5 Identification of Toxic Blooms of Cyanobacteria in Estuarine Habitat

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Abstract

Our entire environment is surrounded with millions of hidden microscopic worlds with diverse life forms. One such microscopic life forms are these photosynthetic bacteria, evolved over 2 billion years ago, known as cyanobacteria. They are found ubiquitously. We will be focusing on their presence particularly in an Estuarian Ecosystem. These photosynthetic cyanobacteria can also produce toxins when they grow excessively and can also cause harm to the environment, plants and animals living under water. They can also cause harmful effects to humans as well sometimes. These toxins producing cyanobacterial blooms can be identified by different studies.

Keywords

Super blooms of cyanobacteria · Nuisance phytoplankton blooms · Estuaries · Cyanotoxin(s) · Microcystin · Nodularin · Toxicogenic cyanobacteria

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5.1 Introduction

We are surrounded by hidden microscopic worlds filled with fascinating life forms. Thousands of microbial organisms live within a single drop of water. A pond ecosystem is composed of microbial wildernesses that ages over 2 billion years, that are yet to be explored. These organisms were the first to live by photosynthesis. These organisms used sunlight to make sugars and then these sugars gave power to the cell. A by-product of photosynthesis is oxygen, and 2 billion years ago, these organisms became so abundant that they completely changed the Earth's atmosphere. It went from the one that was very oxygen poor, to the oxygen-rich atmosphere that we live in today. These microbes are called cyanobacteria (Albrecht et al. 2017). They are just as abundant today as they were 2 billion years ago. Cyanobacteria can be found all over the world. There are currently around 3000 described species with different morphologies and habitats from freshwater ponds to arctic oceans. They even live in soil. These cyanobacteria are also known as photosynthetic bacteria (United States Environmental Protection Agency 2020).

5.1.1 Estuaries

It is an ecosystem by itself. They are simple terms known as the transition area where the freshwater (river or stream) meets the ocean. Here the salinity of water increases. It is a semi-enclosed body of water with an opening to the ocean and feed by freshwater. They are also known as the "nursey of the sea" as they provide shelter for aquatic habitat as it provides a supportive environment for the wild to raise their young (Elliott and McLusky 2002). They have the producers which are the phytoplankton which is a part of the food chain. They are known for the brackish water which is the combination of salt and fresh water. They are important as they help in filtering the sediment and pollutants from the water before it flows into the ocean. In addition, they buffer the ocean and land which helps decrease the effect of flooding and storm surges (Elliott and McLusky 2002; Perillo 1995). These estuaries can be classified according to their geological features. The classification includes (Elliott and McLusky 2002; Perillo 1995; Lehman et al. 2022):

1. Coastal plain estuary: Also known as a lagoon, they resemble valleys with gentle slopping bottoms, their depth increases towards the river's mouth. Very commonly encountered throughout the world, for example, The Chesapeake Bay.
2. Tectonic estuary: Created when the tectonic eruption occurs or when the sea fills in the hole or a basin that is formed from sinking land, for example, San Francisco Bay
3. Bar-Built estuary: formed when sand bars build up along the cost line and they partially cut off the water behind them from the sea.
4. Fjord estuary: they are narrow with steep sides and are usually straight and long. Usually found in areas covered by glaciers.

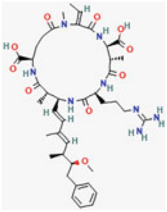
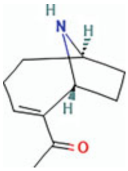
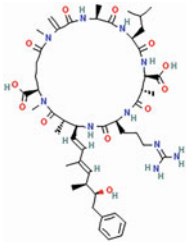
5.1.2 Cyanotoxins

Cyanobacteria are considered the most problematic bacteria in the freshwater ecosystems. These photosynthetic bacteria are ubiquitous, naturally occurring but under certain conditions like presence of high phosphorous and nitrogen (nutrient) content or climate change (excess sunlight/dry or rain/wet conditions) they can grow out of control (overgrow) unchecked by natural cycles in the water. The uncontrolled growth of photosynthetic bacteria in a short period of time are called ‘Blooms of Cyanobacteria’ (Rastogi et al. 2015). Some the effects of these Cyanobacterial Blooms are they consume oxygen and block the sunlight by not letting it penetrate into the waterbody for other plants and animals living underwater. When these Cyanobacterial Blooms start decaying they consume excess oxygen creating hypoxic conditions which results in die-offs of many plants and animals living under water (Bormans et al. 2020). Under favorable conditions i.e., photo rich and nutrient rich environment, some species of these photosynthetic bacteria produce some toxic secondary metabolites or when there is release of gases from the ruptured dead cells of the cyanobacteria it is called a toxin/cyanotoxin (Fristachi et al. 2008).

