case, the tracer is instantly dispersed throughout the tank, and the concentration then steadily declines as it is progressively diluted by incoming fluid. In a PFR all of the tracer added at time 0 would exit the tank at a time equal to the HRT, equivalent to 1 on the dimensionless time scale. As n increases, the pattern of tracer at the tank outlet approaches the PFR case. So as $n \rightarrow \infty$, the hydraulics tend to plug flow.

Procedures for carrying out tracer tests and deriving the number of CSTRs for an existing plant are given in the following section. However, modelled results are provided for three categories of hydraulic characteristics, which should adequately approximate the range of conditions found in practice:

- n = 3, which represents a moderately well mixed tank, which in practice means a tank in which little effort has been made to avoid mixing. Since in practice a tank actually designed for mixing is unlikely to achieve n < 2, n = 3 is probably the worst case
- n = 6, which represents a tank in which steps have been taken to avoid mixing, by design of the inlet and outlet and/or inclusion of baffles. A well-designed contact tank is unlikely to achieve a value of n much greater than 6; and the improvement in performance from n = 6 to, say, n = 9 is much less than from n = 3 to n = 6
- Plug flow. Plug flow can be approached in a pipe in which fully turbulent flow is maintained

The modelling approach used here assumes a constant oxidant concentration; in reality this will not occur because of oxidant decay in natural waters, but the results serve to illustrate the relative performance of the oxidants. Three approaches can be used in interpreting the results:

- the concentration can be estimated from the area under the oxidant decay curve
- an average oxidant concentration can be derived from the arithmetic mean of the dose and residual concentration
- the residual can be used to provide a conservative estimate of concentration and toxin breakdown

The first of these is the most accurate, but it is unlikely that this could be derived in practical situations, so an approximation will be needed from one of the other two approaches. Table 5-6(L2) compares chlorine concentrations from these three approaches, based on a chlorine dose of 2 mg L^{-1}, a residual of 1 mg L^{-1}, 45 minutes contact time at 20°C, and first or second order chlorine decay with suitable rate constants.

Table 5-6(L2) Comparison of chlorine concentrations (mg L^{-1}) derived from the decay curve, arithmetic mean and residual

Hydraulic characteristics	1st order decay	2nd order decay	Arithmetic mean	Residual
n = 3 CSTRs	1.28	1.24	1.5	1
n = 6 CSTRs	1.36	1.31	1.5	1
Plug flow	1.43	1.37	1.5	1

The arithmetic mean overestimates actual values based on the decay curve, and would therefore overestimate the breakdown of toxin, whereas the residual would underestimate toxin breakdown. However, the differences are not large, as illustrated in Table 5-7(L2) using modelled data for m-LR removal with chlorine. This assumes first order chlorine decay, a second order rate constant for m-LR breakdown of 72 $M^{1}.s^{-1}$, and chlorination conditions as above.

Table 5-7(L2) Comparison of M-LR removal using decay curve, arithmetic mean and residual chlorine concentrations

Hydraulic characteristics	% removal using residual (1 mg L^{-1})	% removal using decay curve	% removal using arithmetic mean (1.5 mg L^{-1})
n = 3 CSTRs	85	90 (1.28 mg L^{-1})	92
n = 6 CSTRs	89	94 (1.36 mg L^{-1})	96
Plug flow	93	>95 (1.43 mg L^{-1})	>95

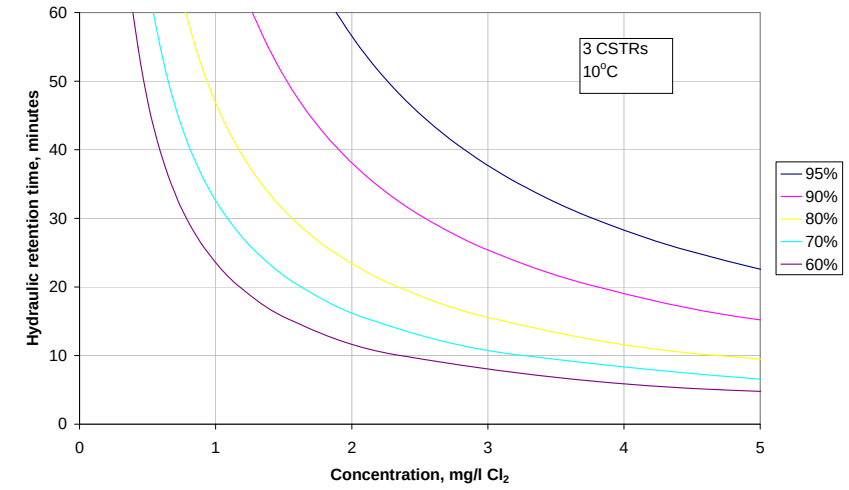
PERFORMANCE

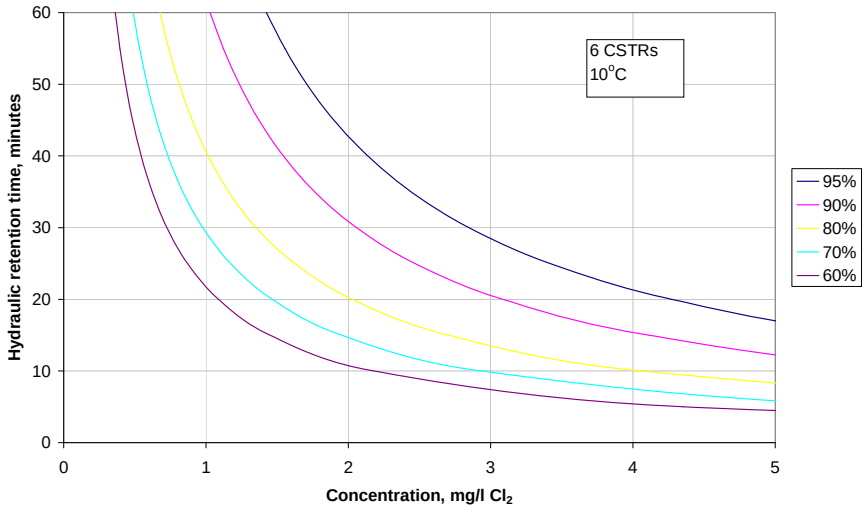
Graphical information is provided below for chlorine and permanganate to allow toxin breakdown to be estimated for existing or proposed plant, after first identifying appropriate hydraulic characteristics and oxidant concentrations as described above. The approach assumes a constant oxidant concentration to illustrate relative performance of each oxidant. Rate constants used are consistent with those given in Table 5-5(L2). Results are given in Figures 5-10(L2) to 5-13(L2).

Less detailed information on chlorine dioxide and chloramine is also provided, mainly to indicate that these are largely ineffective for toxin degradation under practical conditions.

Results for chlorine dioxide are given in Figure 5-15(L2). Under plug flow conditions chlorine dioxide concentrations of 1 mg L^{-1} and 2 mg L^{-1} would require 1 day and 0.5 days respectively to achieve 80% removal at 20°C. A very high concentration of 5 mg L^{-1} would require 7 hours contact time to achieve 90% removal.

Modelling of m-LR breakdown indicates that under plug flow conditions monochloramine concentrations of 1 mg L^{-1} and 2 mg L^{-1} would require more than 35 days and 15 days respectively to achieve 50% removal. A very high concentration of 5 mg L^{-1} would require more than 7 days contact time to achieve 50% removal and over 20 days to achieve 90% removal. Different hydraulic conditions or lower temperature would give less effective removal.





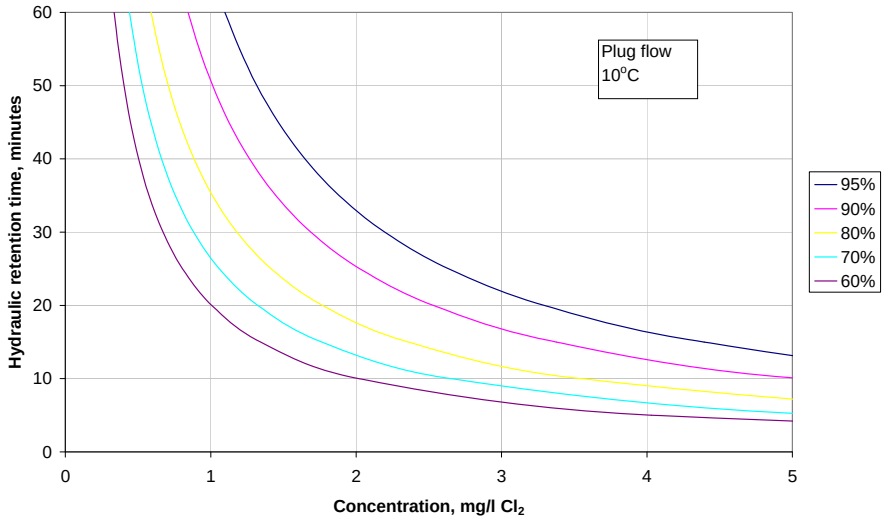


Figure 5-11(L2) Effectiveness of chlorine for m-LR degradation at 10°C

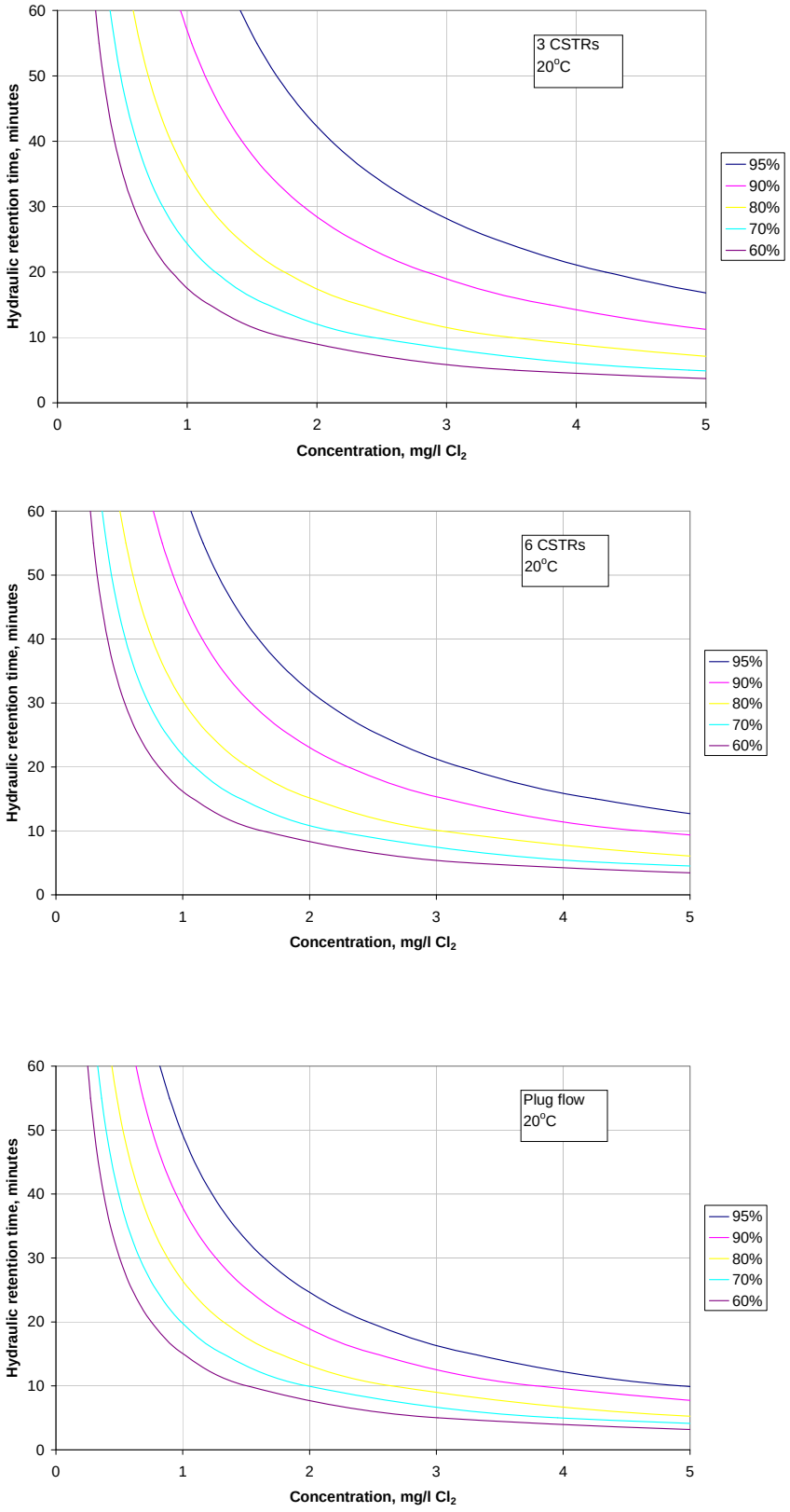


Figure 5-12(L2) Effectiveness of chlorine for m-LR degradation at 20°C

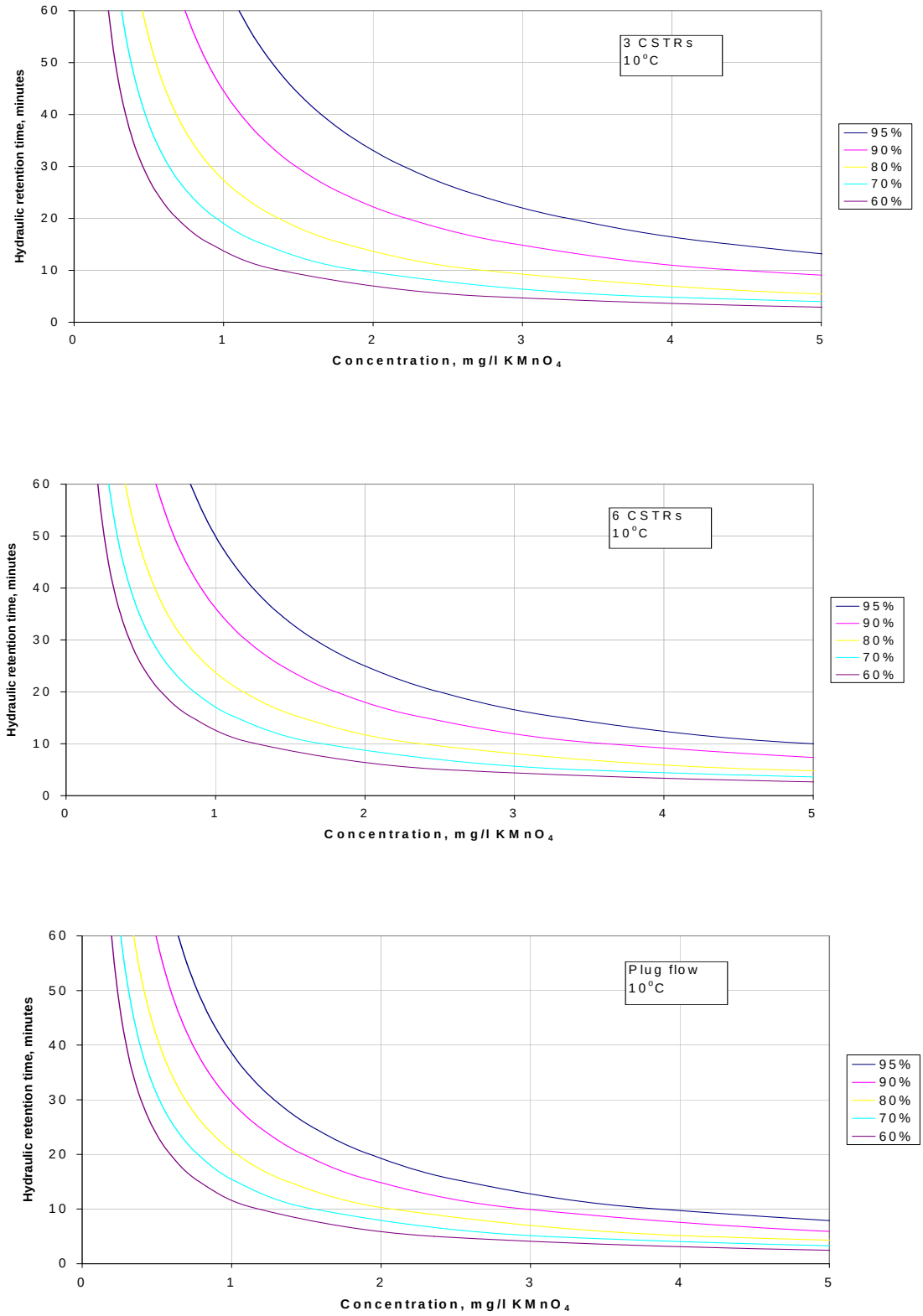


Figure 5-13(L2) Effectiveness of permanganate for m-LR degradation at 10°C

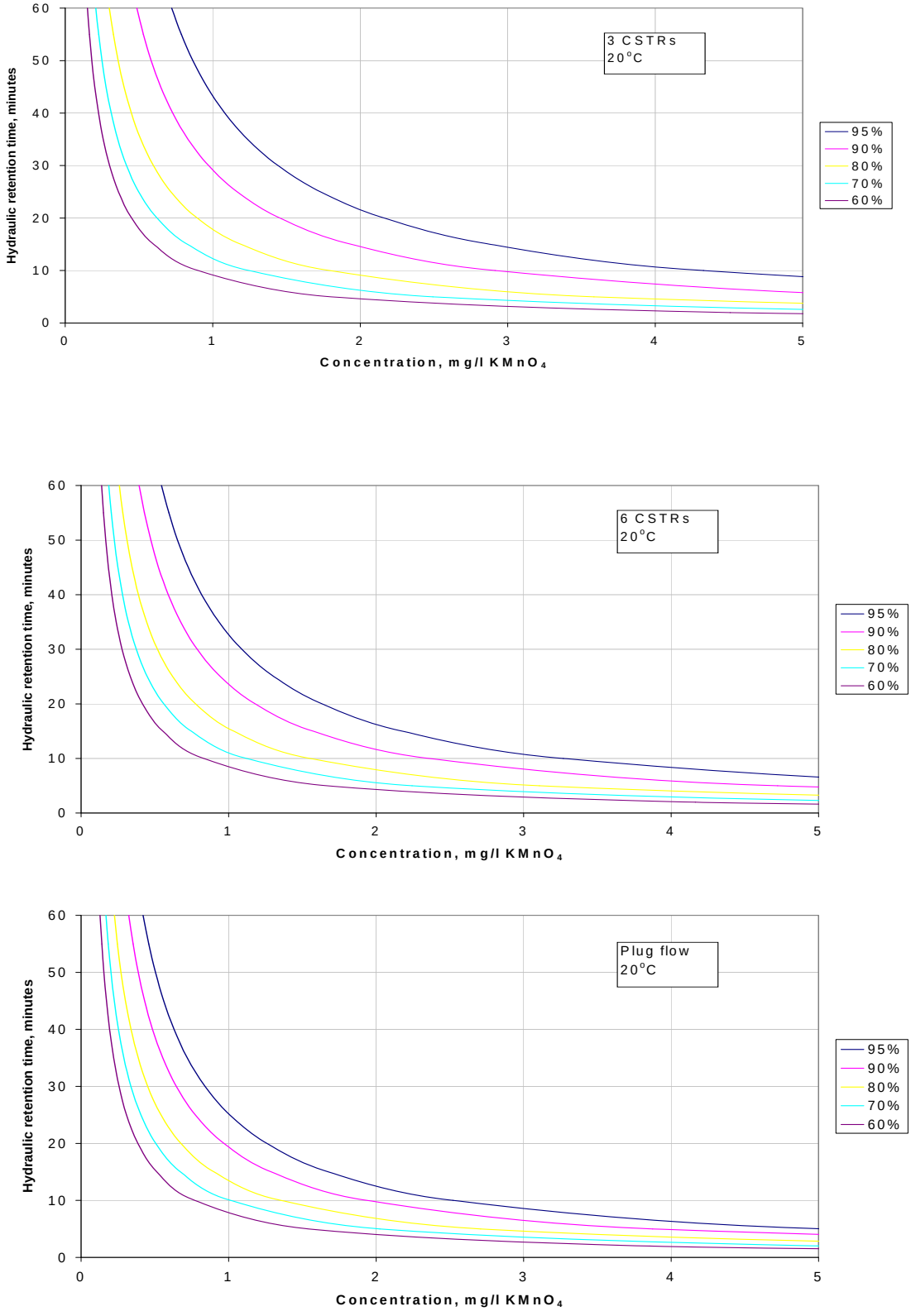


Figure 5-14(L2) Effectiveness of permanganate for m-LR degradation at 20°C

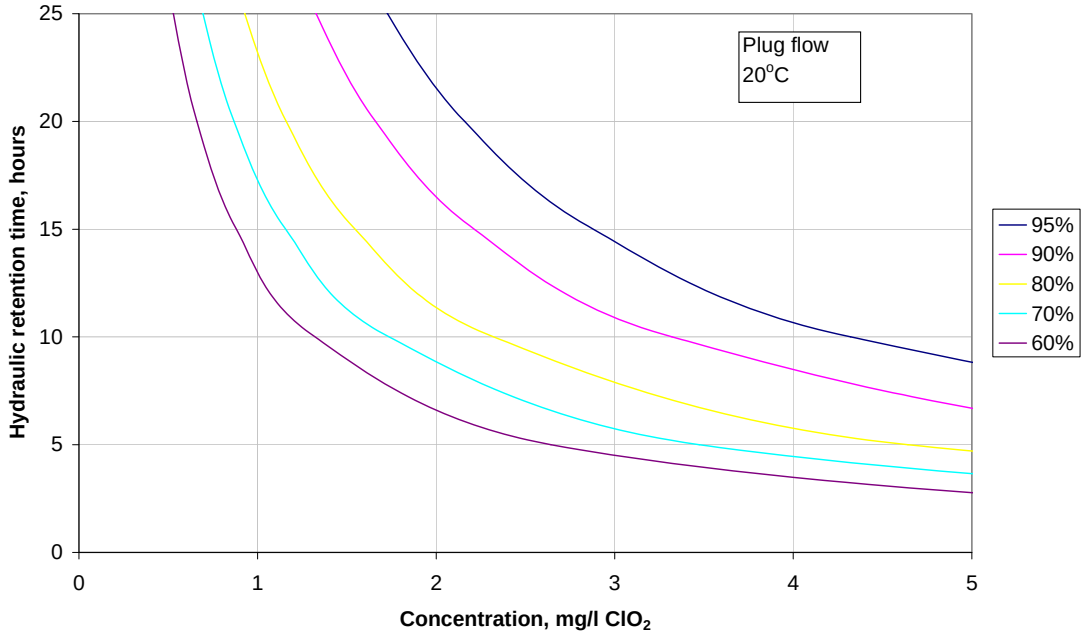


Figure 5-15(L2) Performance of chlorine dioxide for m-LR breakdown

TRACER TESTS FOR OXIDANT CONTACTORS

The value of n of a real tank can be deduced from a tracer test. Lithium chloride is often used, because there is normally little background lithium so small concentrations can be used without needing to take a tank out of service. A chlorine spike is sometimes used to test in-service disinfection contact tanks. If a tank is out of service, other options include sodium chloride (monitored by conductivity) and dye (monitored by UV absorption at the appropriate wavelength). The method is to add an instantaneous dose of tracer to the tank inlet, well mixed with the flow, and monitor for the tracer at the outlet for at least three HRTs. Listing the tracer results as a series of discrete data points of time, t_i, and concentration, C_i, separated by time interval Δt_i (Δt_i does not have to be constant), the value of n is deduced from the mean residence time:

