TOXIC CYANOBACTERIA

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Summary

The number of waterbodies suffering from eutrophication is increasing around the world and cyanobacteria are becoming a problem because of this. Many species of cyanobacteria (blue-green algae) can produce toxins. Some have biomedical properties; however, many can cause human and environmental health problems. Human health problems range from skin and respiratory tract irritation to liver damage, gastric and neurological problems. Some toxins bioaccumulate in seafood and some may bioaccumulate on salad vegetables from irrigation. Several are tumour promoters. Due to lack of animal data, there is little knowledge of the chronic health effects.

The ecophysiology of cyanobacteria can provide them with a substantial advantage over other phytoplankton. Some species are nitrogen fixers, while others possess gas vesicles which enable them to alter their vertical position in the water column to reach their optimal growth conditions. Others can develop akinetes or spores which can remain viable until conditions become more favourable. Some may harbour other organisms, such as pathogenic bacteria.

Bloom formation can not only cause problems for human health, but can also be detrimental to the environment. Eutrophication can result in massive increases in biomass and turbidity. Using their gas vesicles, cyanobacteria can move towards the surface blocking the light causing massive plant death. Decomposition uses up the dissolved oxygen, causing fish kills and zooplankton losses. This results in reduced competition and dominance of cyanobacteria.

There are various methods available for the control and management of cyanobacteria. The most effective, long-term strategy is nutrient reduction, which can be achieved by improved point and diffuse source management. Engineering techniques and dam construction can also have a major impact on reducing the opportunities for cyanobacteria to dominate. Short-term methods involve physical and chemical treatment, particularly of drinking water resources.

1. Introduction

Cyanobacteria are true bacteria, often referred to as ‘blue-green algae’ because of their similar morphology, habitat and photosynthetic ability to green algae. There are many different species of cyanobacteria occurring as single cells, filaments or colonies. Some contain gas vesicles which enable them to migrate within the water column to suit their light requirements. When in excessive numbers, or ‘blooms’, they can affect the environment detrimentally. Many are also capable of producing toxins which can harm human health.

These toxins include skin irritants, and others which can cause damage to the liver and nervous system. They can affect humans directly when present in recreational waters or in the drinking water supply. They can also have indirect effects through consumption of plant and animals affected by cyanotoxin uptake and through their effects on livestock, aquatic organisms and wildlife.

Blooms can occur in fresh, brackish or saltwaters and occur naturally, but are more likely to be enhanced by human interference such as enrichment of waters with nutrients. This enrichment, or 'eutrophication' primarily comes from municipal wastewater, agricultural runoff and the damming of rivers which provides long water retention times, stability of the water column and increased light exposure. World-wide trends show that the levels of eutrophication are increasing. However, eutrophication is not all bad news, as it can also have some positive economic benefits. This article will provide information about cyanobacteria and their causes. In addition, it will discuss the various toxins produced and the effects of these on both human and environmental health. Finally, it will discuss what controls are in place now and what may be required to reduce the risks in the future as well as some of the benefits provided by eutrophication.

2. What are cyanobacteria?

Blue-green algae are also known as cyanobacteria or Cyanophyceae. They are procaryotes (organisms without a nucleus) and were the first organisms to use water as both a proton and an electron donor. Their early photosynthetic activity released free oxygen into the atmosphere and it is believed that they are the evolutionary forerunners of modern plant and algal chloroplasts. They occur naturally in all waters as part of the phytoplankton. They can also be found in moist soils and in symbiotic relationships in some dry soils. In small numbers they are not significant, but under ideal conditions, they cause problems. Several cyanobacteria can produce earthy or musty odours and tastes which are caused by the compounds geosmin and methylisoborneol, which affect the taste and smell of drinking water. Of more importance, many also produce toxins.

2.1. Morphology

Cyanobacteria are an ancient group of gram-negative bacteria with a cell wall consisting of one or several layers of peptidoglycan or murein forming an outer and inner membrane. They are unicellular and can occur as single cells, filaments or mainly as colonies. They are self-nourishing with most using photosynthesis for energy (photoautotrophs). Reproduction is by binary fission, or filaments may break into fragments called hormogonia, which separate and develop into new colonies. In contrast to eukaryotic microalgae, cyanobacteria do not have a nucleus nor do they have membrane-bound sub-cellular organelles. Photosynthetic cyanobacteria contain chlorophyll *a* and carotenoids as well as phycobilins or pigments.

As in other filamentous or colony forming bacteria, cyanobacterial cells may be joined by their walls or by mucilaginous sheaths but each cell remains an independent unit of life. The sheaths can provide protection for other microorganisms. They are often deeply pigmented giving bloom colours ranging from gold, yellow, brown, red, green, blue, violet to blue-black. The sheaths can be secreted around hairlike growths or trichomes.