5.1.3 Toxic Blooms of Cyanobacteria in Estuaries

Ideally toxigenic cyanobacteria occur mostly in freshwater habitats. Since an estuary is an area where freshwater meets ocean, it is ideally a mixture of freshwater as well as saline water (A semi–enclosed body of water were saltwater mixes with freshwater). Estuaries are usually referred to have Brackish water which shows growth of toxigenic cyanobacteria. The blooms of these toxigenic cyanobacteria are found in majority of the estuaries throughout the world. They cause harmful effects to the estuarine environment, to animals, humans as well as spoil recreational opportunities along the coast line. Not all blooms are harmful (Fristachi et al. 2008). Blooms known as “Nuisance blooms” can discolor water, smell bad, and cause the water or fish to taste bad. Nuisance blooms are not usually dangerous to people, pets, and livestock because they do not produce toxins. However, they can discourage people from visiting beaches, drinking tap water, or eating fish from water with an ongoing bloom (Paerl 1988). On the other hand, “Super Blooms” of cyanobacteria that produce biotoxins that are harmful and cause global health issues are caused by certain toxicogenic cyanobacteria (Miller et al. 2010). These toxic cyanobacterial blooms can affect the water in different ways. One of them is by taste and odor, they create water that may tase like mud or moldy water. The cyanotoxins are compounds that may cause health problems aby affecting the liver, kidney or nerves system effects in humans (Brown et al. 2018). There are three different classes of cyanobacteria: Neurotoxin (synthetically or naturally occurring toxins can directly damage/affect the nervous system), Hepatotoxin (synthetically or naturally occurring toxins that damage the liver) & Dermatoxins (synthetically or naturally occurring toxins that damages the skin cells).

Table 5.1 Toxins produced by cyanobacteria in Estuaries

Sl. no.	Cyano-bacteria	Toxin produced	Chemical structure	Toxin category	References
1.	*Nodularia spumigena*	Nodularin		Hepatotoxin	Paerl (1988)
2.	*Oscillatoria* sp.	Anatoxin-a		Neurotoxin	Paerl (1988)
3.	*Microcystis aeruginosa*	Microcystin		Hepatotoxin	Gibble and Kudela (2014)

Usually, toxic blooms of species of *Nodularia* and *Oscillatoria* were initially observed, identified and studied in estuarian ecosystems (Paerl 1988). Later, studies from the 2000s have confirmed presence of toxic blooms of species of Microcystis as well. This is because usually *Microcystis* are intolerant to saline conditions (Fristachi et al. 2008). But there were many studies that reported the presence of *Microcystis* sp. in estuarine habitat can be because of wet climate (Lehman et al. 2022). The wet climatic conditions decrease the salinity of water as there is continuous mixing of freshwater to the saline water with in turn reduces the salinity and creates a favorable environmental condition for the growth of blooms of *Microcystis* sp. as well. Some signs of toxin producing cyanobacteria are (1) they can make the estuary pigmented, (2) they make the environment that they are present very unpleasant by releasing gases/ toxins that give unpleasant odor, taste etc. (3) while collection of water sample there might be presence of tiny, floating, green particles. Though the above-mentioned signs may be considered as the preliminary characteristics to suspect if a particular estuary has toxin producing blooms of cyanobacteria or not (United States Environmental Protection Agency 2020; Rastogi et al. 2015). Because one cannot tell if a water body has a toxin producing blooms of cyanobacteria just by looking at it. To find out what species of toxin producing cyanobacterial bloom is living in that particular estuary a microscopic analysis is required. The Table 5.1

below discusses the toxin produced by the most prominent species of cyanobacteria that produce toxins in estuarian ecosystem. Frequency and Severity ranking of presence of toxin producing cyanobacteria is as follows Freshwater > Estuarine (Brackish) > Marine (Fristachi et al. 2008).