Equation 1

$$\bar{t} \approx \frac{\sum(t_i C_i \Delta t_i)}{\sum(C_i \Delta t_i)}$$

and the variance

Equation 2

$$\sigma^2 \approx \frac{\sum\left(t_i^2 C_i \Delta t_i\right)}{\sum(C_i \Delta t_i)} - \left(\bar{t}\right)^2$$

Then

Equation 3

$$n = \frac{(\bar{t})^2}{\sigma^2}$$

The concept of CSTRs in series requires n to be an integer, so the value found from Equation 3 must be rounded.

An example is given below based on data provided in Table 5-8(L2).

Table 5-8(L2) Example data for calculation of n

t_i (minutes)	Δt_i (minutes)	C_i (mg L^{-1})	$C_i\Delta t_i$	$t_i C_i \Delta t_i$	$t_i^2 C_i \Delta t_i$
0	0	0	0	0	0
1	1	0.1	0.1	0.1	0.1
3	2	0.3	0.6	1.8	5.4
5	2	0.5	1.0	5.0	25.0
6	1	0.7	0.7	4.2	25.2
7	1	1.0	1.0	7.0	49.0
9	2	0.9	1.8	16.2	145.8
10	1	0.5	1.0	5.0	50.0
12	2	0.2	0.4	4.8	57.6
13	1	0.1	0.1	1.3	16.9
15	2	0	0	0	0
			$\Sigma (C_i\Delta t_i)$= 6.7	$\Sigma (t_i C_i \Delta t_i)$=45.4	$\Sigma t_i^2 Ci\Delta t_i$=375

Mean residence time = 45.4/6.7 = 6.8 minutes
Variance = (375/6.7) – 46.2 = 9.8
Number of CSTRs (n) = $6.8^2/9.8$ = 4.7 (=5)

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CHAPTER 6 INCIDENT MANAGEMENT PLANS (LEVEL 1)

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 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 lead to the development and implementation of incident management plans (IMPs), based on alert level frameworks (ALFs), in several countries regularly affected by toxic cyanobacteria, in particular 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 (e.g. with the appropriate health authority, 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, e.g. cell number, cell biovolume or chlorophyll-a concentration. Each value represents a level of risk to drinking water, and will therefore result 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. Links to several frameworks are given below.

ALF developed by Burch, 1993

ALF developed by the World Health Organisation, 1999

CIMF developed by Van Baalen and Du Preez 2001

Australian national protocol for the monitoring of cyanobacteria and cyanotoxins, DRAFT, developed by Burch et al., 2003

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) [1]

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, consumption patterns, etc. 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.

For more details on the actions to be taken at each level follow this link

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	≥ 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.	≥ 2,000[2] & < 6,500 cells mL^{-1} *Microcystis aeruginosa* *-or-* the total biovolume of all cyanobacteria ≥ 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 (ie 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	≥ 6,500 cells mL^{-1} *Microcystis aeruginosa* *-or-* the total biovolume of all cyanobacteria ≥ 0.6 mm^3/L [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,

	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.*	effectiveness of available 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, 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.	≥ 65,000 cells m L^{-1} *Microcystis aeruginosa* *-or-* the total biovolume of all cyanobacteria ≥ 6 mm^3/L (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 (e.g. 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 (≥ 6 mm^3 L^{-1}) (rounded up from 5.7) is approximately equivalent to those numbers of *M. aeruginosa* for Level 3

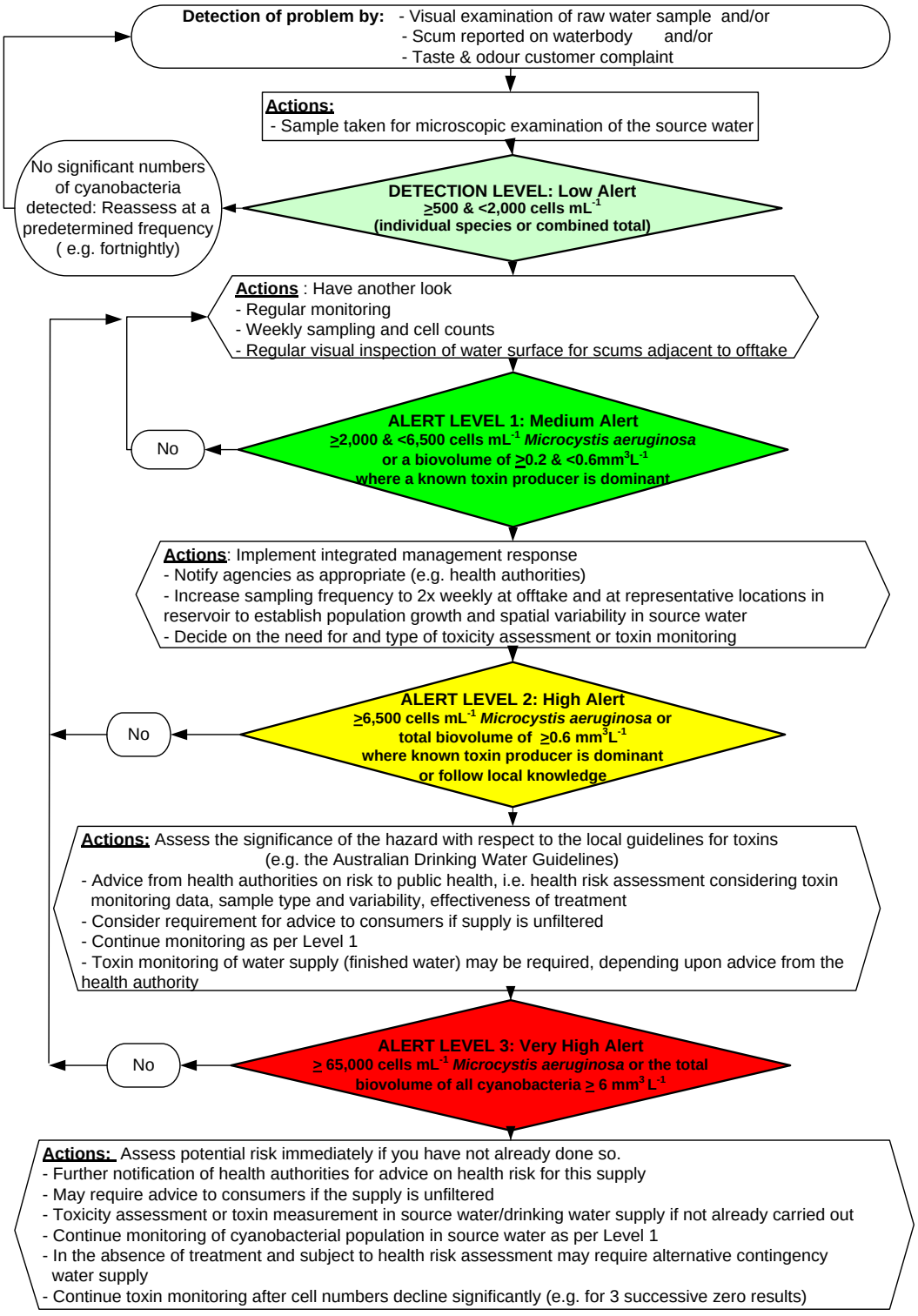

Figure 6-1 Flow chart of the Alert Levels Framework for management of cyanobacteria in drinking water

ALERT LEVELS FRAMEWORK USING CYANOBACTERIAL IDENTIFICATION AND ENUMERATION AS PRIMARY TRIGGER (DU PREEZ AND VAN BAALEN 2006) [2]

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) programme, 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 (***follow this link for details 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 µg 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 µg 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 µg L^{-1} for eight consecutive days or exceeds 5 µg 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.

For more details on the actions to be taken at each level follow this link

ALERT LEVELS FRAMEWORK USING CHLOROPHYLL-A CONCENTRATION AS THE PRIMARY TRIGGER (DU PREEZ AND VAN BAALEN 2006) [2]

For this ALF the primary trigger is chlorophyll-a concentration, while the secondary and tertiary triggers are the same as for 2) 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).

For more details on the actions to be taken at each level follow this link

For an example of a decision matrix that may be used in the application of the preferred ALF, follow this link

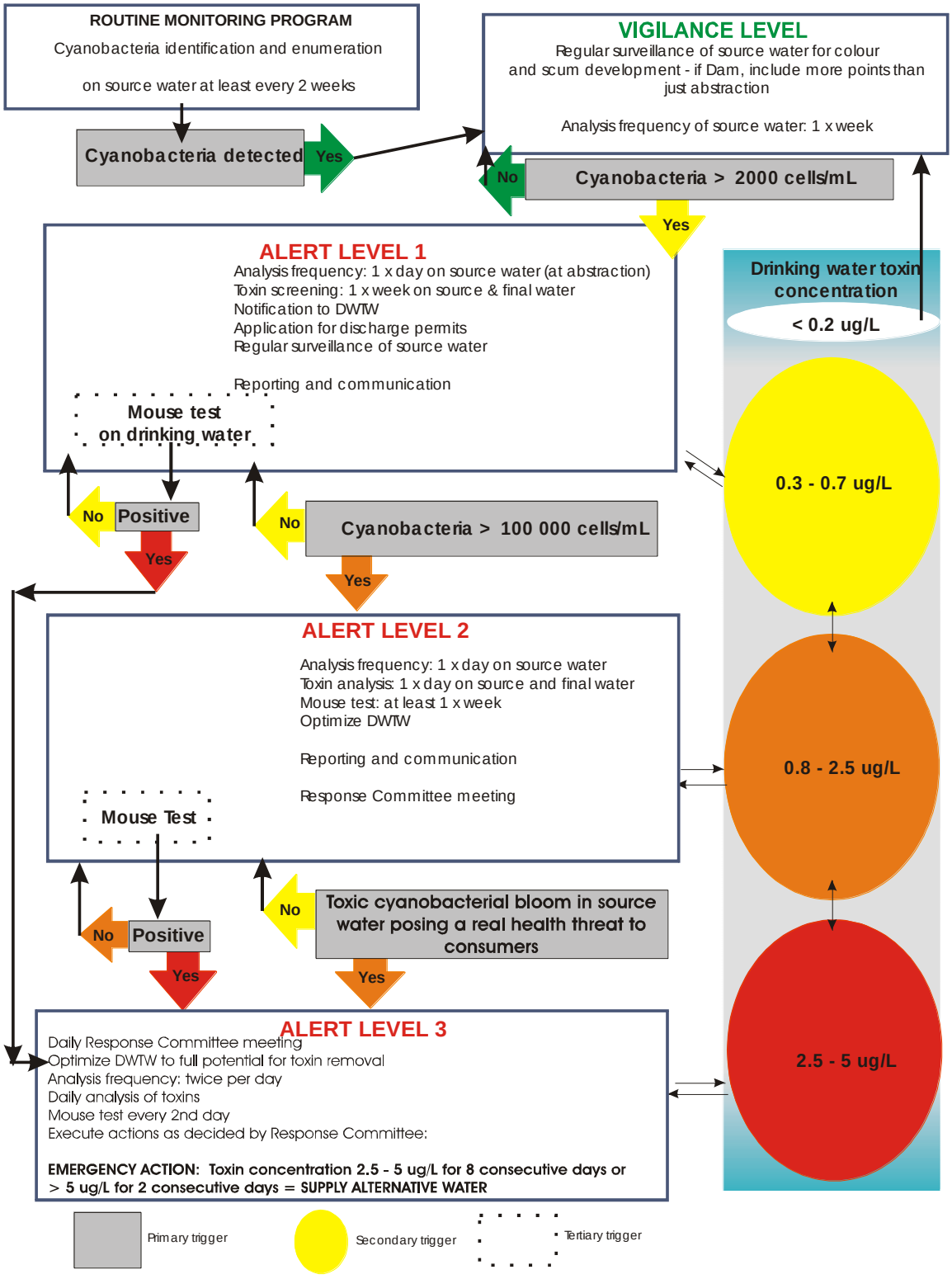

Figure 6-2 Alert Levels Framework using cyanobacterial concentration as primary trigger

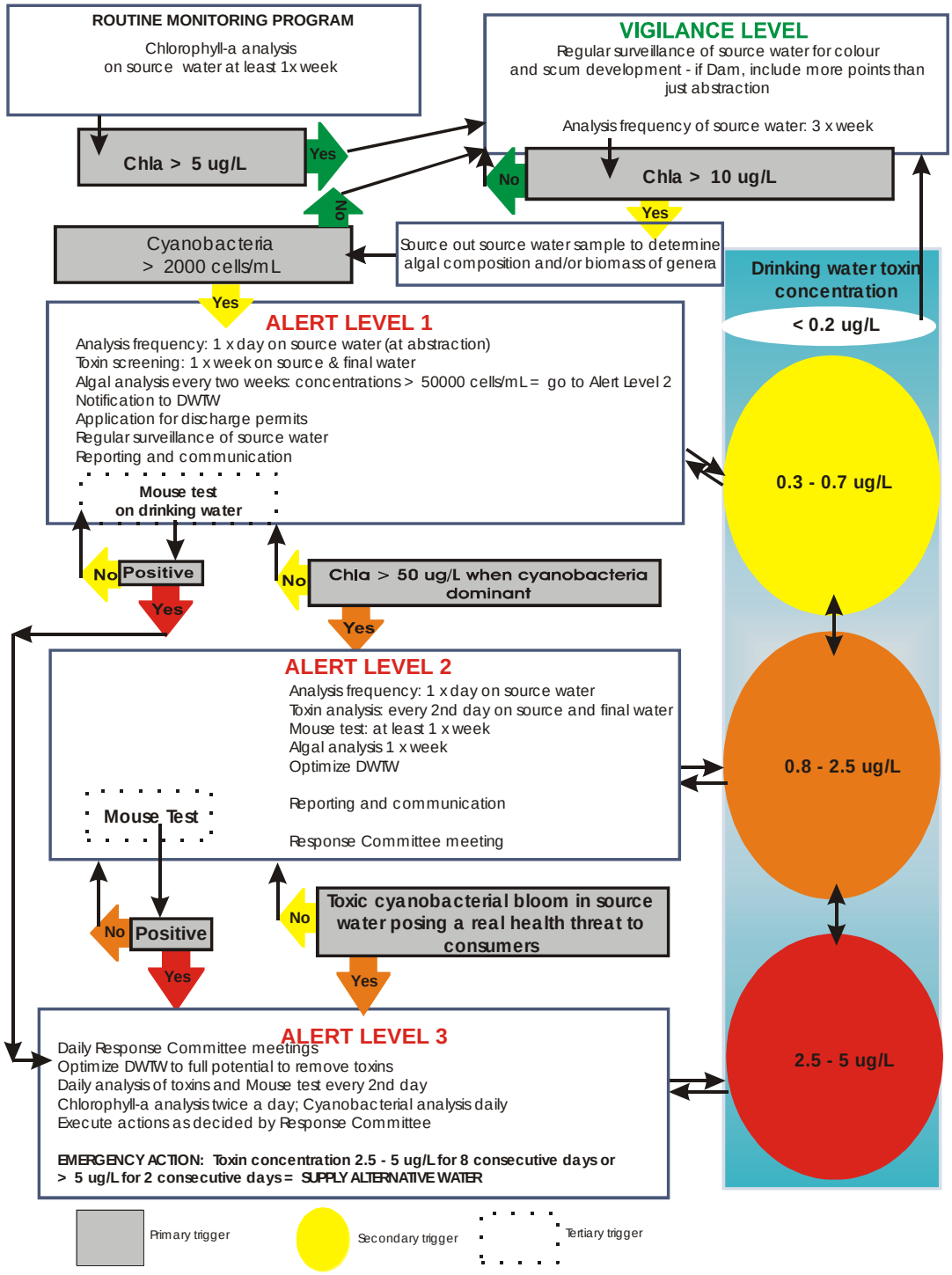

Figure 6-3 Alert Levels Framework using chlorophyll-a concentration as primary trigger

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.

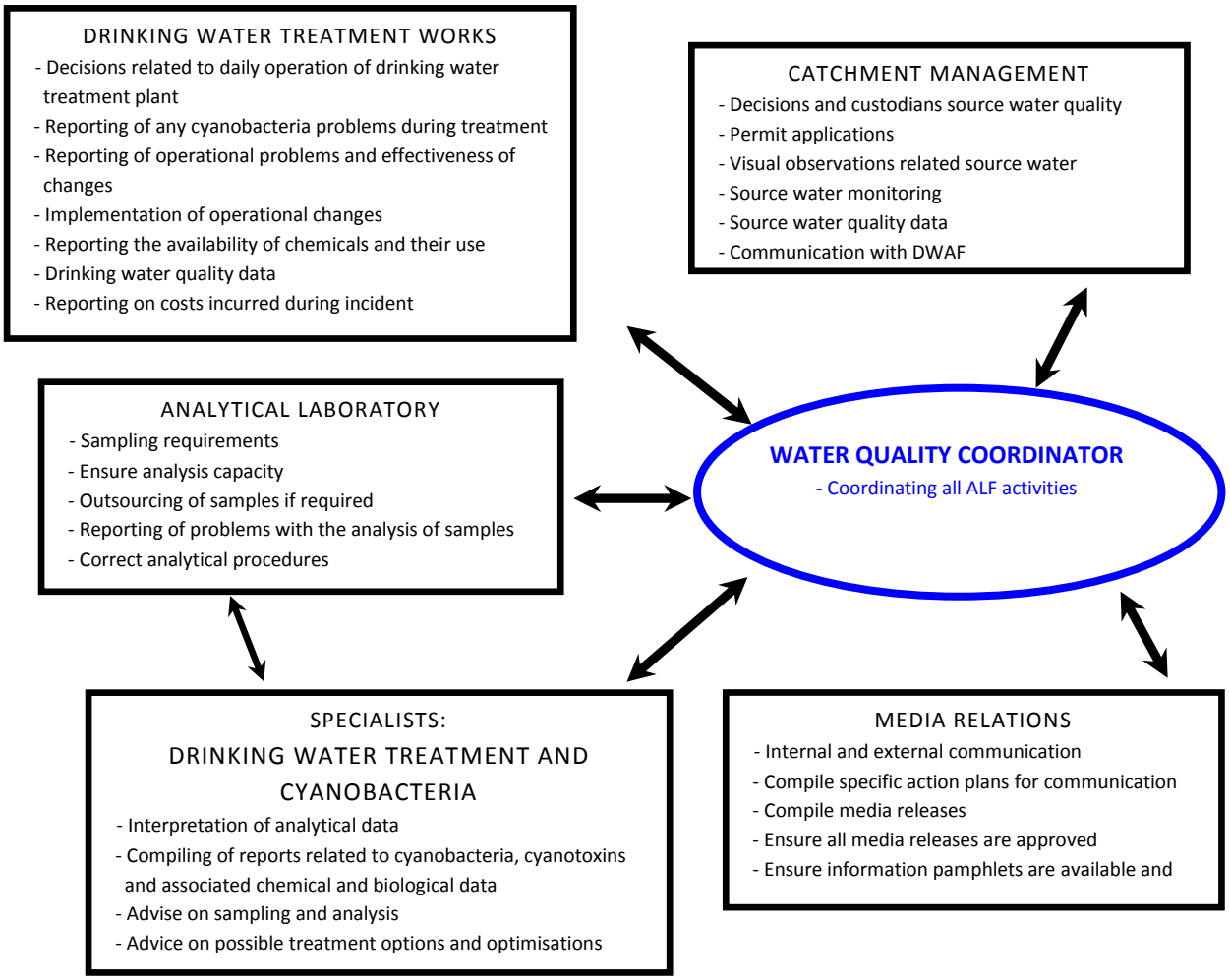

Figure 6-4 Possible communication channels for an ALF [2]

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 [3]. 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 [3] 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 [3]

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 IMS 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.

