

Review on Freshwater Blue-Green Algae (Cyanobacteria): Occurrence, Classification and Toxicology

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Cyanobacteria are one of the most diverse groups of gram-negative photosynthetic prokaryotes. Cyanobacteria are prokaryotes have a simple cell structure with a real nucleus Prokaryotic. Their body is made from a single cell, often clustered cells as colonies of different shapes. Cyanobacteria are typically much larger than bacteria in size, it contain many types of pigments such as carotenoids and phycocyanin. A characteristic of water soluble pigment in cyanobacteria gives the group of cyanobacteria their blue green coloration. Cyanobacteria living in individuals places in fresh and salt water, and some other types live in moist soil. The water distinctive bluish colour is results for cyanobacteria blooms when it dies. Researchers found that only about 10% of all blooms types are considered toxins producer. However, it is still ambiguous what triggers that cause to produce its toxin. This review highlights the occurrence of algal cyanobacteriablooms, its classification and toxicology.

Key words: blue green algae- cyanobacteria- blooms- cyanotoxins- toxins.

Occupying a wide range of terrestrial and aquatic environments all over the world, cyanobacteria or blue-green algae constitute one of the largest groups of photosynthetic prokaryotes. Cyanobacteria, representing some of the most ancient life form on earth, and are one of the most studied organisms worldwide characterized them as unusual prokaryotic microorganisms that can perform oxygenic photosynthesis^{1,2}. They can also synthesize chlorophyll *a* similar to eukaryotic algae and plants, cyanobacteria use H₂O as an electron donor for the production of oxygen. The oldest fossil

findings of cyanobacteria can be dated back to approximately 3500 million years ago. On the last decades, cyanobacteria blooms had given special consideration over worldwide because of it is highly effects on water environments by increasing anthropogenic input of nitrogen and phosphorus^{3,4}. To date, cyanobacteria are still largely present in oceans, freshwaters, and soils. Most cyanobacteria live in water as phytoplankton. Cyanobacteria are often called "blue-green algae" and are named after the blue pigment phycocyanin, which, together with chlorophyll *a* and other pigments, is used to capture light for photosynthesis. Cyanobacteria is important factor used for life evaluation such as phototrophic organisms, they can use the energy of light to produce organic matter (CH₂O) and oxygen (O₂) out of water (H₂O) and carbon dioxide (CO₂)

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through photosynthesis: $\text{H}_2\text{O} + \text{CO}_2 = \text{CH}_2\text{O} + \text{O}_2$. Cyanobacteria were the first organisms capable of oxygenic photosynthesis; thus, they are considered largely responsible for the rise in atmospheric O_2 over two billion years ago⁵. The rise in atmospheric oxygen made the evolution of life that is dependent on oxygenic respiration possible. Furthermore, Raven and Allen (2003) compared ribosomal RNA from cyanobacteria with DNA inside the chloroplasts of eukaryotes and revealed that all photosynthetic eukaryotes derived their photosynthetic capabilities from cyanobacteria through endosymbiosis⁶. Water quality in freshwater lakes and reservoirs is mostly conducted on a regular basis; it can be determined by measuring the existence organism in water which provides the necessary information about long term of water quality. Moreover, some organisms are producing toxins which also effect water quality. Cyanobacteria used sometimes as indicators of water quality because they are light sensitive and can regulate their buoyancy; it is essential property to detect it over other organisms⁷. The occurrence of freshwater blue-green algae and its identifications and toxicology will be discussed in this review.

Overview of algae

Algae are a wide range of plants heterogeneous in all shapes, sizes, and physiological functions, except that they contain chlorophyll pigments and others. Algae include members of the nucleus of a primitive prokaryote such as blue-green algae. Some algae are microscopic, whereas others reach up to several meters in length. Algae are abundant in salt water and fresh and stagnant pools of water, lakes, and in humid places or on the rocks. Large numbers of algae are also found on soil. The presence of algae in surface water has been a long-standing issue all over the world because of their adverse effects on the treatment process and quality of drinking water⁸. The removal and control of algae in the water treatment industry are important global issues, especially in tropical and semi-tropical zones. The presence of algae in surface water causes many problems regarding colour, odour, taste, and toxic compounds, posing potential hazards to human and animal health⁹. Furthermore, algae widely affect the drinking water which mostly required a treatment process¹⁰. Traditionally, some treatment process involved such as pre-oxidation

by chlorine dioxide, ozone, chlorine, or permanganate is usually used to improve algae removal in the coagulation process¹¹⁻¹³.