Some of the impacts of toxin producing cyanobacteria on waterbody health and ecosystem viability are because of the following. Though the factors responsible for bloom formation and toxin production still remains not well understood. Some of the researchers consider that Chemical factors, Hydrological factors, Light and Temperature may be involved factors in bloom formations and toxin production. Invasion of water milfoil with bacterial blooms are occurred in estuarine habitats. In brackish water cyanobacterial blooms producing toxins get accumulated in fish and leads to its death. Fish kills in estuaries can be associated with cyanobacterial blooms (Fristachi et al. 2008).

5.1.3.1 Microcystis

Microcystis is a cyanobacterium that is the most prevalent generator of freshwater blooms on all continents except Antarctica, and these blooms are typically linked with some degree of toxins (Sellner et al. 2003). Microcystis is classified as a toxic CHAB (cyanobacterial harmful algal bloom) because some species produce potent hepatotoxins known as microcystins, which induce cancer and tumor growth in the livers of mammals and humans. It also causes surface scum, which obstructs recreation, lessens dissolved oxygen content, reduces aesthetics and causes odor and taste issues in drinking water. Survival and growth rate of rotifers, cladocera and copepods are affected by microcystins found in zooplankton food or dissolved in the water column (Lehman et al. 2010). Microcystis, Phormidium, Anabaena genera, Planktothrix, Nostoc and Oscillatoria are the primary producers of Microcystins, with Microcystis being the first known producer (Lopes and Vasconcelos 2011). In fresh and brackish eutrophic environments such as lakes, rivers and estuaries, they can generate cyanobacterial hazardous algal blooms and dominate phytoplankton populations. Natural toxins produced by blooms can induce liver cancers, neurological toxicity and developmental toxicity. Neurotoxin β-methylamino-L-alanine, lipopolysaccharides and the metabolite microcystin are among the natural toxins (Acuña et al. 2020).

5.1.3.2 Nodularia

Nodularia spumigena is a brackish water heterocytous cyanobacterium that has been observed blooming in estuary lakes throughout Europe, South Africa, Canada, the Mediterranean, Australia, the United States and New Zealand (Kaur et al. 2015). Toxic *N. spumigena* blooms have regularly been documented in the Baltic Sea in Europe, and as a result, nodularin is regarded as one of the most numerous naturally occurring compounds in this brackish-water environment (Lopes and Vasconcelos 2011). Nodularin is the most well-known toxin generated by the brackish water cyanobacteria *Nodularia spumigena* (Lopes and Vasconcelos 2011). It is a tumor promoter and probable carcinogen that creates the cyclic pentapeptide hepatotoxin nodularin, which is like microcystins, significantly inhibits protein phosphatases

1 and 2A (Lopes and Vasconcelos 2011). Although nodularin intoxication has not resulted in any human deaths, it has been linked to a range of stock and domestic animal poisoning incidents (Lopes and Vasconcelos 2011).

5.1.3.3 Oscillatoria

Oscillatoria is a common species of bacteria found in freshwater systems across the globe that have been associated with disease and, in some cases, death in animals and humans. Ingestion of polluted water or food products exposes people to cyanobacteria. The presence of these bacteria in the gut exposes the body to their toxins, such as lipopolysaccharide (LPS), to B cells in the gut-associated lymphoid tissue. On the other hand, the effect of Oscillatoria sp. LPS on B cell activation is unclear (Swanson-Mungersona et al. 2017) (Table 5.2).

5.2 Materials

- Glass slides
- Forceps
- Dropper
- Coverslips
- Bacterial culture
- Inoculation loop
- 70% ethanol
- Immersion oil
- Microscope

5.3 Methods

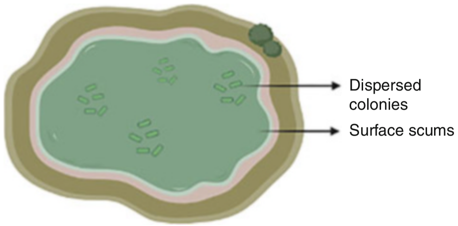
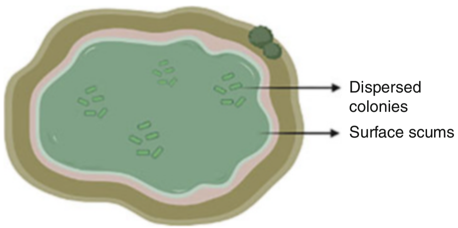
5.3.1 Microscopic Analysis (Hayet et al. 2021)

The identification of cyanobacteria is usually carried out by using a compound light microscope. Collection of water sample containing suspected toxin producing cyanobacteria from the estuarian water body will be the preliminary step.