For an example of a cyanobacteria management plan for Humbug Scrub Reservoir and treatment plant follow this link:

Humbug Scrub Reservoir Algal Management Plan

CHAPTER 6 INCIDENT MANAGEMENT PLANS (LEVEL 2)

OVERVIEW OF THE DEVELOPMENT OF ALERT LEVELS FRAMEWORKS

ALF, BURCH, 1993

In 1993 Burch [4] developed one of the first comprehensive management frameworks based on cyanobacterial cell numbers in the source water. Alert Level 1 is triggered when low numbers (500 to 2000 cells mL^{-1}) are detected in the source water, Alert Level 2 when there are moderate numbers (2000 to 15000 cells mL^{-1}) and Level 3 when there are persistently high numbers (> 15000 cells mL^{-1}), which are toxic. During the Alert Level 1 and Alert Level 2 phases the water supply is considered to be of acceptable quality, but at Alert Level 3 it is considered to be unsafe. The Burch model is further useful to drinking water utilities as it also describes some operational actions (e.g. altering off-take depth, the deployment of booms, the use of PAC, etc.) that could be undertaken, as well as the analyses (e.g. cyanobacteria identification, cyanotoxins analysis) and the consultation that should be undertaken. The Burch model thus formed a generic framework, which could be or has been adapted by many drinking water utilities to include in their specific incident management plans.

Return to level 1

ALF, WHO, 1999

In 1999 the World Health Organisation [5] proposed an Alert Levels Framework for cyanobacteria which is also triggered by different cyanobacterial concentrations in the source water, which are then translated into a Vigilance Level, an Alert Level 1 and an Alert Level 2, with appropriate actions and responses. The Vigilance Level is activated when cyanobacteria are detected at low concentrations. The main actions initiated at this level are an increase in monitoring activities and inspection of the source water at the intakes. Alert Level 1 is activated when the cyanobacterial cell concentration is > 2000 cyanobacteria cells/mL, or the chlorophyll-*a* concentration of the source water exceeds 1 µg L^{-1}. At these cell or chlorophyll-a concentrations it is considered possible that the WHO guideline for microcystin-LR could be exceeded in the source water. At this alert level the main interventions include the expansion of the monitoring program to include cyanotoxin analysis, the feasibility of reducing the intake of cyanotoxins from the source water, an assessment of the capacity of the drinking water treatment works to remove cyanobacteria and cyanotoxins and possible early communications with public health authorities. Alert Level 2 is activated when the cyanobacterial cell concentration exceeds 100 000 cells mL^{-1}, the chlorophyll-a concentration of the source water exceeds 50 µg L^{-1} and the cyanobacteria are toxic. The main actions during this alert level include continuing with the monitoring program and treatment optimisations, consideration of activating alternative water supply plans, increased communication with health authorities and more extensive media releases.

Return to level 1

CIMF, VAN BAALEN AND DU PREEZ, 2001

Van Baalen and Du Preez [6] developed a Cyanobacterial Incident Management Framework (CIMF) for drinking water utilities based on the principles of the Burch [4] and WHO [3] models, but adding additional criteria to make it more practical for day-to-day application by drinking water treatment managers. The Van Baalen and

Du Preez CIMF model consists of various action levels, namely: Routine monitoring ↔ Vigilance Level ↔ Alert Level 1 ↔ Alert Level 2 ↔ Alert Level 3. Between each action alert there are primary triggers (phytoplankton identification and enumeration), secondary triggers (cyanotoxin concentration) and tertiary triggers (mouse bioassay test results), which allow for "movement" (step-up or step-down) between the action alerts.

Return to level 1

DRAFT NATIONAL PROTOCOL FOR THE MONITORING OF CYANOBACTERIA AND CYANOTOXINS, BURCH *ET AL.*, 2003

In 2003 Burch *et al.* [7] developed a national protocol for the monitoring of cyanobacteria and cyanotoxins in surface fresh waters for use in Australia. This protocol includes an Alert Levels Framework for drinking water supply, information on cyanobacteria, cyanotoxins, sampling procedures and analysis procedures for cyanobacteria and cyanotoxins. The Alert Levels Framework primarily uses the cyanobacterial biomass as trigger between the alert levels, ranging from a Detection Level (cyanobacteria > 500 cells mL^{-1}), to Alert Level 1 (cyanobacteria > 2000 cells mL^{-1}), to Alert Level 2 (cyanobacteria > 5000 cells mL^{-1}), and finally to Alert Level 3 (cyanobacteria > 50000 cells mL^{-1}). Biovolumes for the cyanobacteria are also included as trigger values should cell counts not be available. Cyanotoxin analyses are also required throughout the framework and are necessary to assess the risk to the consumer.

Return to level 1

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

DETAILED ACTIONS OF ALF, NEWCOMBE *ET AL.*, 2009 [1]

LEVELS OF THE FRAMEWORK

DETECTION LEVEL

This level encompasses the early stages of bloom development, where cyanobacteria are first detected at low levels in raw water samples. The cell numbers for this level are somewhat arbitrary, ≥ 500 cells mL^{-1} and < 2,000 cells mL^{-1}. Taste and odours may become detectable in the supply, although this does not necessarily indicate the presence of toxic cyanobacteria. If a routine monitoring program is not in place, this is the appropriate time to sample and dispatch the samples to a laboratory for confirmation of the presence of cyanobacteria. If there is no routine program the recommendation for monitoring is to commence weekly sampling and cell counts at a representative location(s) in the water body. The presence of low population densities of cyanobacteria could still mean there is the potential for the formation of localised surface scums, and operators should regularly inspect raw water offtakes for scums or discoloured water.

ALERT LEVEL 1

Alert Level 1 represents the level at which the cyanobacterial population is established, and localised high numbers may occur.

The threshold for this level is a cell number ≥ 2,000 cells mL^{-1} and < 6,500 cells mL^{-1} of *Microcystis aeruginosa* for a sample taken at the source water intake for the drinking water supply, or a total biovolume of all cyanobacteria of ≥ 0.2 and < 0.6mm^3 L^{-1} where a known toxin producer is dominant (Table 6-1).

The variability around a cell count result of 2,000 cells mL^{-1} is likely to be in the range of 1,000-3,000 cells mL^{-1}. This is based upon a likely precision of ±50% for counting colonial cyanobacteria such as *Microcystis aeruginosa* at such low cell densities. For counting filamentous cyanobacteria such as *Anabaena circinalis* the precision is likely to be much better at these cell densities (~±20%), giving an actual likely cell density in the range of 1,600-2,400 cells mL^{-1} for a reported result of 2,000 cells mL^{-1} (see Chapter 3).

The definition for Level 1 is relatively conservative and has been chosen to indicate a point that represents a cell density providing a buffer, or time margin, of 4-6 days before the guideline for toxin in raw water could be exceeded (i.e. Level 2 conditions) if the population is toxic and is actively growing. This is based upon a population doubling rate of 4 days which is equivalent to a growth rate of μ=0.17 d^{-1}.

Alert Level 1 may require notification and consultation with health authorities and other agencies for ongoing assessment of the status of the bloom. Contact with health authorities may be made initially when this level is reached, but may not need to be made weekly if local conditions deem this unnecessary. For instance, if the dominant cyanobacterium present is not known to be problematic based on prior testing and experience (e.g. *Aphanocapsa* sp.), this alert level can be adjusted to suit the local situation.

The requirement for information on toxicity assessment at this level will depend upon advice and discussion with health authorities. It will also depend upon circumstances such as: whether the cyanobacteria are known toxigenic species, past history of toxicity, nature of the supply and associated water treatment, local sensitivity in relation to this supply, etc. This consultation should be initiated as early as possible and continue after the results of toxicity testing and/or toxin analysis become available.

The bloom population should be sampled to establish the extent of its spread and variability. Special samples (concentrated scums and/or grab samples representative for the raw water intake) should be collected and dispatched for toxicity testing or toxin analysis.

This level may warrant operational intervention in drinking water supply, such as the deployment of booms adjacent to offtakes, or changing the depth of drinking water abstraction. Mixing or destratification may be useful in some circumstances to reduce cyanobacterial growth. Treatment with algicides may be an emergency measure in some situations and should be restricted to reservoirs only; its applicability also depends upon local environmental regulations.

ALERT LEVEL 2

Alert Level 2 is the next stage at slightly higher cell numbers of potentially toxic cyanobacteria. The threshold for Level 2 (in the absence of toxin information) is cell numbers and/or biovolume that could indicate the potential for a toxin hazard at or above the guideline level if:

1. the population was highly toxic, and
2. all toxins were released and water treatment is ineffective for their removal.

This level is characterised in general terms by an established bloom with moderately high numbers showing a trend upwards over several successive samples at sampling frequencies of at least twice per week. The cyanobacterial population is likely to have developed to the extent that localised surface scums may form where scum-forming species are prevalent.

Two thresholds definitions for Level 2 (Table 6-1) are:

- Cell numbers $\geq$ 6,500 cells mL^{-1} for *Microcystis aeruginosa* or
- Total biovolume of other cyanobacteria of $\geq$ 0.6 mm^3 L^{-1}, where a known toxin producer is dominant or for local conditions (Note that this is given at 1 significant figure)

The cell numbers for Level 2 ($\geq$ 6,500 cells mL^{-1}) are the preliminary "hazard surrogates" given in the Australian Drinking Water Guidelines (ADWG) for toxic *Microcystis aeruginosa* equivalent to the microcystin guideline of 1.3 µg L^{-1} (Fact Sheet 17a) [8]. The approximate biovolume of 0.6 mm^3 for other cyanobacteria (toxigenic or of unknown toxicity status) is equivalent to 6,500 cells mL^{-1} of *M. aeruginosa*. This biovolume of cyanobacterial cells could be equivalent to the ADWG guideline for microcystins if the cyanobacteria was found to be toxic and to produce microcystins. Furthermore, it is assumed that for blooms and populations of uncharacterised cyanobacteria, the hazard from toxicity is unlikely to exceed the worst case for an equivalent biovolume of highly toxic *Microcystis aeruginosa* containing microcystin. Therefore using this biovolume as indicator of potential toxin hazard in the first instance should allow protection from significant risk while further assessments are made.

As more information about toxicity of different cyanobacteria becomes available it is also possible to develop more specific definitions of Alert Levels for different species of toxic cyanobacteria.

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 health assessment and depend upon circumstances such as availability and performance of water treatment, consumption patterns, etc. It is also possible that an operator may decide to issue advice or a notice at cell numbers lower than these thresholds.

It may be acceptable to continue to supply drinking water from source water even with a positive toxicity result, dependent upon a risk assessment by the health authorities that may recommend specific action to protect more susceptible population groups. The operational interventions at this level are the same as those for Alert Level 1.

ALERT LEVEL 3

The threshold definition for Alert Level 3 is cell numbers of $\geq$ 65,000 cells mL^{-1} of the toxic species *M. aeruginosa* (i.e. toxins confirmed by analytical or bioassay techniques) in the raw water adjacent to the offtake. Alert Level 3 is alternatively defined by the total biovolume of other toxic cyanobacteria $\geq$ 6 mm^3 L^{-1} (see Table 6-1). The cell number for Level 3 represents ten times the Australian Drinking Water Guidelines for toxic *Microcystis aeruginosa* (Fact Sheet 17a) [8] of 6,500 cells mL^{-1}, and is also equivalent to approximately 13 µg L^{-1} microcystin-LR. This describes an established toxic bloom with high cell numbers and possibly localised scums. The sampling program will have indicated that the bloom is widespread with no indication of a cyanobacterial population in decline in the short term. Conditions in Level 3 are indicative of a significant increase in the risk of adverse human health effects from the water if it were untreated, or treated by an ineffective system, even for short-term exposure.

The cell count in Level 3 can be a trigger for the immediate notification to health authorities, but this would only be in a situation where this has not occurred earlier (at Level 1 or 2). This would occur where there was no prior information from an ongoing monitoring program, and treatment is limited or its performance for toxin removal is untested. This could be a scenario where a one-off sample or result is the initial discovery of a major bloom in the source water. By definition the circumstances for Level 3 are that there is some potential for adverse public health outcomes if these high numbers are present in the source water or supply combined

with the nature of the water treatment, the population sensitivity, and their consumption patterns. High cell numbers also mean there is potential for much higher localised concentrations, i.e. surface scums and, depending upon the position of the offtake, this could then mean that very high cell numbers could be entering the supply for short periods and this may not be captured by the monitoring program.

If activated carbon (powdered or granular) or an advanced oxidation process such as ozone is available in the treatment process, it is likely it will be needed at this level. The treated water should be monitored for the specific cyanotoxins occurring in the source water to confirm their removal.

The application of algicides in this phase can potentially enhance problems for treatment by releasing high concentrations of dissolved toxins as a result of cell rupture. Where coagulation and filtration systems generally remove cell-bound toxins, dissolved toxin is more likely to break through the treatment system (Chapter 5).

If water treatment is unsatisfactory for toxin removal, and toxins are present in supply at concentrations significantly above the guideline then Level 3 may result in the activation of a contingency water supply plan that is appropriate for the operator and the system. This may involve switching to an alternative supply for human consumption, or in some circumstances the delivery of safe drinking water to consumers by tanker or in bottles. More extensive media releases and even direct contact with appropriate advice to customers may be necessary. Where advice is provided to the public because of a cyanobacterial hazard to human health it may be appropriate to indicate that the water would be suitable for purposes such as washing, laundry, toilet flushing etc. Closure of a public drinking water supply because of a cyanobacterial hazard in source water is not likely to be justified since potential hazards from disruption of supply (public hygiene and fire-fighting, etc.) are likely to be worse than the cyanobacterial hazard.

Monitoring of the bloom should continue, to determine when it is in decline, so that normal supply can be resumed. Monitoring is usually only warranted at 3-7 day intervals. Experience suggests that the toxicity of a cyanobacterial population can change, but it is unlikely to become completely non-toxic or to decline in a period of a few days.

The sequence of actions at Level 3 should follow through to deactivation of an emergency with advice and media releases to confirm this. It is possible that the collapse of a bloom, or management action such as flushing and control of scum, could lead to a rapid decline from Level 3 back to Level 1 or beyond. Likewise the sequence might escalate rapidly, bypassing Level 1 & 2, if adequate monitoring and early warning information is not available.

CUSTOMER AND MEDIA INFORMATION

Providing information to consumers and media liaison are important aspects of managing water quality problems associated with cyanobacterial blooms. Information should be prompt and concise with detail about reasons for changes to supply and explanation for any differences in water quality. It is important for all of the agencies involved to provide coordinated and consistent advice.

The Alert Levels Framework suggests a number of points where media releases could be issued. These are in situations where consumers may experience changes in water quality, e.g. due to changes in source water quality, switching to another source water, changes in treatment, implementation of a contingency plan, or warning notices for recreational use of the source water.

The approach to releasing information will depend on the nature of the supply and the problem. For example, in major urban water supplies with sophisticated treatment infrastructure, it may not be necessary to advise

consumers, as water quality changes will not be evident. In circumstances with limited treatment, as is often the case in rural or remote areas, or if the bloom occurs in a multiple use water resource (for instance, those also used for recreation) it is important to inform consumers of the extent of the problem as part of the management strategy.

Return to level 1

DETAILED ACTIONS FOR THE ALERT LEVELS FRAMEWORK USING CYANOBACTERIAL IDENTIFICATION AND ENUMERATION AS PRIMARY TRIGGER (DU PREEZ AND VAN BAALEN 2006) [9]

ROUTINE MONITORING LEVEL

Routine monitoring refers to monitoring of the primary trigger namely cyanobacterial identification and enumeration, which is performed on the source water sample from the abstraction point at least once every two weeks. If the analysis can be performed more frequently, that would be an advantage. When a drinking water treatment works is prone to experiencing cyanobacterial/algal-related problems, or has a history of problems in their source water during summer and autumn months, it is recommended that cyanobacterial identification and enumeration analysis is included in the routine source water monitoring program.

ANALYSIS

Cyanobacterial identification and enumeration should be performed on the source water at least once every two weeks. It would be an advantage if this was performed more frequently.

STEPPING–UP ACTIVATION

When cyanobacteria are detected during the routine cyanobacterial analysis then the alert is stepped-up to the Vigilance Level

VIGILANCE LEVEL

REGULAR SURVEILLANCE OF SOURCE WATER

The reservoir, lake or river from which the source water is abstracted should be surveyed for the development of colour and scum associated with a cyanobacterial bloom (excessive cyanobacterial growth). The first site that should be examined is the area around the abstraction point. However, areas close to the shore are usually good places to detect increased algal growth because of the concentration effect in shallow waters. The reason for “looking” for scum development in other areas of a reservoir is that many cyanobacterial species can concentrate in the top layers of the water because of the presence of gas vacuoles and can quite easily be transported by the wind from one location in a dam to another. Therefore, even though cyanobacteria may not be spotted at the abstraction point, this situation can easily change over a short period of time (within hours) by a change in the wind direction whereby a bloom present in another area of the dam will concentrate in the abstraction area.

In a river, the bloom develops as the water moves downstream and then appears at an abstraction point for a short period (pulse or plug flow). In some slow-flowing rivers frequent monitoring supports the detection of increases in cyanobacterial concentration over time. When a river has weirs or naturally-impounded areas it is more likely that cyanobacteria- and algal-related problems will occur there, if they are going to occur at all. People abstracting water along the rivers can also establish a network between companies, and the local community (then it is important to select a central coordinator), whereby the upstream users can notify the downstream users if a “pocket” of high cyanobacterial or algal biomass is seen moving downstream.

ANALYSIS

Cyanobacterial identification and enumeration should be performed at least once per week on the source water.

STEPPING–UP ACTIVATION

When the cyanobacterial concentration of the source water exceeds 2000 cells mL^{-1}, the alert must be stepped-up to Alert Level 1.

STEPPING–DOWN ACTIVATION

When cyanobacteria are not detected for 14 consecutive days during the routine cyanobacterial analysis of the source water then the alert is stepped-down to the routine monitoring level.

ALERT LEVEL 1

REGULAR SURVEILLANCE OF SOURCE WATER

Increase the surveillance of the reservoir (as described under Vigilance Level), from which the source water is abstracted to at least once a week for the development of colour and scum associated with a cyanobacterial bloom (excessive cyanobacterial growth).

ANALYSIS

Cyanobacterial identification and enumeration analysis must be performed daily on the source water at the abstraction point.

CYANOTOXIN SCREENING/ANALYSIS

Cyanotoxin screening refers to the determination of cyanotoxin concentration. It is important to perform a cyanotoxin analysis on the source and the final water. The more comprehensive the better, as appropriate management is more effective when the data are more representative. Results from the source water will indicate if there are any cyanotoxins present and results from the final water will indicate how well the process is performing in removing these toxins (if at all) as well as the potential risk to the consumer.

The frequency of analysis should be at least once per week. If the drinking water utility does not have the capacity to perform cyanotoxin analysis it is important to outsource the samples to laboratories that have that capacity.

MOUSE TEST BIOASSAY

If feasible, a mouse test bioassay is performed to establish whether a water sample has a toxic effect on a mouse. A mouse test bioassay is performed at least on the drinking water during cyanobacterial dominance in the source water. The main objective with the mouse test bioassay is to confirm that no other cyanotoxins are present.