Trichomes are made up of two different types of cells: the blue-green photoautotrophic cells and the larger, colourless heterocysts where nitrogen fixation occurs. The heterocysts can occur at the end of the filament or they may alternate with the photoautotrophic cells. Several sheaths which enclose more than two trichomes form what appears to be branching. The typical movement of cyanobacteria is a gliding motion when the trichomes may go into oscillation, which gave rise to the genus, *Oscillatoria*. Their movement is believed to be due to slime extrusion through small pores in the cell wall combined with contractile waves in one of the surface layers of the wall. The drawings in Figure 1 depict the various morphologies of cyanobacteria.

2.2. Environment

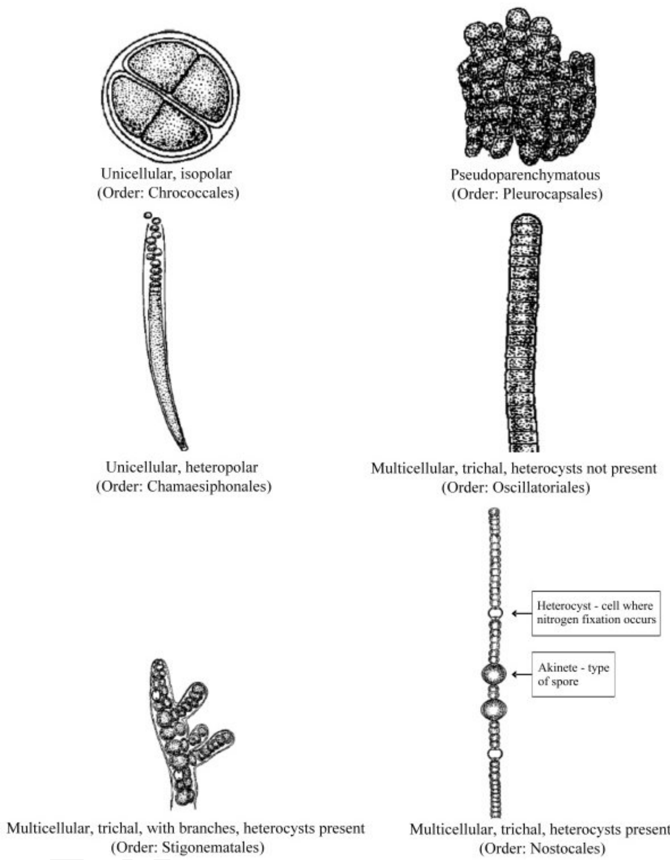

Figure 1 – Basic morphology of cyanobacteria Unicellular, isopolar (Order: Chrococcales) Pseudoparenchymatous (Order: Pleurocapsales) Unicellular, heteropolar (Order: Chamaesiphonales) Multicellular, trichal, heterocysts not present (Order: Oscillatoriales) Multicellular, trichal, with branches, heterocysts present (Order: Stigonematales) Multicellular, trichal, heterocysts present (Order: Nostocales) Source: Chorus, I., & Bartram, J. (Eds.) (1999). *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*: World Health Organization.

Cyanobacteria can be found in many different environments. The majority are from fresh or brackish waters with others from marine environments. The water in which they can grow can range from ultra-oligotrophic to hypertrophic. In the majority of cases, conditions which favour excessive growth include: high temperatures over 25°C; long sunny days; high levels of nutrients (mainly phosphorus and nitrogen); low flow in streams or rivers; and calm wind conditions allowing cells to migrate to the surface. Many marine cyanobacteria grow in lime-rich substrates such as coral or molluscs. They can inhabit near-surface epilimnic waters or deep, euphotic, hypolimnic waters. Others may attach to rocks, plants, or sediment. Some species can thrive in environments where no other microalgae can exist, such as the hot springs of Yellowstone or under ice in Antarctica. Less common are those species which live in the soil forming symbiotic associations with animals and plants. Wherever they grow, they are an important link in the cycling of nutrients.

2.3. Competitive advantages over other phytoplankton

It is impossible to set effective water management strategies without an understanding of the environmental factors responsible for cyanobacterial growth. Cyanobacteria are considered to be 'ecostrategists' in planktonic ecosystems, inhabiting particular niches of aquatic ecosystems. In order to understand their behaviour, knowledge of their properties and reactions is required.