5.3.1.1 Experimental Protocol

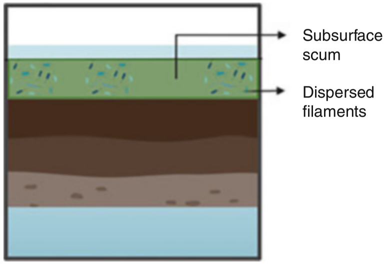
1. Take a clean glass slide and wipe the surface with 70% ethanol
2. Mount the cyanobacterial culture(sample) on the slide using a dropper
3. Make a uniform smear with an inoculation loop or forceps
4. The microscopic slide culture is covered with a glass coverslip
5. View the slide through the microscope at 10×, 40× objective lens and using oil immersion at a magnification of 100× lens of the compound light microscope. The schematic representation of the protocol is given in Fig. 5.1.

Table 5.2 Identification of cyanobacterial toxic blooms of Estuarine habitat and their mode of action

Genus	Toxin produced	Bloom morphology	Biological toxicity	Mode of action	References
Microcystis	Microcystins	Surface scums and dispersed colonies 	Hepatotoxic	Inhibitors of protein phosphatases 1, 2A and 3, tumor promoter, genotoxicity	Paerl (1988); Swanson-Mungersona et al. (2017)
Nodularia	Nodularin	The bloom was first noticed as a thick layer of brilliant blue-green surface scum and dispersed colonies concentrated by wind-driven advection along the lakeshore 	Hepatotoxic	Inhibitors of protein phosphatases 1, 2A and 3, tumor promoter	Paerl (1988); Mcgregor et al. (2012)
Oscillatoria	Lipopolysaccharides (LPS)		Cytotoxic, dermatotoxic, gastroenteritis	Dermonecrotic, protein kinase C activator, and potent tumor promoters	Paerl (1988); Swanson-Mungersona et al. (2017)

(continued)

Table 5.2 (continued)

Genus	Toxin produced	Bloom morphology	Biological toxicity	Mode of action	References
		Subsurface layers and dispersed filaments 			

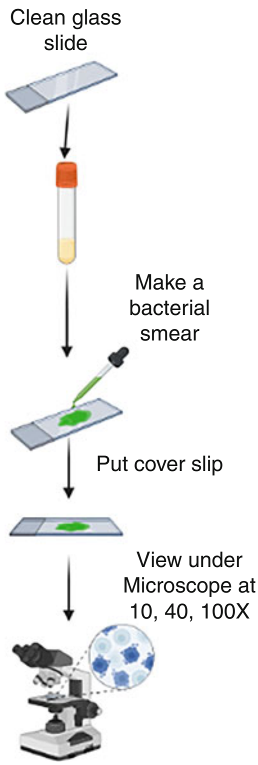


Fig. 5.1 Schematic representation of microscopic identification of cyanobacteria

5.4 Observations/Interpretations

- *Microcystis* colonies are more densely clustered. Cells are spherical shaped, enclosed in a mucilaginous sheath and non-filamentous. Each cell has gas vesicles that often appear blackish blue.
- *Nodularia* trichomes were solitary, cylindrical and straight to slightly flexuous. Akinetes were common, discoid-subspherical mostly single or in pairs. Heterocysts were also compressed discoid.
- *Oscillatoria* trichomes (threads of cells) often appear as dense, opaque ribbons, which oscillate under the microscope. The cells are short cylinders. Trichomes are straight or irregularly twisted.

5.4.1 General Interpretation of Cyanobacteria

Morphologically cyanobacteria are unicellular, Soft, green gelatinous, membranous, filamentous with a true or false type of branching, repeatedly branched, rounded cylindrical, heterocysts or non-heterocysts, trachomatous, perennation (hormogones and akinetes) and multicellular with carbon dioxide fixation (CO_2) in vegetative cells and nitrogen fixation (N_2) in heterocysts (Shiels et al. 2019).

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