NOTIFICATION TO DRINKING WATER TREATMENT WORKS (DWTW)

The manner in which the "Notification to DWTW" will be executed will proactively be defined by the Response Committee, which would in turn be determined by the size and communication structures of the drinking water utility. The responsibilities of the various role-players must be defined as in the decision matrix, which forms part of the Incident Management Plan of the drinking water treatment works. The notification should be documented and traceable and ideally should include the following:

- Background information including historical data related to previous incidents
- Current trends in the relevant water quality data related to the specific DWTWs
- Prediction in terms of immediate and short-term possibilities of cyanobacterial bloom formation
- Recommendations for possible actions (e.g. ensure sufficient coagulant is available, ensure staff are aware and ready to react at short notice, ensure all steps in process are optimised and are in working condition, etc.) that can be taken into consideration in order to prepare for a cyanobacterial incident
- Reference to the ALF that has been developed for the specific DWTW.

DISCHARGE PERMITS

Should a cyanotoxin incident occur, it is likely that a decision will be taken not to recycle filter backwash water or sludge supernatant back to the head of the DWTW but to store the water on-site in holding dams or to discharge the waste water into the river or reservoir/dam below the point of abstraction. No discharges are permitted without a valid permit. It is also recommended that the process of obtaining a discharge permit be initiated in a proactive manner as this can be a very lengthy process.

REPORTING AND COMMUNICATION

The communication and reporting that must be initiated will have been defined proactively by the Response Committee, which would in turn be determined by the size and the communication structures of the water utility. At this Alert Level there should already be some communication between the water quality coordinator, the specialist on cyanobacteria and drinking water treatment, the analytical laboratory staff and the DWTW Manager.

STEPPING–UP ACTIVATION

When the cyanobacterial concentration in the source water exceeds 100 000 cells mL^{-1} then actions should be stepped-up to Alert Level 2.

OR

When the cyanotoxin (microcystins or nodularin or cylindrospermopsin) concentration in the drinking water exceeds 0.7 µg L^{-1} then actions should be stepped-up to Alert Level 2.

OR

When the mouse test bioassay is positive for cyanotoxins in the drinking water then actions should be stepped-up to Alert Level 3.

STEPPING–DOWN ACTIVATION

When the cyanobacterial concentration in the source water decreases to below 2000 cells mL^{-1} for at least 14 consecutive days, the cyanotoxins (microcystins or nodularin or cylindrospermopsin) concentration in the drinking water is < 0.2 µg L^{-1} for 14 consecutive days and the mouse test bioassay is repeatedly negative for the drinking water, then actions should be stepped-down to the Vigilance Level.

Note: When stepping-up or -down from one Alert Level to the next it is important always to use the primary trigger (in this ALF: cyanobacterial concentration in the source water) as default analysis to determine which actions to take. However, should the cyanotoxin concentration exceed the concentration limits of the Alert Level in which it is operating based on the primary trigger then the secondary trigger (cyanotoxin concentration) overrides the primary trigger and the actions should be performed at the Alert Level specified by the secondary trigger. Similarly, should the mouse test bioassay be positive, then the tertiary trigger (mouse test bioassay) overrides the primary trigger and the actions should be performed at the Alert Level specified by the tertiary trigger. Should the concentration of the secondary trigger decrease to lower Alert Levels (or should the tertiary trigger be repeatedly negative) then actions should revert back to the appropriate Alert Level as dictated by the results of the primary trigger.

ALERT LEVEL 2

REGULAR SURVEILLANCE OF SOURCE WATER

Increase the surveillance of the reservoir, lake or river from which the source water is abstracted. This should be surveyed at least weekly at the abstraction point and surrounding area for the development of colour and scum associated with a cyanobacterial bloom (excessive cyanobacterial growth).

ANALYSIS

Cyanobacterial identification and enumeration analysis must be performed daily on the source water at the abstraction point.

CYANOTOXIN SCREENING/ANALYSIS

Cyanotoxin analysis is performed daily on the source water and the drinking water (also see Section under Alert Level 1). If the drinking water utility does not have the capacity to perform cyanotoxin analysis it is important to outsource the samples to laboratories that have the required capacity

MOUSE TEST BIOASSAY

Mouse test bioassay is performed at least once a week on the drinking water (also see Section under Alert Level 1).

OPTIMISATION OF THE DRINKING WATER TREATMENT PROCESS

The optimisations that should be considered fall into the following broad categories: 1) actions on the abstraction of the source water (e.g. manipulation of the abstraction depth), 2) optimisation of the conventional treatment process (e.g. stop pre-treatment with oxidants, optimisation of coagulation, flocculation, sedimentation, filtration and flotation processes, optimisation of disinfection) and 3) the use of advanced treatment processes (e.g. ozone, powdered activated carbon etc.). If the possible optimisation process that could be implemented has already been done during the development of the ALF, then the main

focus would be to ensure that the actions are implemented and are functioning optimally to ensure that the drinking water utility can effectively remove cyanobacteria and cyanotoxins from the source water as soon as the cyanobacteria numbers increase.

RESPONSE COMMITTEE MEETING

A meeting of the Response Committee is convened at Alert Level 2. At their first meeting it is important 1) to familiarise each member with the Incident Management Plan, 2) to clarify their roles and responsibilities and 3) to update contact information. The Response Committee discusses the current situation based on the available data, determines the appropriate actions that must be taken and identifies any problems that may hinder the implementation of those actions. Dates for feedback and follow-up meetings are set. Formal minutes of the meeting are kept.

DISCHARGE PERMITS

If the discharge permit has not been received from the relevant governmental authority, the Response Committee decides on the course of action to obtain it (see comments under Alert Level 1).

REPORTING AND COMMUNICATION

The reporting and communication focus on internal reporting and communication to ensure that information is shared and any actions are speedily taken and implemented.

STEPPING–UP ACTIVATION

When the cyanobacterial concentration in the source water consistently exceeds 100 000 cells mL^{-1}, and scum forms in the source water and the cyanobacteria have been shown to be toxic then actions should be stepped-up to Alert Level 3.

OR

When the cyanotoxin (microcystins or nodularin or cylindrospermopsin) concentration in the drinking water is between 0.8 and 2.5 µg L^{-1} for more than 14 days, then actions should be stepped-up to Alert Level 3.

OR

When the cyanotoxins (microcystins or nodularin or cylindrospermopsin) concentration in the drinking water exceeds 2.5 µg L^{-1} for more than 4 days, then actions should be stepped-up to Alert Level 3.

OR

When the mouse test bioassay is positive for cyanotoxins in the drinking water then actions should be stepped-up to Alert Level 3.

STEPPING–DOWN ACTIVATION

When the cyanobacterial concentration in the source water decreases to below 100 000 cells mL^{-1} for at least 14 consecutive days, the cyanotoxins analyses (microcystins or nodularin or cylindrospermopsin) concentration in the drinking water is < 0.8 µg L^{-1} for 14 consecutive days and the mouse test bioassays is repeatedly negative for the drinking water then actions should be stepped-down to Alert Level 1.

ALERT LEVEL 3

REGULAR SURVEILLANCE OF SOURCE WATER

Surveillance (see also Vigilance Level) of the reservoir, lake or river from which the source water is abstracted should be undertaken at least daily at the abstraction point and surrounding area for the development of colour and scum associated with a cyanobacterial bloom (excessive cyanobacterial growth).

ANALYSIS

Cyanobacterial identification and enumeration analysis must be performed twice a day (early morning and late afternoon) on the source water at the abstraction point. A depth profile of the cyanobacterial cell concentration in the source water column must be determined (e.g. when abstracting from a dam), and thereafter a series of profiles (at least 4) over a 24 hour period must be performed to optimise the abstraction, as the cyanobacterial cell concentrations may show diurnal depth variation.

CYANOTOXIN SCREENING/ANALYSIS

Cyanotoxin analysis is performed daily on the source water and the drinking water (also see Section under Alert Level 1). If the drinking water utility does not have the capacity to perform cyanotoxin analysis it is important to outsource the samples to laboratories that have the required capacity.

MOUSE TEST BIOASSAY

A mouse test bioassay can be performed on the drinking water on every alternative day (also see Section under Alert Level 1).

OPTIMISATION OF THE DRINKING WATER TREATMENT PROCESS

The following processes must function at their optimal capacity: 1) the abstraction of source water (e.g. manipulation of the depth of abstraction or the use of an alternative source), 2) the conventional treatment process (e.g. stop pre-treatment with oxidants, optimisation of coagulation, flocculation, sedimentation, filtration and flotation processes; optimisation of disinfection), 3) the use of advanced treatment processes (e.g. ozone and powdered activated carbon) and the discarding of filter backwash water.

RESPONSE COMMITTEE MEETING

The Response Committee should meet daily during this Alert Level to evaluate the success of measures implemented and to decide if further actions should be taken. Special attention should be given to solving optimisation problems that are being experienced, alternative actions that can be implemented and to communication with external role-players (Department of Health, Department of Water Affairs, customers and the general public). Formal minutes of the meeting are kept.

DISCHARGE PERMITS

If the discharge permit has not been received from the relevant governmental authority, the Response Committee decides on the course of action to obtain this (see comments under Alert Level 1).

REPORTING AND COMMUNICATION

Reporting and communication focus on both internal and external stakeholders (Department of Health, Department of Water Affairs, customers and the general public) to ensure that information is shared and any actions are speedily taken and implemented.

EMERGENCY ACTION

When the cyanotoxin (microcystins or nodularin or cylindrospermopsin) concentration in the drinking water is between 2.5 and 5 µg L^{-1} for more than 8 days then an alternative drinking water source must be supplied.

OR

When the cyanotoxin (microcystins or nodularin or cylindrospermopsin) concentration in the drinking water exceeds 5 µg L^{-1} for more than 2 days then an alternative drinking water source must be supplied.

STEPPING–DOWN ACTIVATION

When cyanobacterial scum formation in the source water is not evident for at least 14 consecutive days, the cyanotoxin (microcystins or nodularin or cylindrospermopsin) concentration in the drinking water is less than 2.5 µg L^{-1} for 14 consecutive days and the mouse test bioassays are repeatedly negative for the drinking water then actions should be stepped-down to Alert Level 2.

CLOSING PROCEDURE

When the conditions as described for Alert Level 1 occur after a cyanobacterial incident, then the Response Committee should close the incident. This would include a formal report describing the incident, the actions that were taken and the recommendations for improvements to the CIMF as well as preventative actions. All role-players must receive the final communication of the closure of the incident.

Return to level 1

DETAILS OF ALERT LEVELS FRAMEWORK USING CHLOROPHYLL-A CONCENTRATION AS THE PRIMARY TRIGGER (DU PREEZ AND VAN BAALEN 2006) [2]

ROUTINE MONITORING LEVEL

Routine monitoring refers to monitoring of the primary trigger namely chlorophyll-a concentration, which is performed on the source water sample from the abstraction point at least once every week. If the analysis can be performed more frequently that would be an advantage. When a drinking water treatment works is prone to experiencing cyanobacterial/algal-related problems, or has a history of problems during summer and autumn months in the source water it is recommended that chlorophyll-a is included in their routine source water monitoring program.

ANALYSIS

Chlorophyll-a analyses should be performed at least once per week on the source water. It would be an advantage if this were done more frequently.

STEPPING–UP ACTIVATION

When the chlorophyll-a concentration detected during routine monitoring exceeds 5 µg L^{-1} then the alert is stepped-up to the Vigilance Level.

VIGILANCE LEVEL

REGULAR SURVEILLANCE OF SOURCE WATER

The reservoir, lake or river from which the source water is abstracted should be surveyed for the development of colour and scum associated with a cyanobacterial bloom (excessive cyanobacterial growth). The first site that should be examined is the area around the abstraction point. However, areas close to the shore are usually good places to detect increased algal growth because of the concentration effect in shallow waters. The reason for “looking” for scum development in other areas of a reservoir is that many cyanobacterial species can concentrate in the top layers of water (because of the presence of gas vacuoles) and can quite easily be transported by the wind from one location in a dam to another. Therefore, even though cyanobacteria may not be spotted at the abstraction point, this situation can easily change over a short period of time (within hours) by a change in the wind direction whereby a bloom present in another area of the dam will concentrate in the abstraction area.

In a river, the bloom develops as the water moves downstream and then appears at an abstraction point for a short period (pulse or plug flow). In some slow-flowing rivers frequent monitoring supports the detection of increases in cyanobacterial concentration over time. When a river has weirs or naturally-impounded areas it is more likely that cyanobacteria- and algal-related problems will occur there, if they are going to occur at all. People abstracting water along the rivers can also establish a network between companies, and the local community (then it is important to select a central coordinator), whereby the upstream users can notify the downstream users if a “pocket” of high cyanobacterial or algal biomass is seen moving downstream.

ANALYSIS

Chlorophyll-a analysis must be performed on the source water at least three times a week. If the analysis can be performed more frequently that would be an advantage. Cyanobacterial identification and enumeration analysis should be performed on the source water sample if the chlorophyll-a concentration exceeds 10 µg L^{-1}. If the drinking water utility does not have the capacity to perform the cyanobacterial identification and enumeration analysis, it is important that the sample be outsourced to a laboratory that does have the required capacity.

STEPPING–UP ACTIVATION

When the chlorophyll-a exceeds 10 µg L^{-1} and the cyanobacterial concentration of the source water exceeds 2000 cells mL^{-1} then the alert must be stepped-up to Alert Level 1.

STEPPING–DOWN ACTIVATION

When the chlorophyll-a concentration detected in the source water is less than 5 µg L^{-1} for 14 consecutive days then the alert is stepped-down to the Routine Monitoring Level.

OR

When no cyanobacterial concentration is detected in the source water sample then the alert is stepped-down to the Routine Monitoring Level.

ALERT LEVEL 1

REGULAR SURVEILLANCE OF SOURCE WATER

Surveillance (as described under Vigilance level) of the reservoir, lake or river from which the source water is abstracted, should be conducted at least twice a week for the development of colour and scum associated with a cyanobacterial bloom (excessive cyanobacterial growth).

ANALYSIS

Chlorophyll-a analysis must be performed daily on the source water at the abstraction point. Cyanobacterial identification and enumeration analysis should be performed at least every two weeks on a source water sample. If the drinking water utility does not have the capacity to perform the cyanobacterial identification and enumeration analysis, it is important to outsource the sample to a laboratory that does have the required capacity.

CYANOTOXIN SCREENING/ANALYSIS

Cyanotoxin screening refers to the determination of cyanotoxin concentrations. It is important to perform a cyanotoxin analysis on the source and the final water. The more comprehensive the better, as appropriate management is more effective when the data are more representative. Results from the source water will indicate if there are any cyanotoxins present and the final water will indicate how well the process is performing in removing these toxins (if at all) and also indicate the potential risk to the consumer.

The frequency of analysis should be at least once per week. If the drinking water utility does not have the capacity to perform cyanotoxins analysis it is important to outsource the samples to laboratories that have the required capacity.

MOUSE TEST BIOASSAY

If feasible a mouse test bioassay is performed to establish whether a water sample has a toxic effect on a mouse. A mouse test bioassay is performed at least on the drinking water during cyanobacterial dominance in the source water. The main objective with the mouse test bioassay is to confirm that no other cyanotoxins are present.

NOTIFICATION TO DRINKING WATER TREATMENT WORKS (DWTW)

The manner in which the "Notification to DWTW" will be executed will be proactively defined by the Response Committee, which would in turn be determined by the size and communication structures of the drinking water utility. The notification should be documented and traceable and ideally should include the following:

- Background information including historical data related to previous incidents
- Current trends in the relevant water quality data related to the specific drinking water treatment works
- Prediction in terms of immediate and short-term possibilities of cyanobacterial bloom formation
- Recommendations for possible actions (e.g. ensure sufficient coagulants are available, ensure staff are aware and ready to react at short notice, ensure all steps in process are able to be optimised and are in working condition, etc.) that can be taken into consideration in order to prepare for a cyanobacterial incident
- Reference to the ALF that has been developed for the specific drinking water treatment works.

DISCHARGE PERMITS

Should a cyanotoxin incident occur, it is likely that a decision will be taken not to recycle filter backwash water back to the head of the drinking water treatment works but to store the water on-site in holding dams or to discharge the filter backwash water into the river or reservoir/dam below the point of abstraction. No discharges are permitted without a valid permit. It is also recommended that the process of obtaining a discharge permit be initiated in a proactive manner as this can be a very lengthy process.

REPORTING AND COMMUNICATION

At this Alert Level there should already be some communication between the water quality coordinator, the specialist on cyanobacteria and drinking water treatment the analytical laboratory staff and the drinking water treatment works manager.

STEPPING–UP ACTIVATION

When chlorophyll-a exceeds 50 µg L^{-1} and cyanobacteria are dominant in the source water and their concentration exceeds 50 000 cells mL^{-1} then the alert must be stepped-up to Alert Level 2.

OR

When the cyanotoxin (microcystins or nodularin or cylindrospermopsin) concentration in the drinking water exceeds 0.7 µg L^{-1} then actions should be stepped-up to Alert Level 2.

OR

When the mouse test bioassay is positive for cyanotoxins in the drinking water, then actions should be stepped-up to Alert Level 3.

STEPPING–DOWN ACTIVATION

When the chlorophyll-a concentration detected in the source water is less than 10 µg L^{-1} and the cyanobacterial concentration in the source water decreases to below 2000 cells mL^{-1} for at least 14 consecutive days, the cyanotoxin analysis (microcystins or nodularin or cylindrospermopsin) concentration in the drinking water is < 0.2 µg L^{-1} and the mouse test bioassays is negative for the drinking water, then actions should be stepped-down to the Vigilance Level.

Note: When stepping-up or -down from one Alert Level to the next it is important always to use the primary trigger (in this ALF: chlorophyll-a concentration in the source water) as the default analysis to determine which actions to take. However, should the cyanotoxin concentration exceed the concentration limits of the Alert Level in which it is operating (based on the primary trigger) then the secondary trigger (cyanotoxin concentration) over-rides the primary trigger and the actions should be performed at the Alert Level specified by the secondary trigger. Similarly, should the mouse test bioassay be positive, then the tertiary trigger (mouse test bioassay) over-rides the primary trigger and the actions should be performed at the Alert Level specified by the tertiary trigger. Should the concentration of the secondary trigger decrease to lower Alert Levels (or the tertiary trigger be negative repeatedly) then actions should revert back to the appropriate Alert Level as dictated by the results of the primary trigger.

ALERT LEVEL 2

REGULAR SURVEILLANCE OF SOURCE WATER

Surveillance (see also Vigilance Level) of the reservoir (dam), lake or river from which the source water is abstracted, should be conducted daily at the abstraction point and surrounding area for the development of colour and scum associated with a cyanobacterial bloom (excessive cyanobacteria growth).

ANALYSIS

Chlorophyll-a analysis must be performed daily on the source water at the abstraction point. Cyanobacterial identification and enumeration analysis should be performed once a week on a source water sample. If the drinking water utility does not have the capacity to perform the cyanobacterial identification and enumeration analysis, it is important to outsource the sample to a laboratory that does have the required capacity.

CYANOTOXIN SCREENING/ANALYSIS

Cyanotoxin analysis is performed every second day on the source water and the drinking water (see also Section under Alert Level 1). If the drinking water utility does not have the capacity to perform cyanotoxin analysis, it is important to outsource the samples to laboratories that have the required capacity.

MOUSE TEST BIOASSAY

If feasible the mouse test bioassay is performed at least once a week on the drinking water (see also Section under Alert Level 1).

OPTIMISATION OF THE DRINKING WATER TREATMENT PROCESS

The optimisations that should be considered fall into the following broad categories: 1) actions on the abstraction of source water (e.g. manipulation of the depth of abstraction), 2) optimisation of the conventional treatment process (e.g. stop pre-treatment with oxidants, optimisation of coagulation, flocculation, sedimentation, filtration and flotation processes, optimisation of disinfection) and 3) the use of advanced treatment processes (e.g. ozone and powdered activated carbon).

It is recommended that the possible optimisation process be identified and tested in a proactive manner during the development of the IMP for the specific drinking water utility. If this has been done, the main focus would then be to ensure that the actions are implemented and are functioning optimally so that the drinking water utility can effectively remove cyanobacteria and cyanotoxins from the source water whenever the cyanobacterial concentrations increase. This will also reduce the risk of reaching Alert Level 3.

RESPONSE COMMITTEE MEETING

A meeting of the Response Committee is convened at Alert Level 2. The structure, roles and responsibilities of each member of the Response Committee would have been defined proactively during the development of the CIMF for the specific drinking water treatment works. However, this would be dependent on the size and the communication structures of the drinking water utility.

At the first meeting it is important 1) to familiarise each member with the IMP, 2) to clarify the roles and responsibilities and 3) to update contact information. The Response Committee discusses the current situation based on the available data, the appropriate actions that must be taken and identifies any problems that may hinder the implementation of the actions. Dates for feedback and follow-up meetings are set. Formal minutes of the meeting are kept.

DISCHARGE PERMITS

If the discharge permit has not been received from the relevant governmental authority, the Response Committee decides on the course of action to obtain this (see comments under Alert Level 1).