2.3.1. Light pigments

The majority of cyanobacteria are aerobic photoautotrophs, requiring only water, carbon dioxide, inorganic substances and light for growth. Although some species are able to survive long periods in complete darkness and others show ability for heterotrophic nutrition, their metabolism is principally through photosynthesis. Like algae, they contain chlorophyll *a* to enable them to use the energy from the sun to synthesize carbohydrates from carbon dioxide and water, releasing oxygen as a by-product. However, unlike other phytoplankton, they also contain other pigments such as the phycobiliproteins. These include allophycocyanin (blue), phycocyanin (blue) and sometimes phycoerythrin (red), enabling them to use the green, yellow and orange ranges of the light spectrum which is hardly accessed by other phytoplankton. In spite of their slow growth in comparison to green algae, long retention times combined with the use of this wide light spectrum, allow them to outcompete in conditions which are unsuitable for other algae.

Cyanobacteria which form surface blooms seem to have a higher tolerance for high light intensities. Others are more sensitive to long periods of strong light, however, intermittent periods of strong light intensity can provide optimal growth. Many also have the ability to grow in low light conditions which enables them to continue growing whilst in the 'shadow' of other phytoplankton. As they continue to grow, they can dominate the phytoplankton, overshadowing other algal species causing their death.

2.3.2. Nutrients

Increases in the levels of phosphorus can accelerate the rate of eutrophication, impairing the quality of water for drinking, recreational and industrial use. Total phosphorus concentrations of less than 0.1 mg L^{-1} are sufficient to induce a cyanobacterial bloom. The growth of most other phytoplankton is reduced by nitrogen limitation; however, nitrogen-fixing cyanobacteria are able to outcompete other organisms under low nitrogen to phosphorus ratios.

Cyanobacteria also have a strong affinity for phosphorus with each cell capable of storing enough to perform two to four cell divisions. This corresponds to a 4 to 32 fold increase in biomass. High concentrations of total phosphate can indirectly support cyanobacteria by providing a high carrying capacity for phytoplankton, which in turn causes high turbidity and low light availability. Cyanobacteria which have a preference for low light can dominate under these conditions.

2.3.3. Nitrogen Fixation

The ability to fix nitrogen provides cyanobacteria with the simplest nutritional requirements of all living organisms. Using the enzyme nitrogenase, they can convert nitrogen into ammonium, which can be used directly by the adjoining filamentous cells and by other aquatic flora. Nitrogen fixation is common among the filamentous, heterocyst forming genera. The process only occurs in anaerobic conditions so the organism must maintain oxygen-free areas for it to occur. To overcome this problem, heterocysts have a thickened glycolipic cell wall that slows the rate of diffusion of oxygen into the cell. A by-product of nitrogen fixation is the production of hydrogen, which reduces any oxygen that does diffuse into the cell, or it may also be expelled through the cell wall. There are also some symbiotic bacteria which associate with the heterocysts, consuming oxygen and creating oxygen-free spaces. Other cyanobacteria which do not form heterocysts, such as Trichodesmium, are also capable of nitrogen fixation, although the method is little understood.

A low nitrogen to phosphorus ratio may favour the growth of certain cyanobacteria. Optimum ratios for algae are 16 to 23:1 on a molar basis, whereas bloom-forming, nitrogen-fixing cyanobacteria have an optimum ratio of 10 to 16:1. Hepatotoxic strains produced more toxins in high concentrations of phosphorus, but for anatoxin-a production, phosphorus had no effect. Non-nitrogen fixing species produce more toxins under nitrogen-rich conditions.

2.3.4. Akinetes

Some cyanobacteria produce akinetes, which are structures similar to spores. When the cyanobacteria die, due to unfavourable environmental conditions, the akinetes, which are resistant to heat, freezing and dessication, may lie in the sediments for months or years. As the dam or lake refills with water, the spore seed the water allowing the cyanobacteria to dominate again.

2.3.5. Stratification

The euphotic zone is the zone which extends from the surface to the depth at which 1 percent of the surface light intensity can be detected. This is the zone in which photosynthesis can occur and it may be shallower or deeper than the mixed epilimnion of a stratified waterbody. Many planktonic algae and cyanobacteria have little movement and can only be photosynthetically active when circulation maintains them in the euphotic zone. In eutrophic water, the turbidity caused by excessive phytoplankton biomass makes the euphotic zone shallower than the epilimnion, resulting in the phytoplankton spending part of the daylight period in the dark.