REPORTING AND COMMUNICATION

Reporting and communication focus on internal reporting and communication to ensure that information is shared and actions are speedily taken and implemented.

STEPPING–UP ACTIVATION

When the cyanobacterial concentration in the source water consistently exceeds 100 000 cells mL^{-1}, are toxic and with scum forming in the source water, then actions should be stepped-up to Alert Level 3.

OR

When the cyanotoxin (microcystins or nodularin or cylindrospermopsin) concentration in the drinking water is between 0.8 and 2.5 µg L^{-1} for more than 14 days then actions should be stepped-up to Alert Level 3.

OR

When the cyanotoxin (microcystins or nodularin or cylindrospermopsin) concentration in the drinking water exceeds 2.5 µg L^{-1} for more than 4 days then actions should be stepped-up to Alert Level 3.

OR

When the mouse test bioassay is positive for cyanotoxins in the drinking water then actions should be stepped-up to Alert Level 3.

STEPPING–DOWN ACTIVATION

When the chlorophyll-a concentration detected in the source water is less than 50 µg L^{-1} and the cyanobacterial concentration in the source water decreases to below 50 000 cells mL^{-1} for at least 14 consecutive days, the cyanotoxin analysis (microcystins or nodularin or cylindrospermopsin) concentration in the drinking water is less than 0.8 µg L^{-1} and the mouse test bioassays are negative for the drinking water, then actions should be stepped-down to the Alert Level 1.

ALERT LEVEL 3

REGULAR SURVEILLANCE OF SOURCE WATER

Surveillance (see also Vigilance Level) of the reservoir, lake or river from which the source water is abstracted, should be conducted at least daily at the abstraction point and surrounding area for the development of colour and scum associated with a cyanobacterial bloom (excessive cyanobacteria growth).

ANALYSIS

Chlorophyll-a analysis must be performed twice a day (early morning and late afternoon) on the source water at the abstraction point. Cyanobacterial identification and enumeration analysis must be performed daily on the source water at the abstraction point. A depth profile of the cyanobacterial cell concentration in the source water column must be determined if applicable (e.g. if water is abstracted from a dam), thereafter a series of at least 4 profiles over a 24 hour period must be performed to optimise the abstraction as the cyanobacterial cell concentrations may show diurnal depth variation.

CYANOTOXIN SCREENING/ANALYSIS

Cyanotoxin analysis is performed daily on the source water and the drinking water (also see Section under Alert Level 1). If the drinking water utility does not have the capacity to perform cyanotoxin analysis it is important to outsource the samples to laboratories that have the required capacity.

MOUSE TEST BIOASSAY

A mouse test bioassay can be performed on the drinking water at every alternative day (also see Section under Alert Level 1).

OPTIMISATION OF THE DRINKING WATER TREATMENT PROCESS

The following processes must function at their optimal capacity: 1) the abstraction of source water (e.g. manipulation of the depth of abstraction or the use of an alternative source), 2) the conventional treatment process (e.g. stop pre-treatment with oxidants, optimisation of coagulation, flocculation, sedimentation, filtration and flotation processes, optimisation of disinfection), 3) the use of advanced treatment processes (e.g. ozone and powdered activated carbon) and the discarding of filter backwash water.

RESPONSE COMMITTEE MEETING

The Response Committee should meet daily during this Alert Level to evaluate the success of measures implemented and to decide if further actions must be implemented. Special attention should be given to solving optimisation problems that are being experienced, alternative actions that can be implemented and to communication with external role-players (Department of Health, Department of Water Affairs, customers and the general public). Formal minutes of the meeting are kept.

DISCHARGE PERMITS

If the discharge permit has not been received from the relevant governmental authority, the Response Committee decides on the course of action to obtain this (see comments under Alert Level 1).

REPORTING AND COMMUNICATION

Reporting and communication focus on both internal (relevant role-players) and external role-players (Department of Health, Department of Water Affairs, customers and the general public) to ensure that information is shared and any actions are speedily taken and implemented.

EMERGENCY ACTION

When the cyanotoxin (microcystins or nodularin or cylindrospermopsin) concentration in the drinking water is between 2.5 and 5 µg L^{-1} for more than 8 days then an alternative drinking water source must be supplied.

OR

When the cyanotoxin (microcystins or nodularin or cylindrospermopsin) concentration in the drinking water exceeds 5 µg L^{-1} for more than 2 days then an alternative drinking water source must be supplied.

STEPPING–DOWN ACTIVATION

When cyanobacterial scum formation in the source water is not evident for at least 14 consecutive days, the cyanotoxin (microcystins or nodularin or cylindrospermopsin) concentration in the drinking water is less than 2.5 µg L^{-1} for 14 consecutive days and the mouse test bioassays are repeatedly negative for the drinking water then actions should be stepped-down to Alert Level 2.

CLOSING PROCEDURE

When the conditions as described for Alert Level 1 occur after a cyanobacterial incident, then the Response Committee should close the incident. This would include a formal report describing the incident, the actions that were taken and the recommendations for improvements to the ALF, as well as preventative actions. All role-players must receive the final communication of the closure of the incident.

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RESPONSE COMMITTEE FOR THE ALF

The application of an ALF requires a co-ordinated effort from all stakeholders. It is recommended that a Response Committee is formed to ensure the ALF is applied effectively and in a timely manner.

A typical Response Committee can comprise members with the following ability/authorisation:

- Water Quality Coordinator (Coordinator of the ALF)
- Management Representative from the drinking water utility (authority to make highest level decisions)
- Person responsible for the day-to-day management of the drinking water utility and who has authority to make decisions
- Person responsible for the sludge disposal plant and has who authority to make decisions
- The drinking water utility chemist (to advise on water quality optimisation)
- Analytical laboratory representative (responsible for analysis of samples)
- Catchment management representative (responsible for discharge permits and catchment monitoring)
- Communication representative (responsible for external communication - media, other companies, Department of Health, newspapers, etc.)
- Specialist on drinking water treatment
- Specialist on cyanobacteria and cyanotoxins

It must be stressed that there is no fixed composition of representation on the Response Committee as it will depend on the size, reporting structure and the communication lines of the specific structures of the drinking water utility. One representative can also fulfill more than one of the functions listed above.

AGENDA FOR THE RESPONSE COMMITTEE MEETING

An example of a basic agenda for a Response Committee meeting is as follows:

- Welcome
- Brief situation summary by the Water Quality Coordinator.
- Brief overview of the Alert Levels Framework
- Clarification of roles and responsibilities as required by ALF
- Feedback by the specialist on Cyanobacteria:
  - Graphs with cyanobacterial concentrations during the current season and graphs with concentrations of previous seasons (if available)
  - Prediction on cyanobacterial biomass/growth for the remainder of the season and the risk of the occurrence of cyanotoxins. Input from Catchment Management Representative
  - Indication of the company's standing on the Alert Levels Framework
- Water Quality Coordinator feedback:
  - The company's standing on the Alert Levels Framework

  - Feedback on measures that have been implemented to date. (Make sure that these are in line with recommendations provided in the Alert Levels framework)
  - Highlight problem areas
- Feedback by the drinking water treatment works representatives:
  - Identification of envisaged optimisation problems
  - Recommendations on what should be done operationally to reduce the risk of going to a higher Alert Level
- Open-floor discussion on:
  - The optimisation actions that should be applied, which must be in line with the ALF
  - Alternative measures that are available but which are not included in the ALF
- Feedback from the media relations representative:
  - Clarification of communication channels as documented in the ALF
  - Presentation of available communications documentation
  - Identification of information needs (with sources and timing)
  - Confirm communication channels for the benefit of all
- Summary by water quality coordinator the main actions to be taken, and their links to the ALF
- Date of the next meeting
- Meeting Close

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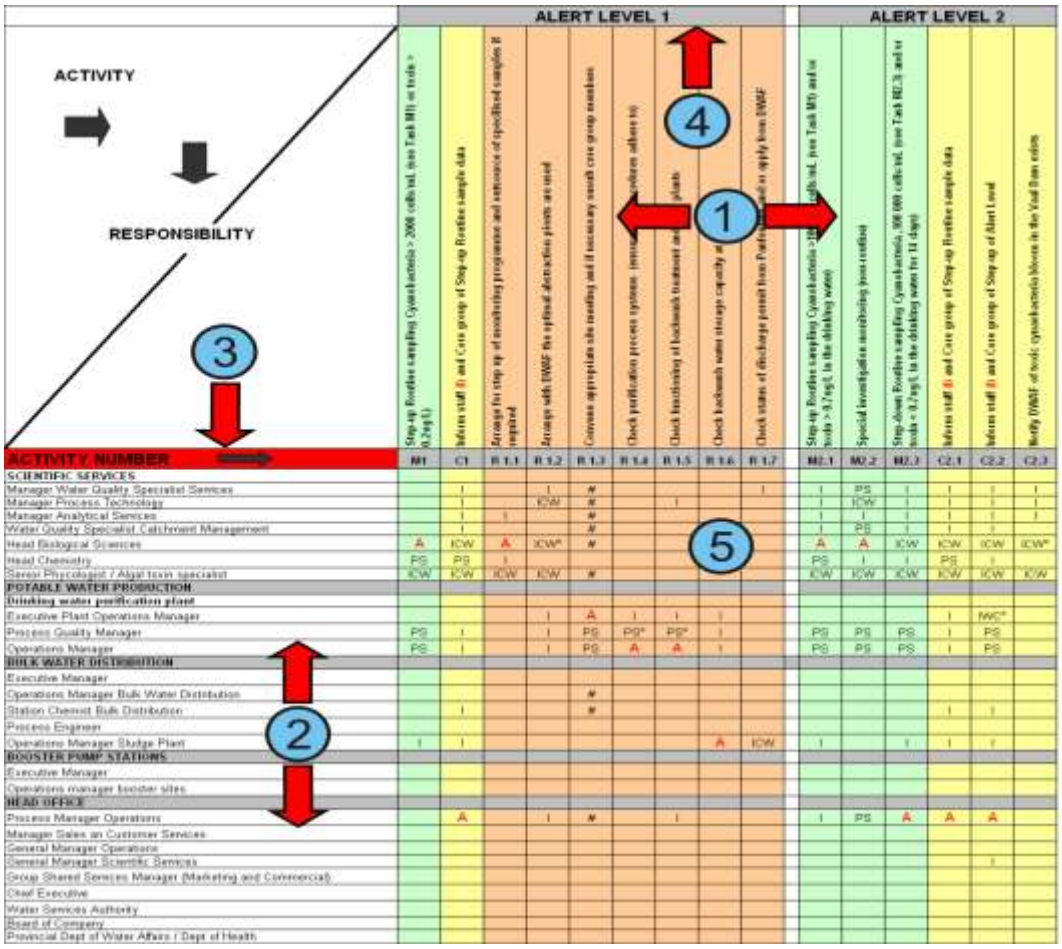
DECISION MATRIX FOR THE ALF

Table 6-1(L2) Example of a decision matrix for an Alert Levels Framework



1: Indicates the list of activities that needs to be performed

2: Indicates responsible persons and the respective departments and sites

3: Indicates specific activities in respect of monitoring, communication and remedial action.

M (Green) = Monitoring activities

C (Yellow) = Communication activities

R (Light brown) =Remedial activities

As an example activity R1.1:

R = Indicates that the activity falls under remedial actions.

R1 = Indicates that this activity is included under Alert Level 1 of the remedial action.

R1.1 = Indicates the first activity under Alert Level 1 of the remedial actions.

Increasing numbers i.e. R2.**1**, R2.**2** to R2.**6** indicates the sequence in which activities should be performed.

4: Indicates the Alert Levels 1, 2 or 3.

5: Indicates who is responsible for what. This requires that the specific activity is read from the horizontal axis '1' and the responsibility of the respective persons indicated on the vertical axis '2' is found where the two lines intersect '5'.

Return to level 1

HUMBUG SCRUB RESERVOIR ALGAL MANAGEMENT PLAN

Management of *Anabaena circinalis* blooms using natural limitation

Table of contents

1 Humbug Scrub reservoir statistics

- Supplied solely by a local catchment, does not receive any pumped water from the River Paradise
- Total catchment area: 12,294 hectares
- Reservoir capacity: 26,800 megalitres
- Maximum depth: 36 m
- Area of waterspread: 280 hectares

2 Incident criteria for *Anabaena circinalis*, saxitoxin and geosmin

Direct Supply Reservoir		
Anabaena circinalis in reservoir (cells/mL)	**≤ 1,999**	N/A
	2,000 – 19,999	TYPE 2 (DH)
	≥ 20,000	TYPE 1 (DH)
Saxitoxin in reservoir (µg/L)	**≥ 1**	TYPE 2 (DH)
	≥ 3	TYPE 1 (DH)
Water Treatment Plant Inlet		
Anabaena circinalis WTP inlet (cells/mL)	**≤ 499**	N/A
	500 – 999	TYPE 3 (HUMBUG SCRUB)
	1,000 – 1,999	TYPE 2 (DH)
	2,000 – 19,999	TYPE 1 (DH)
	≥ 20,000	PRIORITY TYPE 1 (DH)
Total geosmin WTP inlet (ng/L)	**≤ 9**	N/A
	10 - 79	TYPE 3 (HUMBUG SCRUB)
	≥ 80	TYPE 2 (HUMBUG SCRUB)
Saxitoxin WTP inlet (µg/L)	**≥ 1**	TYPE 1 (DH)
	≥ 3	PRIORITY TYPE 1 (DH)
Water Treatment Plant Outlet and Distribution System		
Total geosmin WTP outlet /	**≤ 9**	N/A

distribution (ng/L)	**10-29**	TYPE 3 (HUMBUG SCRUB)
	≥ 30	TYPE 1 (HUMBUG SCRUB)
Saxitoxin WTP outlet / distribution (µg/L)	**≥ 1**	PRIORITY TYPE 1 (DH)

3 Preventive measures in place for algae, toxins and taste & odour compounds

System component	Current preventive, management and contingency measures for algae, toxins and taste & odour compounds in the Humbug Scrub system
Catchment	• Control of nutrient inputs from catchment
Reservoir	• Variation of offtake level • Use of aerator when required
Water Treatment Plant	• Filtration process • Flocculation process / DAFF • PAC dosing • Disinfection (chlorine)
System capacity	• Increase water storages at Pansy Hill and Mt Coke Tank to full capacity

4 Variable offtake

Management recommendation:

Variable offtake to be set to the lowest level during a cyanobacterial bloom in order to minimise algal cells and associated metabolites such as geosmin from entering the WTP.

Humbug Scrub reservoir offtake depths:

Full Supply = EL 211.00 (33.10m on depth sight board)
- No 4 Offtake = EL 201.25 (15m)
- No 3 Offtake = EL 184.54 (21m)
- No 1 & 2 Offtake = EL 177.75 (30m)

Rationale:

Anabaena circinalis tends to form surface scums, and highest cell numbers tend to be distributed through the upper layer of water.

Considerations:

Should a major rainfall event occur resulting in significant catchment runoff, the offtake depth is routinely raised to reduce the risk of *Cryptosporidium* entering the WTP. These protozoa are carried with the inflowing water along the bottom of the reservoir, frequently short-circuiting towards the dam wall/WTP inlet tower. Should there be a concurrent algal bloom and rainfall event, a considered approach has to be taken, weighing up the potential risks from *Cryptosporidium* and from algal cells at the WTP inlet. In this situation, it is generally advisable to raise the offtake depth as far as practicable to reduce the *Cryptosporidium* risk at the

WTP inlet. However, as algal cell numbers have been shown to vary significantly at the offtake depending on wind strength and direction (i.e. elevated cell numbers during persistent easterly winds), the most appropriate offtake depth should be selected following thorough assessment of prevailing conditions at the time.

5 Aerator operation

Management recommendation:

Aerator to be left on during algal blooms to prevent stratification and anoxia in the hypolimnion (leading to release of P, Fe, Mn).

Rationale:

Management of previous algal bloom incidents has shown that the aerator in Humbug Scrub reservoir should be left on during algal blooms, as a strong link between the aerator being turned off and release of soluble iron and manganese from the sediments was demonstrated during these past events. The dissolved metals subsequently enter the WTP and tend to precipitate out in the filtered water storage. Anoxic conditions are also conducive to the release of phosphorus from sediments.

Considerations:

During previous bloom events it was discovered that under persistent easterly winds elevated algal cell numbers and subsequently higher geosmin concentrations were entering the WTP, even when the offtake was set at the lowest level. This situation was traced back to an interaction between aerator operation and prevailing wind direction driving algal cells down into deeper waters (offtake depth). Turning off the aerator for short periods of time (i.e. not more than three days) should be considered to mitigate this situation should it occur.

In relation to the release of soluble manganese from sediments when the aerator is turned off, investigations have shown that it is possible to dose potassium permanganate for manganese removal without compromising algal treatment in the WTP as algal cells are not damaged by this process.

The aerator should be turned off in case of a major rainfall event resulting in significant inflow from the catchment. This is to minimise the risk of *Cryptosporidium* being entrained in the aerator plume and being carried into the upper layers of the water column (WTP offtake level during rain events).

Note that the WTP needs to be informed whenever the aerator is turned on or off.

6 Reservoir and WTP inlet monitoring program

Routine and non-routine monitoring is essential for the application of control measures such as changing offtake levels, use of the aerator and PAC, as well as the assessment of the efficiency of the control measures that are in place.

Please refer to the map at the end of the document for sample point locations.

- **Reservoir (routine – summer)**

Water Quality parameter	Sampling frequency
Phytoplankton (surface all locations)	Twice weekly (Monday/Thursday)
Phytoplankton (Loc 1 at 10m, 20m, 30m depth)	Weekly
Nutrients	Weekly
pH / turbidity / colour	Weekly
Temperature / DO	Weekly
Chlorophyll	Weekly
Fe / Mn	Fortnightly
Microbiological	Monthly
TDS	Monthly
Pesticides	Bi-monthly

- **WTP Inlet – (routine – summer)**

Water Quality parameter	Sampling frequency
Total MIB / geosmin	Weekly
Fe / Mn	Weekly
Odour	Fortnightly
DOC	Fortnightly
Turbidity / colour	Monthly
Cryptosporidium / *Giardia*	Monthly
Microbiological	Monthly
pH	Monthly
Physical	Monthly
Lang Index	Monthly
Nutrients	Monthly
SiO_2	Monthly
Corrosive metals	Monthly
Rare inorganics	Monthly
Aluminium	Monthly
Pesticides	Monthly

- **In-reservoir temperature and water quality monitoring**

A thermistor chain (plus weather station, including wind speed and direction) is installed in the reservoir with data accessible via SCADA and automatic data downloads via GPRS emailed twice a week.

A pontoon fitted with an automated vertical water quality profiler system provides in-situ on-line data (available via a web-based interface) for temperature, turbidity, total cyanobacteria, chlorophyll, pH, conductivity and dissolved oxygen. The pontoon also carries a meteorological station including wind speed, solar radiation and temperature sensors.

- **Non-routine sampling**

To obtain as much information as possible on the status and possible trend in bloom development and to assist in management of the bloom, an increased monitoring regime will need to be initiated, consisting of both increased monitoring frequency and analyses (with fast turnaround times). In particular, increased monitoring will consist of phytoplankton sampling/algal scum enumeration (including direct counts), geosmin

analyses, nutrient sampling and additional temperature/DO profiles at key locations. Consideration should also be given to non-routine cell counts of *Anabaena circinalis* at the WTP inlet to compare with counts at the reservoir offtake. The field cyanobacterial probe can also be deployed to track total cyanobacteria numbers *in-situ*, including monitoring of cyanobacteria at the various offtake levels to determine the best offtake to use at times of very high cell counts. There will also be a need for saxitoxin testing, using the rapid Jellet field test to establish initial toxicity and then the quantitative HPLC method.

- **Field Response Team**

The Field Response Team based within Humbug Scrub Council's Water Quality and Integrated Management Group can be mobilised for additional surveillance on the reservoir if required (for example rapid saxitoxin tests, use of the YSI cyanobacteria sensor).

7 Humbug Scrub water treatment plant

The WTP is a dissolved air flotation, filtration (DAFF) design, which is ideal for the removal of algal cells. It includes a powdered activated carbon (PAC) dosing facility designed to remove taste and odour compounds and algal toxins. It has a nominal capacity of 50 ML day^{-1}, however this is not achievable for extended periods.

The treatment process consists of:

- Powdered activated carbon
- Potassium permanganate dosing for manganese oxidation
- Coagulation, using alum plus cationic polymer
- One stage flocculation
- Six flotation tanks
- Mono media filter beds situated in the flotation tanks
- Filter backwash facilities, including air scour
- Four sludge lagoons
- Clarification plant for filter backwash and lagoon supernatant, prior to recycle
- Chlorination: product water is chlorinated once between the WTP and the product water storages. Water flowing to southern metropolitan area receives trim chlorination. Water for the regional centres is drawn from the trunk main supplying the metropolitan area, with a significant storage at Pansy Hill. After Pansy Hill, the water is again chlorinated at Daydream Valley chlorination station prior to distribution. Water for the Bigville supply zone is drawn from the product water storage at the Humbug Scrub WTP. This water receives a trim dose of chlorine at the treatment plant before distribution.

Contingency in case of cyanobacterial bloom:

- Reduce flow as much as possible and dose PAC based on dissolved geosmin levels at the WTP inlet (obtained through the increased monitoring)
- Prior to receiving geosmin concentration results, a conservative estimate of the potential geosmin concentration can be made using *Anabaena circinalis* cell counts (refer to table in section 9)
- Humbug Scrub WTP can dose PAC at 50mg L^{-1} for a very short period and at reduced flow
- Ensure all tanks are at highest possible level (e.g. Pansy Hill Storage, Mt Coke Tank) before reaching major bloom status (i.e. prior to reaching cell counts of 50,000 cells mL^{-1})

- During times of reduced output from Humbug Scrub WTP during PAC dosing – Tearful Valley WTP will need to supply parts of southern areas normally supplied by the Humbug Scrub WTP
- Humbug Scrub WTP output – 40ML per day at 10mg L^{-1} PAC
- Humbug Scrub WTP cannott run below 20ML day^{-1}
- Implement appropriate changes to supernatant return
- Storages to return to normal operating levels after a bloom to minimise water age in the system. This should be coordinated with Outer Metro Operations.

8 Possible equipment failure

Three key areas have been identified for possible equipment failure that can impact water quality:

- Failure of the aerator
- Failure of the PAC dosing facility
- Complete breakdown of the WTP

Contingency plans need to be in place for all potential failure scenarios, including ensuring that a replacement compressor for the aerator is available in the shortest possible time, the availability of spares for PAC dosing equipment with the aim of reducing outages to the shortest time possible. A complete WTP breakdown would result in a shutdown of the treatment plant.

9 Management of geosmin

GEOSMIN - MANAGEMENT / CONTROL MEASURES				
Reservoir	**Multiple offtake**	**PAC**	**Flocculation/ Filtration**	**Cl_2**
Yes Half-life of geosmin loss due to biodegradation and loss to atmosphere in reservoir = 1 - 2 days. Time in reservoir with WTP shut off depends on product water storage capacity and system demand	**Yes** Barrier under most situations for intact cells where cells unevenly distributed through water column	**Yes** 92% at 20 mg L^{-1}	**Yes** 70% intact cell removal	**No**

Typical PAC doses required to remove geosmin to levels below 10 ng L^{-1}*	
WTP inlet geosmin concentration (ng L^{-1})	**PAC dose (mg L^{-1})**
10-30	4-15
30-100	15-35

*These doses were estimated from many laboratory experiments but the actual doses required will depend strongly on water quality. Site specific testing is recommended

Anabaena circinalis cell numbers and associated potential geosmin concentrations, percent removal required to reach a concentration goal of <10 ng L^{-1}, and estimated PAC doses for 30 minute contact time. All values for PAC doses are estimates as the actual values will depend on mixing and water quality conditions

Anabaena circinalis **(cells mL^{-1})**	**Potential geosmin concentration (ng L^{-1})**	**% removal required to achieve final concentration goal of < 10 ng L^{-1} ***	**PAC dose for dissolved geosmin (mg L^{-1}) (30 min contact time)**
200	9	0	0
400	18	50	5
500	23	60.9	7
1,000	46	80.4	10
2,000	91	90.1	17
3,000	137	93.4	20
4,000	182	95.1	25
5,000	228	96.1	30
7,500	341	97.4	>30
10,000	455	98.0	>30
15,000	683	98.7	>30
20,000	910	99.0	>30
30,000	1365	99.3	>30
40,000	1820	99.5	>30
50,000	2275	99.6	>30
60,000	2730	99.7	>30
80,000	3640	99.8	>30
100,000	4550	99.8	>30
200,000	9100	99.9	>30

* If all geosmin is released from cells

10 Management of saxitoxin

Note that it is highly likely that management actions for geosmin will be necessary before actions are needed for saxitoxins.

- Different variants of the saxitoxins adsorb to different extents on PAC. In the case of saxitoxins, 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 PAC and a contact time of at least 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}

- Chlorination is considered an effective process in the multi-barrier approach to saxitoxin removal, with destruction of toxicity in the range of 75-90% at C.*t* of 20 mg min L^{-1}.

SAXITOXIN - MANAGEMENT / CONTROL MEASURES				
Reservoir	**Multiple offtake**	**PAC**	**Flocculation / Filtration**	**Cl_2**

No Saxitoxin does not biodegrade	**Yes** Barrier under most situations for intact cells where cells unevenly distributed through water column; extracellular toxin expected to move through water column rapidly	**Yes** 60% at 20 mg L^{-1} 64% at 30 mg L^{-1} 30 mg L^{-1} PAC can be dosed at reduced flow but is limited to a few hours	**Yes** Removal of intact cells; however intact cells removed likely to break down in supernatant return	**Yes** 96% Assuming maximum removal contact time of 111 mins. At Humbug Scrub WTP: 13 hr contact time at >1.5 mg L^{-1} Cl_2

Anabaena circinalis cell numbers and associated potential saxitoxin concentrations, percent removal required to reach goals of < 3 µ/L and < 1 µ/L, estimated PAC doses for 30 minute contact time and chlorine contact time values to reach the target percent removal. Note that all values for PAC and chlorine doses are estimates as the actual values will depend on mixing and water quality conditions (from AWQC).

Anabaena circinalis **(cells mL^{-1})**	**Potential STX equivalent concentration (µg L^{-1})**	**% removal required (to reach 3 µg L^{-1})**	**PAC dose for dissolved saxitoxin (mg L^{-1}) * (30 min contact time)**	**Chlorine contact time for dissolved saxitoxin (mg $min\ L^{-1}$) ****	**% removal required (to reach 1 µg L^{-1})**	**PAC dose for dissolved saxitoxin (mg L^{-1}) *** (30 min contact time)**	**Chlorine contact time for dissolved saxitoxin (mg $min\ L^{-1}$) ******
200	0	0	0	0	0	0	0
400	0	0	0	0	0	0	0
500	0.1	0	0	0	0	0	0
1,000	0.1	0	0	0	0	0	0
2,000	0.3	0	0	0	0	0	0
3,000	0.5	0	0	0	0	0	0
4,000	0.6	0	0	0	0	0	0
5,000	0.8	0	0	0	0	0	0
7,500	1.1	0	0	0	9.1	5	2
10,000	1.5	0	0	0	33.3	7	5
15,000	2.3	0	0	0	56.5	15	13
20,000	3.0	0	0	0	66.7	25	21
30,000	4.5	33.3	7	5	77.8	30	33
40,000	6.0	50.0	10	10	83.3	>30	40
50,000	7.5	60.0	20	15	86.7	>30	>45
60,000	9.0	66.7	25	20	88.9	>30	>45
80,000	12.0	75.0	30	20	91.7	>30	>45
100,000	15.0	80.0	>30	35	93.3	>30	>45
200,000	30.0	90.0	>30	45	96.7	>30	>45

* If all saxitoxins are released from cells, estimate

** Dependent on the saxitoxin variants present, applicable to a "typical" Australian toxic bloom, final concentration goal, < 3 µg L^{-1}

*** If all saxitoxins are released from cells

**** Dependent on the saxitoxin variants present, applicable to a "typical" Australian toxic bloom, final concentration goal, < 1 µg L^{-1}

11 Communications

An incident update spreadsheet will be prepared for each Type 1 incident triggered by cyanobacterial cell counts. This spreadsheet will contain information and data relevant for management of the bloom and will be a repository of the latest data/information. The spreadsheet will be emailed to the established stakeholder email group as soon as new information becomes available.

Humbug Scrub stakeholder email group as of October 2009:

Note: this email group can be found in Council's Outlook Global Address book as "Humbug Scrub Update"

Council	Laboratory	Department of Health

A meeting with relevant stakeholders should be organised once *Anabaena circinalis* cell count at location 1 or reservoir average exceeds 50,000 cells mL^{-1}.

As per the Council Water Quality Incident Notification Table, all step changes must be reported to the Department of Health. If the product water before storage geosmin result is >10 ng L^{-1} the Council Customer Call Centre should be notified; consideration should also be given to notification of customers in consultation with Stakeholder Relations, Head of Council and DH.

12 Recommended actions based on cell numbers at reservoir offtake - triggers for actions in WTP and reservoir

Anabaena circinalis **at reservoir offtake (Loc 1,) (cells mL^{-1})**	**Recommended action**
< 500	**Monitoring:** • Continue routine monitoring **Algae in reservoir:** • If *A. circinalis* reservoir average >200 cells mL^{-1}, request direct cell counts **Geosmin:** • If total geosmin at WTP inlet >10 ng L^{-1}, also report dissolved geosmin component • If mostly dissolved geosmin present, geosmin removal required

500 – 1,000	**Monitoring:** • Increase monitoring as per below **Algae in reservoir:** • Sample any scums and consider using YSI cyanobacteria sensor **Geosmin:** • In addition to routine total geosmin at WTP inlet also schedule dissolved geosmin sample. • If mostly dissolved geosmin present, geosmin removal required **Variable offtake:** • Ensure variable offtake is set to lowest level **Treatment:** • Consider PAC to remove taste & odour
1,000 – 2,000	**Monitoring:** • Increase monitoring as per below **Algae in reservoir:** • In addition to routine twice weekly phytoplankton samples (Mondays & Thursdays), consider non-routine phytoplankton samples if *A. circinalis* >1000 cells mL^{-1} at reservoir locations 1, 4 or 5, or the reservoir average exceeds 1000 cells mL^{-1} • Sample any scums and consider using YSI cyanobacteria sensor • Schedule twice weekly phytoplankton depth samples at location 1 at 10m, 20m and 30m if *A. circinalis* >1000 cells mL^{-1} at reservoir location 1 • Schedule regular phytoplankton samples at WTP inlet **Geosmin:** • Geosmin removal required • Paired geosmin samples: WTP Inlet and Plant Outlet PBS **Treatment:** • PAC dosing required to remove geosmin **Communication:** • As per Notification Protocol including Incident Notification Table; all step changes must be reported to DH; if PBS geosmin result is >10 ng L^{-1} notify Council Customer Call Centre; consideration should also be given to notification of customers in consultation with Stakeholder Relations, Head of Council and DH
2,000 – 5,000	**Monitoring:** • Increase monitoring as per below and continue to closely monitor progress of bloom • Consider mobilising Field Response Team for additional surveillance (rapid saxitoxin tests, YSI cyanobacteria sensor) **Algae in reservoir:** • In addition to routine twice weekly phytoplankton samples (Mondays & Thursdays), continue with non-routine phytoplankton samples, including sampling any scums and consider using YSI cyanobacteria sensor **Geosmin:** • Geosmin removal required • Paired geosmin samples: WTP Inlet and Plant Outlet PBS **Toxins:** • Determine whether bloom is toxic using Jellet rapid field test. NOTE: this test requires a concentrated raw water sample (plankton net tow) as limit of detection >100 µg L^{-1} saxitoxin. If positive toxin result, run analysis by HPLC **Treatment:** • PAC dosing required to remove potential toxin and geosmin

	Communication: • As per Notification Protocol including Incident Notification Table; all step changes must be reported to DH; if PBS geosmin result is >10 ng L^{-1} notify Council Customer Call Centre; consideration should also be given to notification of customers in consultation with Stakeholder Relations, Head of Council and DH
5,000 – 10,000	**Monitoring:** • Increase monitoring as per below and continue to closely monitor progress of bloom • Mobilise Field Response Team for additional surveillance (rapid saxitoxin tests, YSI cyanobacteria sensor) **Algae in reservoir:** • In addition to routine twice weekly phytoplankton samples (Mondays & Thursdays), continue with non-routine phytoplankton samples, including sampling any scums and consider using YSI cyanobacteria sensor **Geosmin:** • Geosmin removal required • Paired geosmin samples: WTP Inlet and Plant Outlet **Toxins:** • Determine whether bloom is toxic, analysis by HPLC • Take samples: Plant Inlet, Pre-disinfection and Post-disinfection • Analyse pre-disinfection only if Plant Inlet is >1µg L^{-1} • Analyse post-disinfection only if Pre-disinfection is >1µg L^{-1} **Treatment:** • PAC dosing required to remove potential toxin and geosmin, maximise chlorine CT for saxitoxin removal • Ensure plant is optimised for cell, geosmin and toxin removal **Communication:** • As per Notification Protocol including Incident Notification Table all step changes must be reported to DH; if PBS geosmin result is >10 ng/L notify Council Customer Call Centre; consideration should also be given to notification of customers in consultation with Stakeholder Relations, Head of Council and DH

10,000 – 20,000	**Monitoring:** • Increase monitoring as per below and continue to closely monitor progress of bloom • Mobilise Field Response Team for additional surveillance (rapid saxitoxin tests, YSI cyanobacteria sensor) **Algae in reservoir:** • In addition to routine twice weekly phytoplankton samples (Mondays & Thursdays), continue with non-routine phytoplankton samples, including sampling any scums and consider using YSI cyanobacteria sensor **Geosmin:** • Geosmin removal required • Paired geosmin samples: WTP Inlet and Plant Outlet PBS **Toxins:** • Determine whether bloom is toxic, analysis by HPLC • Take samples: Plant Inlet, Pre-disinfection and Post-disinfection • Analyse pre-disinfection only if Plant Inlet is >1µg L^{-1} • Analyse post-disinfection only if Pre-disinfection is >1µg L^{-1} **Treatment:** • PAC dosing required to remove toxin and geosmin • Ensure plant is optimised for cell, geosmin and toxin removal • Consider adjusting dose rate to increase chlorine CT to maximise saxitoxin removal; also consider impact on THMs in distribution system and notify Outer Metro Operations and DH of changes / possible impacts • If saxitoxin levels >1.0 µg L^{-1} at outlet (prior to chlorination) then undertake further in-plant toxicity analysis of recycle streams (sedimentation, filter back wash, supernatant from lagoon or thickener) to determine source of toxins. Make adjustments to the process as required • If saxitoxins >3.0 µg L^{-1} at WTP outlet, implement contingency plan to supply drinking water **Communication:** • As per Notification Protocol including Incident Notification Table; all step changes must be reported to DH; if PBS geosmin result is >10 ng L^{-1} notify Council Customer Call Centre; consideration should also be given to notification of customers in consultation with Stakeholder Relations, Head of Council and DH
≥ 20,000 **Trigger Type 1 incident**	**Monitoring:** • Increase monitoring as per below and continue to closely monitor progress of bloom • Mobilise Field Response Team for additional surveillance (rapid saxitoxin tests, YSI cyanobacteria sensor) **Algae in reservoir:** • In addition to routine twice weekly phytoplankton samples (Mondays & Thursdays), continue with non-routine phytoplankton samples, including sampling any scums and consider using YSI cyanobacteria sensor **Geosmin:** • Geosmin removal required • Paired geosmin samples: WTP Inlet and Plant Outlet PBS

	Toxins: • Determine whether bloom is toxic, analysis by HPLC • Take samples: Plant Inlet, Pre-disinfection and Post-disinfection • Analyse pre-disinfection only if Plant Inlet is >1µg L^{-1} • Analyse post-disinfection only if Pre-disinfection is >1µg L^{-1} **Treatment:** • PAC dosing required to remove toxin and geosmin • Ensure plant is optimised for cell, geosmin and toxin removal • Consider adjusting dose rate to increase chlorine CT to maximise saxitoxin removal • If saxitoxin levels >1.0 µg L^{-1} at outlet (prior to chlorination) then undertake further in-plant toxicity analysis of recycle streams (sedimentation, filter back wash, supernatant from lagoon or thickener) to determine source of toxins. Make adjustments to the process as required • If saxitoxins >3.0 µg L^{-1} at WTP outlet, implement contingency plan to supply drinking water **Communication:** • As per Notification Protocol including Incident Notification Table; all step changes must be reported to DH; if PBS geosmin result is >10 ng L^{-1} notify Council Customer Call Centre; consideration should also be given to notification of customers in consultation with Stakeholder Relations, Head of Council and DH • Meeting with Stakeholders if cell counts at location 1 or reservoir average >50,000 cells/mL

13 Benthic cyanobacteria

Species of concern: The two benthic cyanobacteria of particular concern in Australia are *Phormidium* spp and *Oscillatoria* spp.

Habit: Benthic algae grow on bottom sediments, on rocks and also grow attached to larger aquatic plants such as water milfoil and reeds. They usually form distinct mats on these substrates and can frequently be found forming a distinct band around the shallow margin of a reservoir. In general, benthic cyanobacteria prefer shallower water bodies with low turbidity.

Water quality issues: The major water quality issue is the production of the taste and odour compounds geosmin and MIB (2 methyl-isoborneol), produced by both *Phormidium* and *Oscillatoria*. There is anecdotal evidence that the benthic cyanobacterium *Geitlerinema* (a relatively new genus) is a potential geosmin producer.

Oscillatoria is not known to be toxic in Australia. While *Phormidium* has been shown to be toxic overseas (including anatoxin-a), there is no evidence to suggest that *Phormidium* in Australia produces the same toxin(s). Tests using *Phormidium* material collected in 2000 showed that mice injected with cell extracts died, however they did not show the usual symptoms associated with cyanobacterial toxins. Cell extracts administered orally did not show any toxicity, nor could the toxin be extracted into water. Furthermore, boiling and chlorination effectively destroyed the toxin, while chloramine had no effect in reducing toxicity. Based on the above, there is evidence of *Phormidium* in Australia producing a cyanobacterial toxin, however the exact type of toxin(s) produced has not been established. It would be fair to assume that elevated geosmin levels would make a water supply undrinkable before toxin from *Phormidium* would reach a level where it would be necessary to stop supply.

Detection: Benthic algal mats impact on water quality when fragments are dislodged and float free in the water column. This can be caused by wind and wave action or variation in flow and water level. It can also occur during periods of high photosynthetic activity when oxygen bubbles form and may carry fragments into the water. As they grow attached to submerged substrates, benthic algal cells are likely to be under stress/ruptured when free-floating and these conditions can lead to spikes in geosmin. In the absence of significant numbers of geosmin-producing planktonic cyanobacterial species such as *Anabaena circinalis*, it is highly likely that any elevated geosmin levels are due to benthic algal activity. Furthermore, in the case of benthic cyanobacteria there is usually very little difference between total and dissolved geosmin figures as most geosmin would be in solution throughout the water column emanating from benthic mats or dislodged floating clumps.

Management: Benthic cyanobacteria are more difficult to manage than planktonic species. Changing the level of a reservoir to expose areas of benthic algal growth is usually of very little benefit as it will take a long time for the mats to desiccate and the likelihood of cells surviving in the body of the mats is very high. $CuSO_4$ dosing would present little benefit, as the $CuSO_4$ in the form it is commonly applied for the control of planktonic cyanobacteria (fine granules) is not effective at depth. A potential option could be the use of $CuSO_4$ in the form of larger pieces which sink to the bottom of the reservoir. However, this would still lead to a very patchy result and would be a quite temporary measure (and likely lead to a spike in geosmin release).

Return to level 1

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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 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 with 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, driven by prevailing breezes, can accumulate in bays around the shore edge. 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 - whether intentional or incidental. Incidental ingestion of water is a particularly high risk 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, i.e. campers intentionally ingesting lake water and therefore toxin, it 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) [1]. 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 etc. 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;

- Select monitoring sites 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 are 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 [1] 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 [1]

Guidance level	Health Risks	Typical Actions
20,000 cyanobacterial cells mL^{-1} *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^{-1} *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 (*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 affects 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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GLOBAL WATER RESEARCH COALITION

WATER QUALITY RESEARCH AUSTRALIA

INTERNATIONAL GUIDANCE MANUAL FOR THE MANAGEMENT OF TOXIC CYANOBACTERIA

Global Water
Research Coalition

International Guidance Manual for the Management of Toxic Cyanobacteria

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GLOBAL WATER RESEARCH COALITION

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).

The members of the GWRC are:

- Anjou Recherche – Water Operations Research Center of Veolia Water (France)
- EAWAG – Swiss Federal Institute for Aquatic Science and Technology
- KWR – Watercycle Research Institute (Netherlands)
- PUB – National Water Agency of Singapore
- SUEZ Environmental – CIRSEE – International Research Center on Water and Environment (France)
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- TZW - Water Technology Center of the German Waterworks Association
- UKWIR - UK Water Industry Research
- Water Environment Research Foundation (USA)
- WQRA - Water Quality Research Australia
- WRC - Water Research Commission (South Africa)
- Water Research Foundation (USA)
- WateReuse Foundation (USA)
- WSAA - Water Services Association of Australia

These organisations have national research programs addressing different parts of the water cycle. They provide the impetus, credibility, and funding for the GWRC. Each member brings a unique set of skills and knowledge to the Coalition. Through its member organisations GWRC represents the interests and needs of 500 million consumers.