2.3.6. Gas vesicles

Those cyanobacteria inhabiting the surface layers of water are planktonic and usually contain cytoplasmic inclusions called gas vesicles. Gas vesicles are hollow protein structures with a hydrophilic outer surface and a hydrophobic inner surface, filled with various gases. Gas vesicles have a density about one tenth that of water, which allows the cyanobacterial cells to regulate their buoyancy within the water column. This allows them to maintain their position in the euphotic zone, providing a competitive advantage, especially in deeper, stratified waters. This buoyancy, combined with few natural enemies, induces low rates of loss of cyanobacterial populations which compensates for their slow growth. The amount of gas alters in response to changes in light, temperature, water density relative to colony size and the amount of carbohydrate in storage.

Microcystis, Anabaena and *Aphanizomenon* have this ability to move in the water column. At the surface, the photosynthetic rate is high, allowing the cells to store large quantities of carbohydrates. Although the cells contain gas vesicles, the density of the carbohydrate is greater, causing the cells to sink. Larger colonies have greater densities than smaller ones and sink at a faster rate. Single cells show little vertical migration. During the night, all colonies may become buoyant and if the surface is turbulent, may be accumulated as stable scums on the shore.

As the colonies sink, they move out of the euphotic zone and stop photosynthesis. Instead they use their stored carbohydrates during respiration to synthesize new gas vesicles, becoming buoyant once again. In temperate climates, this process may take longer. As the weather cools, the rate of photosynthesis becomes more rapid than respiration. When the colonies sink to the bottom, their carbohydrate is used gradually and if they survive the winter, they reascend in the spring as single cells or small colonies. During this stage, toxic cyanobacteria may be difficult to recognise in samples, until the size of the colonies increases during early summer.

Buoyancy regulation can be a substantial advantage, allowing cyanobacteria to outcompete other phytoplankton organisms. However, some species can still dominate in shallow waterbodies where vertical migration is of no advantage. *Microcystis spp*. has the ability to dominate lakes that stratify on a daily basis, particularly in subtropical and tropical regions. It is less sensitive to high light intensities and is efficient in harvesting nutrients from mesotrophic, eutrophic and hypertrophic waters.

In eutrophic systems, the high concentration of organisms causes a lot of competition for resources as well as increased pressure from predators. As a result of the struggle for survival, there is a lower diversity of organisms in eutrophic than in oligotrophic systems. Cyanobacteria have many advantages over the other phytoplankton because of their ability to position themselves for optimal growth.

2.4. Cyanobacterial species

There are about 2 000 cyanobacteria described to date. Most are aquatic, with freshwater species predominant. Some genera like *Nostoc* live on moist soils and others prefer dry habitats. A few cyanobacteria are symbionts of liverworts, ferns, cycads, flagellated protozoa, and algae. A species of *Anabaena* lives in symbiosis with a water fern *Azolla* and supplies it with nitrogen-containing compounds. Lichens and cyanobacteria often form photosynthetic partners. Of the cyanobacteria described, approximately 200 species are considered toxic.

It may be known that a particular species is capable of producing toxins. However, they may not do so all the time. This may be due to several factors:

- A different strain may not be toxic
- The toxic species may not dominate the bloom
- Within a single-species bloom there may be a mixture of toxic and non-toxic strains
- Some strains may be up to three orders of magnitude more toxic than others, resulting in a toxic bloom even when dominated by non-toxic strains.

In Australia, the most common problem genera of cyanobacteria include:
Microcystis spp., Anabaena spp., Nodularia spp., Cylindrospermopsis spp., Planktothrix spp., Spirulina spp., Aphanizomenon ovalisporum, Haphalosiphon hibernicus, Anabaenopsis spp., Nostoc spp. and Phormidium spp. in fresh water and *Nodularia spumigena* and *Lyngbya majuscula* in estuarine and coastal marine water.

3. Causes of bloom

3.1. The eutrophication process

The progression towards hypereutrophication occurs in three stages. The first stage begins with an oligotrophic water body receiving nutrients which creates exponential growth of aquatic organisms. The growth levels off, reaching equilibrium with the nutrient supply. This is referred to as the mesotrophic level. It represents a stable, ecologically sustainable system. Continued increase of nutrients causes the next stage of eutrophication. There may be signs of instability in the system and partial bloom crashes. Point source reduction of nutrients can reverse or halt the progression towards hypereutrophication at this stage. Further increases in nutrients, result in exponential growth of algae and cyanobacteria.

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Biographical Sketches

Jennifer L. Davis recently completed her Bachelor of Science Environmental Health receiving several awards for excellence during her studies. For the last seven months, she has worked as a research assistant at Griffith University, co-authoring with Dr. Glen Shaw three articles in peer reviewed publications. She is currently awaiting her acceptance for a Master of Philosophy, which will research the effect on aquatic organisms of chlorination disinfection byproducts in wastewater.

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