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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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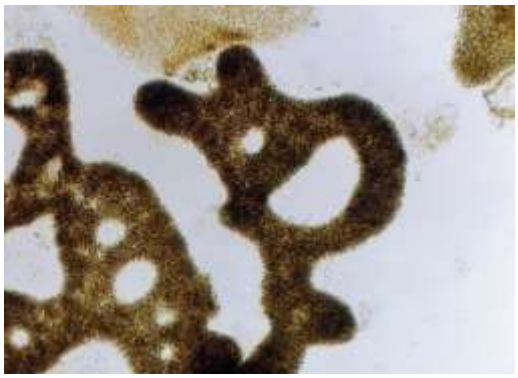
CHAPTER 1 INTRODUCTION

CYANOBACTERIA

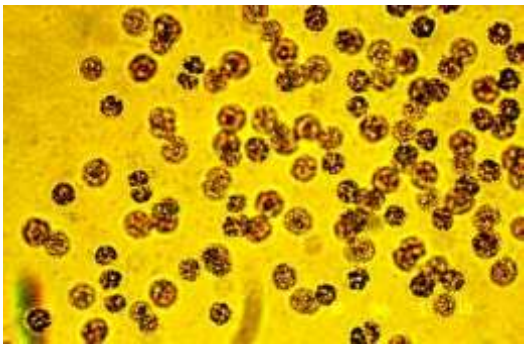
Cyanobacteria, also known as blue-green algae, blue-green bacteria or cyanophytes, are part of a primitive group of organisms which, according to fossil records, have existed for approximately 3.5 billion years [1, 2]. They are not true algae, they are gram-negative bacteria which contain chlorophyll 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



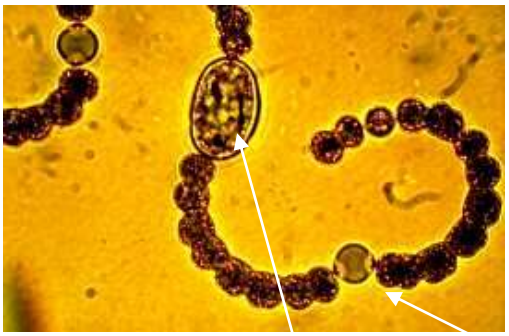
Microcystis

STRAIGHT FILAMENTS

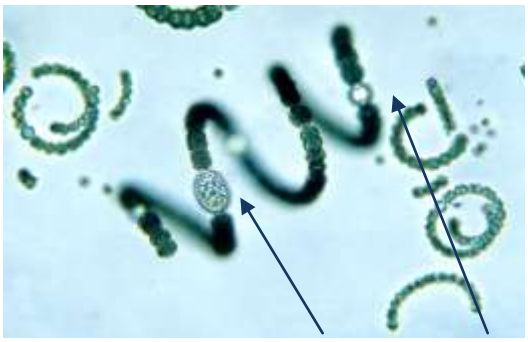
Phormidium

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.

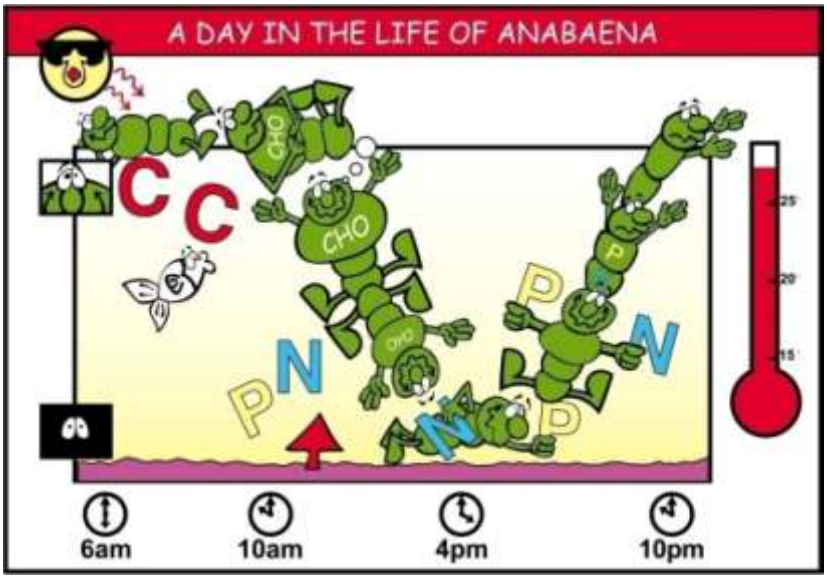


Figure 1-2 A stylised diagram of the daily cycle of buoyancy regulation and vertical migration in a lake by the cyanobacterium *Anabaena*

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.

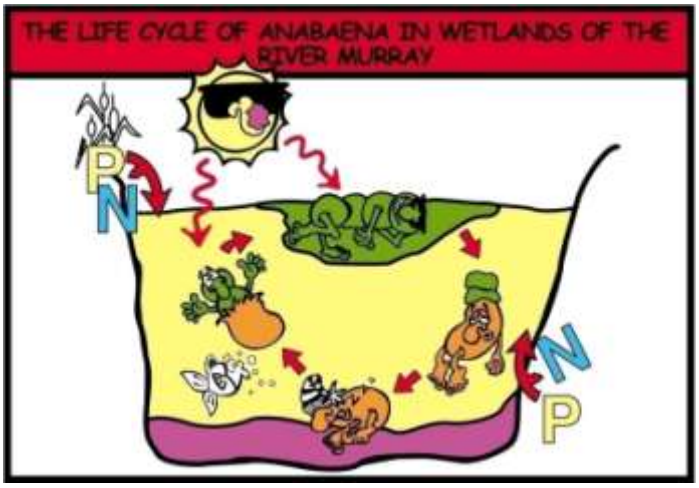


Figure 1-3 The typical life cycle of the cyanobacterium *Anabaena* showing akinete formation and germination

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.

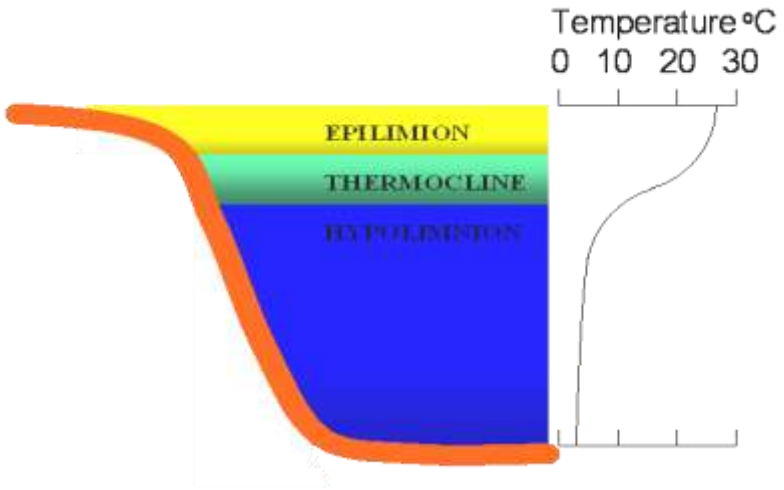


Figure 1-4 Cross section of a thermally stratified lake showing location of the epilimnion and hypolimnion and associated temperature changes

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 Composting	Including the treatment works, pumping stations, wastewater overflow structures and the reticulation system (<250 kilolitres/day) 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.

			Predicted concentrations of cyanobacteria and their metabolites						
Reservoir nutrient status	Total Phosphorus (µg L^{-1})	Scenario modelled:	Bioavailable Phosphorus (µg L^{-1})	*Microcystis aeruginosa* (cells mL^{-1})	Microcystin (Total) (µg L^{-1})	*Anabaena circinalis* (cells mL^{-1})	Geosmin (Total) (ng L^{-1})	Geosmin (Dissolved) (ng L^{-1})	Saxitoxin (Total) (µg 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.

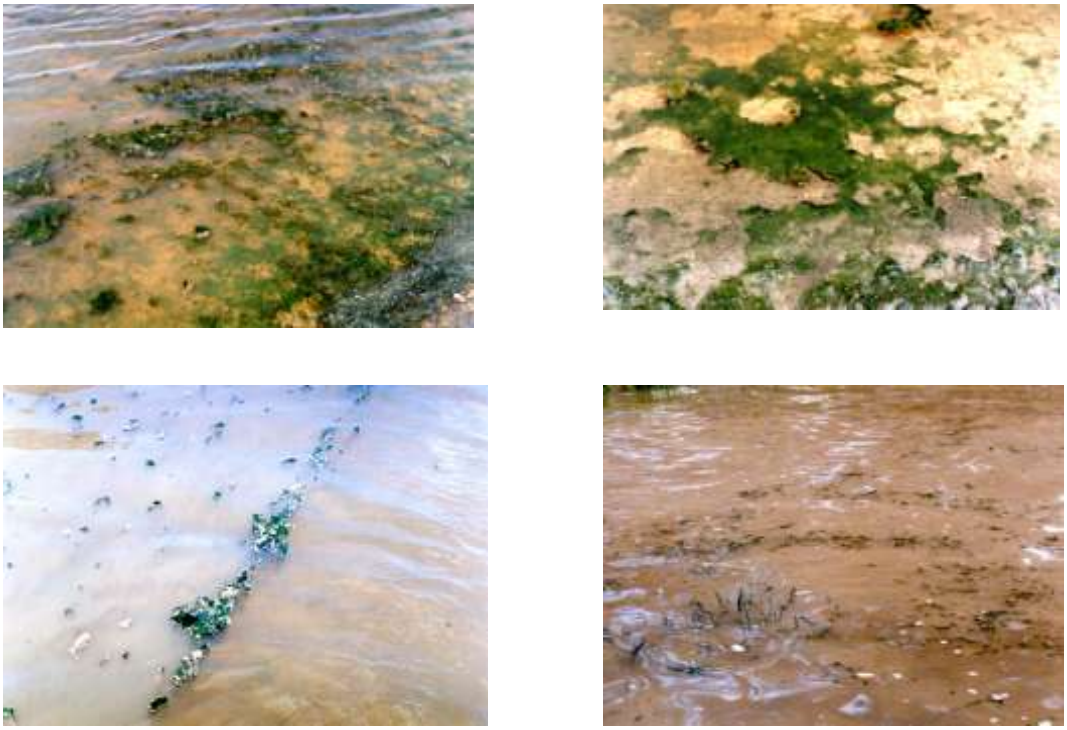


Figure 3-3 Benthic cyanobacteria attached to sediments and rock surfaces, and floating on the surface after breaking free from the substrate

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.

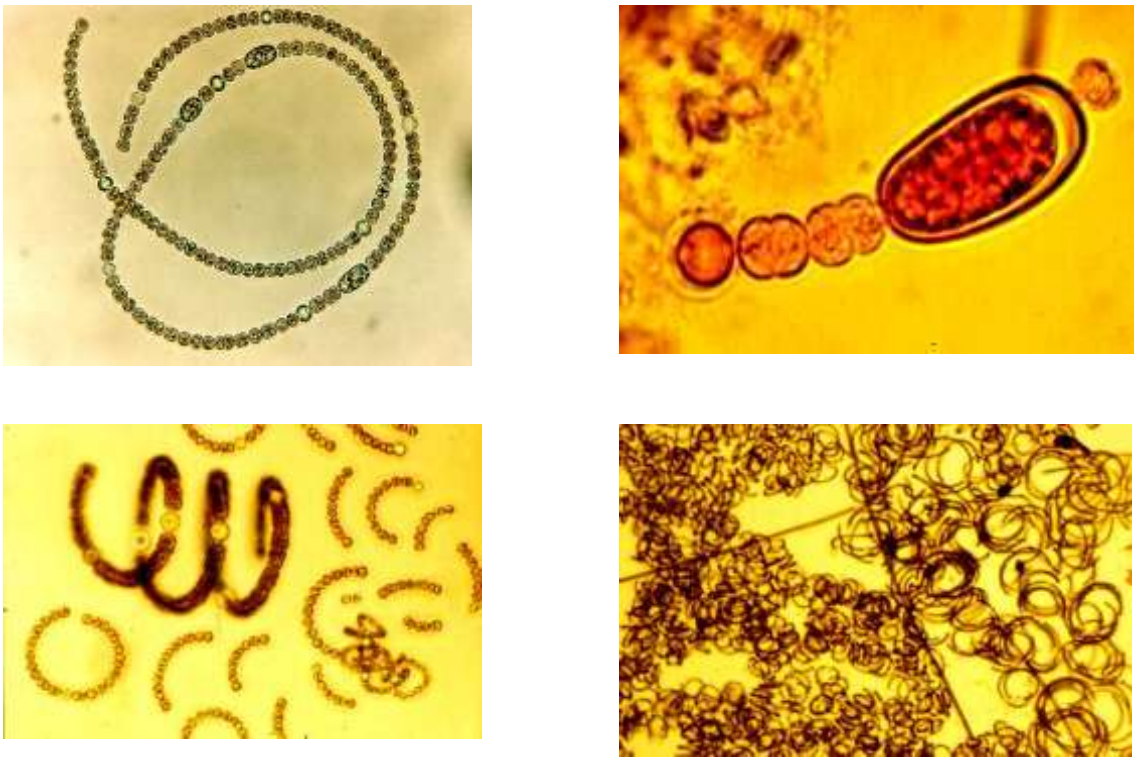


Figure 3-6 Different strains of the same cyanobacterium, *Anabaena circinalis*, several of which are toxic. This figure illustrates the difficulties inherent in microscopic identification for the determination of toxicity

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 ± 20-30%. A precision of ± 30% enables a doubling of a population in successive samples to be detected while a precision of ±20% will enable a statistically significant change to be detected. This level of precision can only be obtained if high analytical effort is employed in the laboratory.

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.

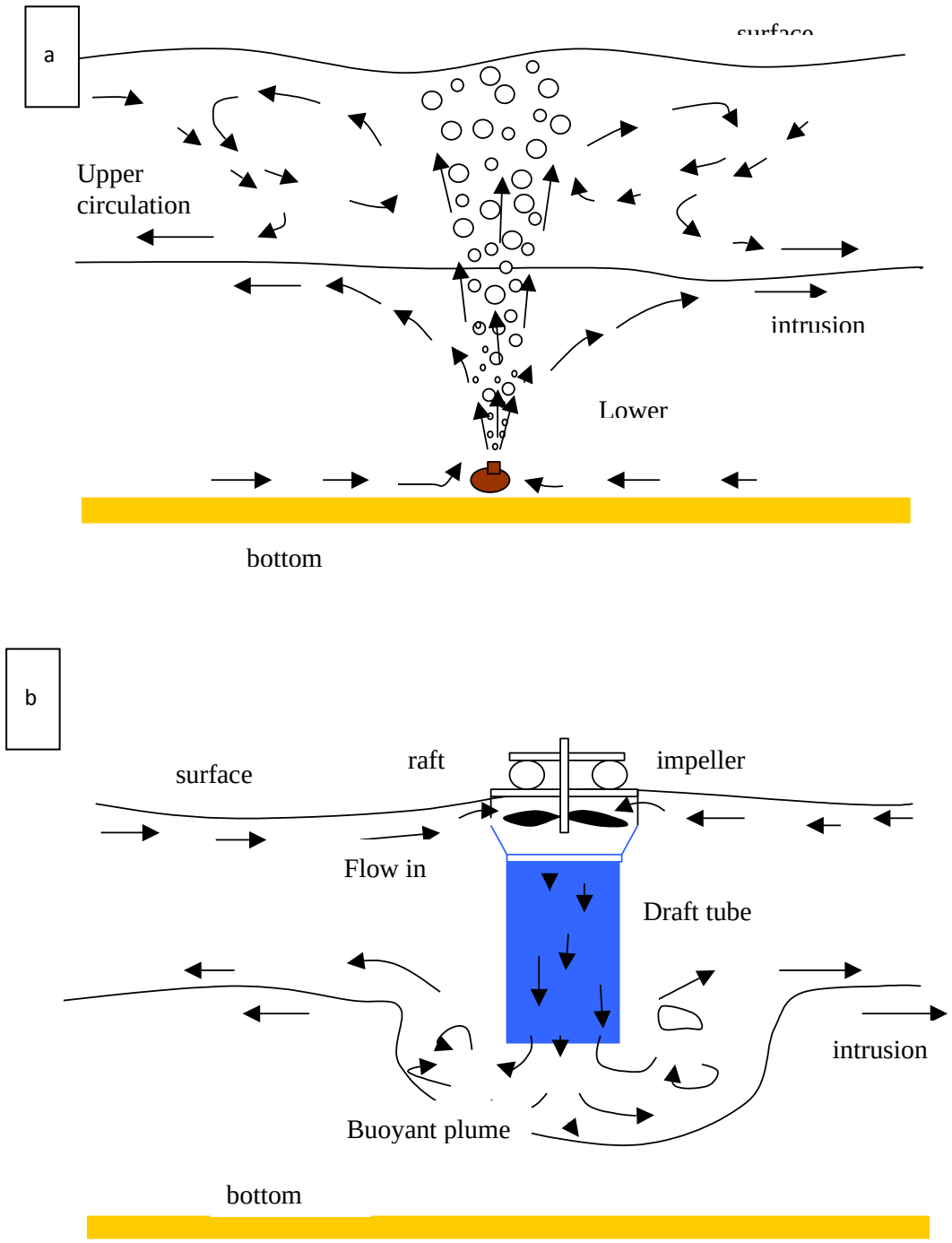


Figure 4-1 Flow and circulation fields created by a bubble plume aerator a) and a surface-mounted mechanical mixer b) in reservoirs

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 ll 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.

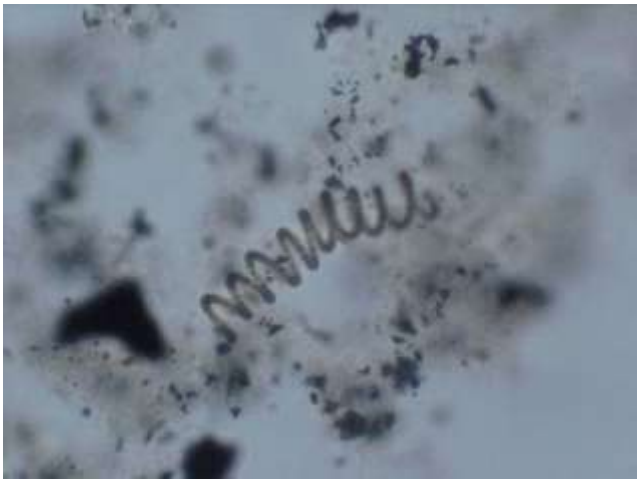


Figure 5-1 *Anabaena* filament encased in an alum floc. Dark areas are powdered activated carbon particles used to remove dissolved tastes and odours and cyanotoxins

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.

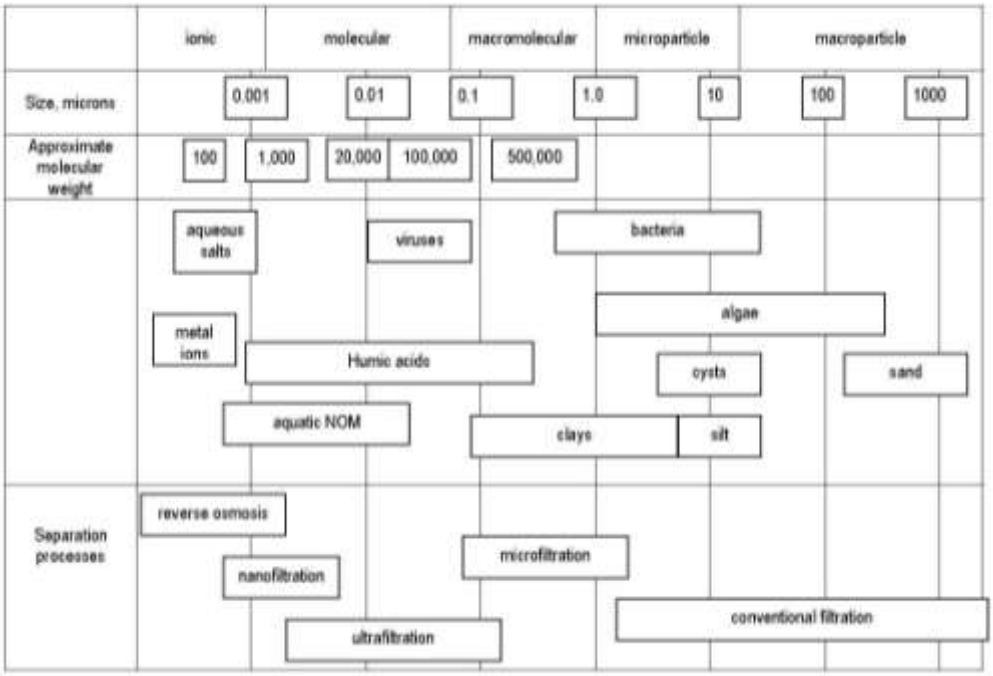


Figure 5-2 Efficiency of various filtration processes

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 $\mu g\ L^{-1}$ 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 $\mu g\ L^{-1}$ 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 $\mu g\ L^{-1}$ STX equivalents, and a finished water goal concentration of <3 $\mu g\ L^{-1}$.

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 $\mu g\ L^{-1}$ for cylindrospermopsin would be 10-20 mg L^{-1} for an inlet concentration 1-2 $\mu g\ L^{-1}$ and 20-30 for an inlet concentration of 3-4$\mu g\ L^{-1}$.

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 ($\mu g\ L^{-1}$)	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.

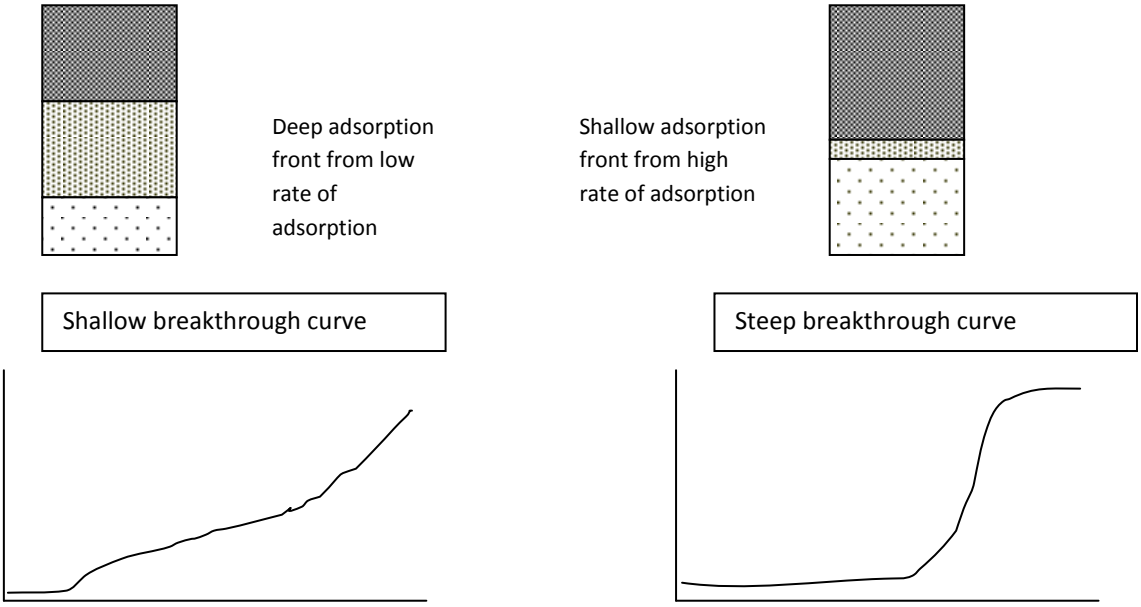


Figure 5-3 Effect of the adsorption front on the shape of the breakthrough curve

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^{o}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 Cl_2

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.

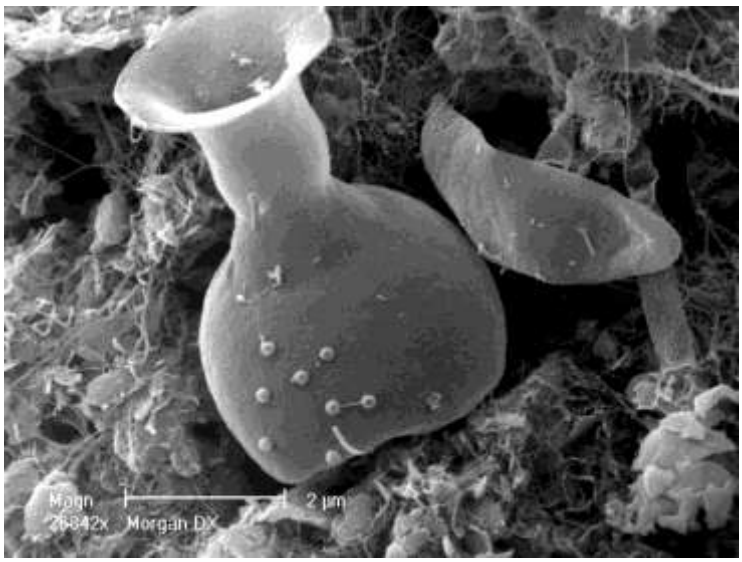


Figure 5-4 Scanning electron micrograph of biofilm on a sand particle from the rapid sand filter at Morgan Water Filtration plant, South Australia

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	≥ 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.	≥ 2,000[2] & < 6,500 cells mL^{-1} *Microcystis aeruginosa* *-or-* the total biovolume of all cyanobacteria ≥ 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	≥ 6,500 cells mL^{-1} *Microcystis aeruginosa* *-or-* the total biovolume of all cyanobacteria ≥ 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.	≥ 65,000 cells mL^{-1} *Microcystis aeruginosa* *-or-* the total biovolume of all cyanobacteria ≥ 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 (≥ 6 $mm^3 L^{-1}$) (rounded up from 5.7) is approximately equivalent to those numbers of *M. aeruginosa* for Level 3

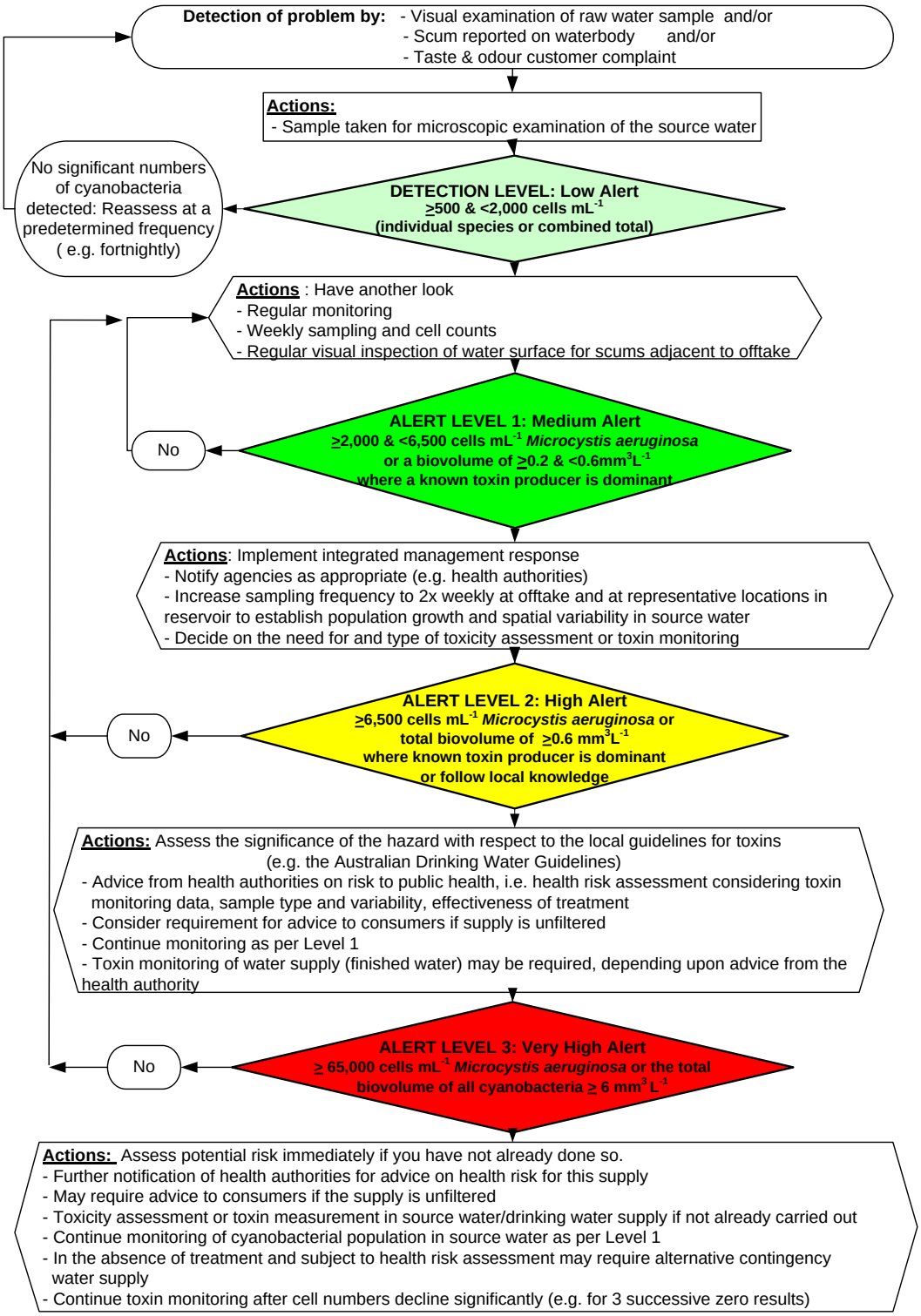


Figure 6-1 Flow chart of the Alert Levels Framework for management of cyanobacteria in drinking water

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 µg 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 µg 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 µg L^{-1} for eight consecutive days or exceeds 5 µg 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).

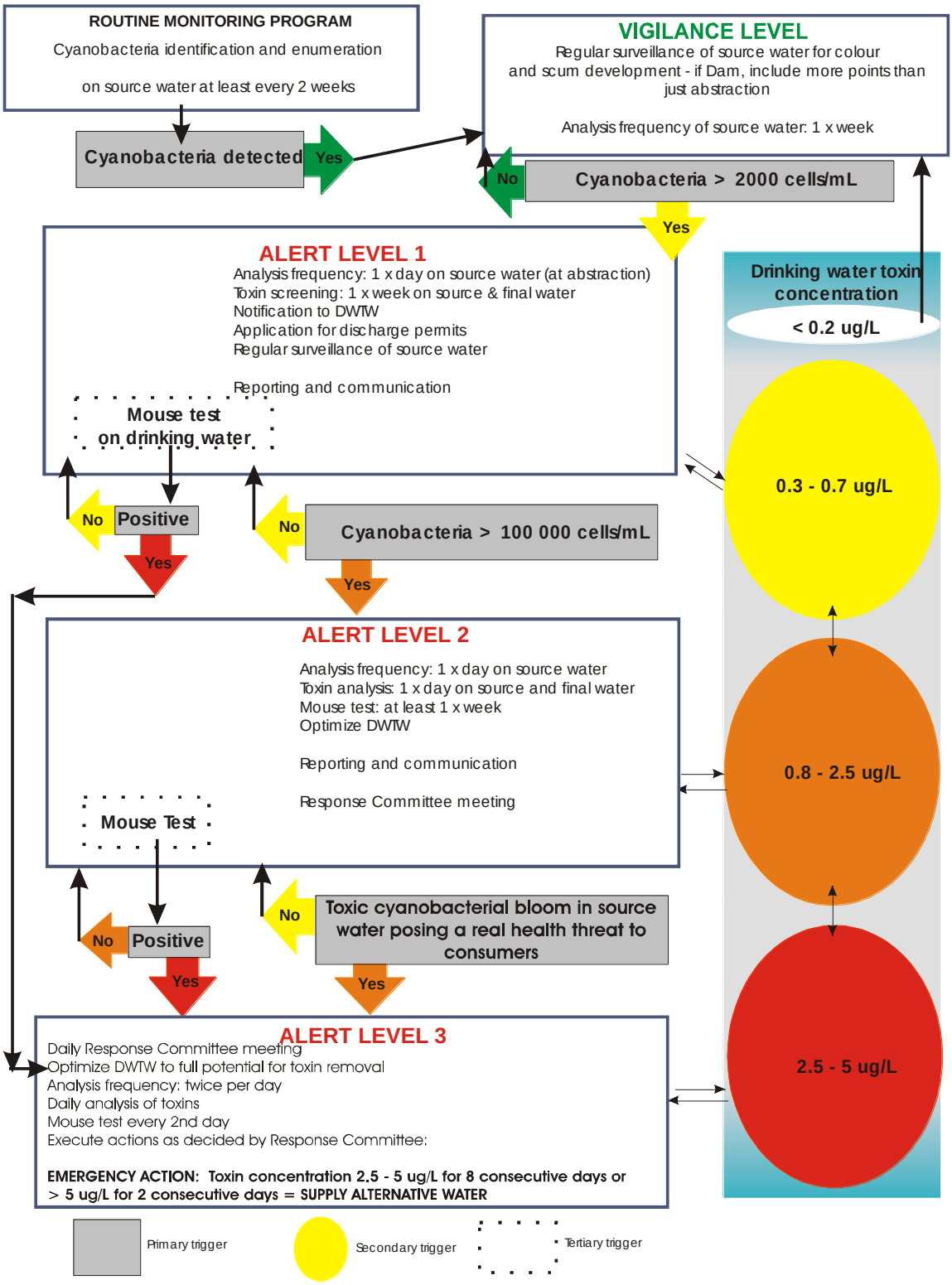


Figure 6-2 Alert Levels Framework using cyanobacterial concentration as primary trigger

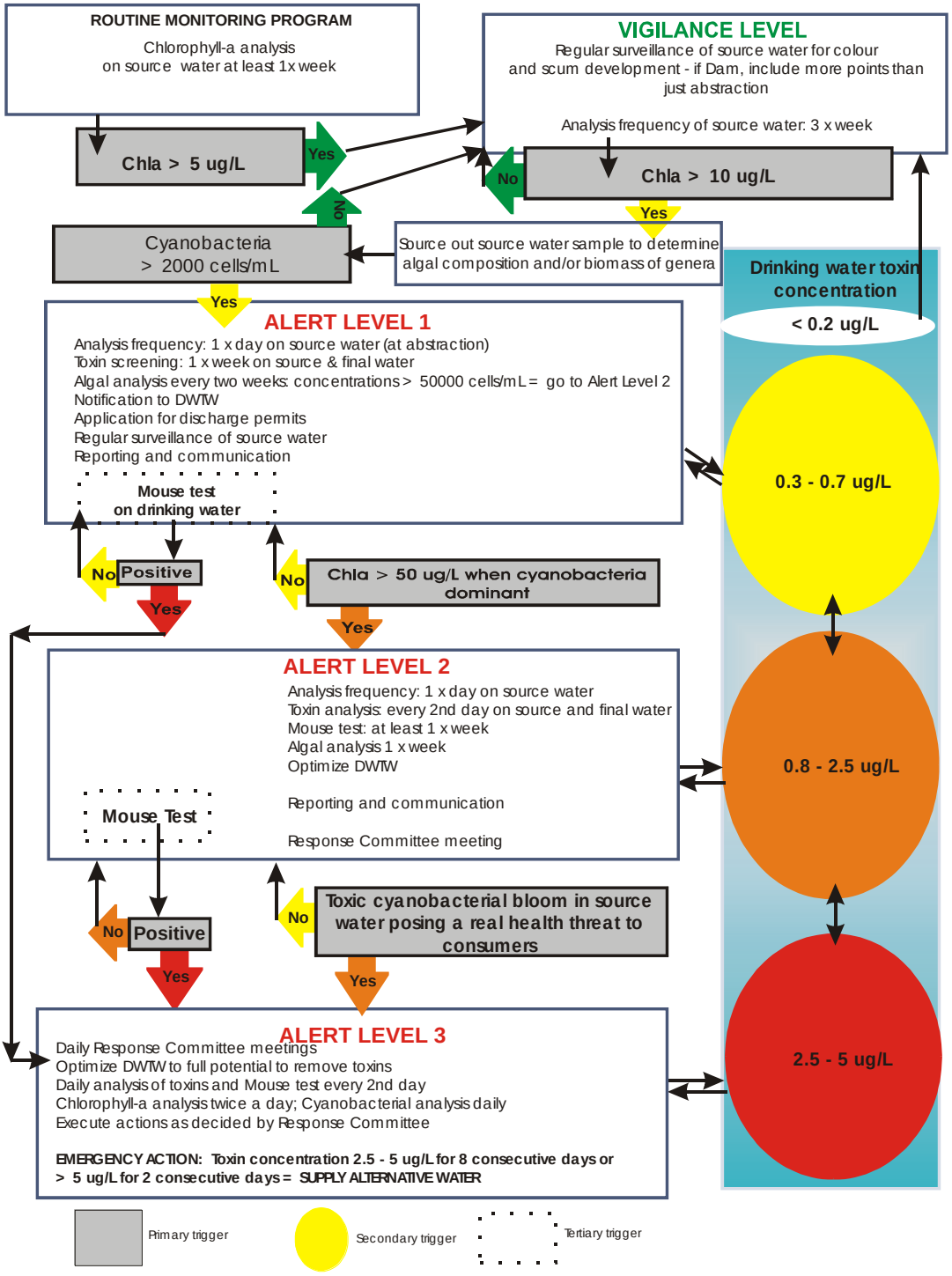


Figure 6-3 Alert Levels Framework using chlorophyll-a concentration as primary trigger

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.

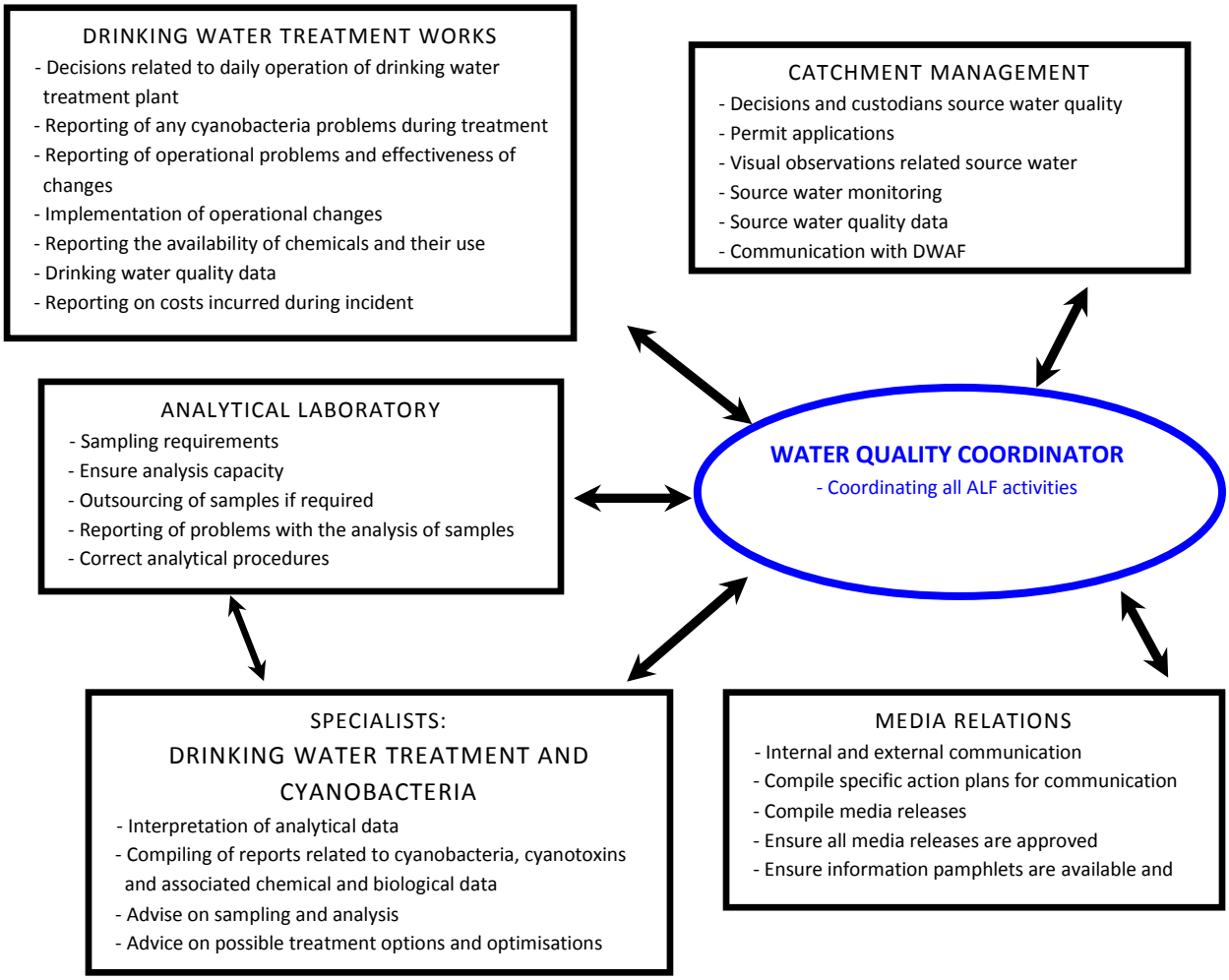


Figure 6-4 Possible communication channels for an ALF [152]

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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