Research found that algae are important factors in most of aquatic ecosystems; they reported that algae are classified as important components of biological monitoring programs. Algae have very short life cycles with rapid reproduction rates which make them an ideal component for water quality assessment of short terms effects. Algal showed wider distribution among geographical regions and ecosystems. Algal divisions are very sensitive to some poisons, and they accumulate pollutants readily. Algae as primary producers are most organisms that directly affected by physical and chemical factors because their metabolism is also sensitive to natural disturbances and differences in environmental. In laboratory, standard methods exist for evaluation of functional and non-taxonomic structural characteristics of algae communities showed that algae are easily cultured, easy and inexpensive in sampling process, and have a minimal effect on local biota¹³⁻¹⁹. Furthermore, as biological indicators algae showed many attributes of spatial and temporal environmental changes, especially as parameter for algae community in structural and functional variables which used as biological monitoring programs. Biological indicators like algae have only recently been included in water quality assessments in some areas of Malaysia. Using algae as parameters in identifying the different types of water degradation have been becomes essential and complementary with other environmental indicators over worlds²⁰.

Actually, more than 10,000 living diatom species are become well known, and also same number approximately of named fossil forms, about over than 90% of the biosphere is employed by plant life, where diatoms make up approximately a quarter by weight. They are hugely abundant in the upper layers of oceans, where they providing a high-grade of nutrition to a variety of creatures from protozoans to baleen whales²¹.

Automated recognition system for algae

Many attempts have been made to automate the identification system for organisms. However, such an attempt has not been done for algal species, especially in Malaysia. Furthermore, image analysis of phycological images has been

conducted for cyanobacterial classification. Work has been conducted on many cyanobacterial genera, such as *Microcystis*, *Anabaena*, *Planktothrix*, *Aphanocapsa*, *Aphanizomenon*, *Coelosphaerium*, *Gloeotrichia*, *Merismopedia*, *Nostoc*, and *Oscillatoria*. A localization method based on image-fusion techniques, high-resolution microscope images, colour interest point extraction, self-organizing map network, and aid of textural features was among the methods used to analyse algal images. Most processes collect various algal samples to be observed and automated and success rate is at most up to 90%²².

The aim in pattern recognition is to use a set of example solutions to solve problems to infer an underlying regularity and to serve as reference for possible solutions in subsequent cases. Among many models proposed over the years, neural network models have exhibited the most optimal nonlinear boundary for classification problems. The capabilities of nonlinear learning in ANN is supported a powerful tool to be used widely for solving many complex applications such as nonlinear system identification, unsupervised classification and optimization, and functional approximation. The multidisciplinary nature of the neural network classification research is believed to generate more research activities and produce more fruitful outcomes in the future^{23,24}.

Identification and classification of algae

Image recognition and identification of fresh water algae has been manually executed, and very few automated systems have been implemented. Manual identification and classification are tedious and time consuming. A classification problem for algae exists because they need to be categorized into predefined groups or division based on a number of observed attributes related to the object shape and measurements. Traditional statistical classification procedures are usually built on Bayesian decision theory, which details the concept of probability by reasoning with uncertain statements. In specific procedures, the probability model is assumed to calculate the posterior probability to guide the classification process. However, one major problem of probability models is effective only if the underlying assumptions are satisfied. The efficiency of this technique is highly demands on the level of conditions and assumptions made during

development process of this model. That is, one must be knowledgeable and experienced in the data properties and model capabilities to successfully apply or create the model²⁵.

The classification of algae into interested groups or division was performed on based of the same rules which used for land plants the classification however organization of groups of algae above the order level has been changed its methods substantially since 1960²⁶. Research begins to use the differences of algae feature in classification process such as cell division, organelle structure and function, and flagellar apparatus. Microscopic images showed a similarities and differences among algal, fungal, and protozoan groups which led scientists to propose new major taxonomic changes that are currently under process. Division-level in classification of algae is used as well as kingdom-level classification but it is tenuous for algae because classes are mostly distinguished by the structure of flagellate cells such as (scales, microtubular roots, angle of flagellar insertion, and striated roots), the cytoplasmic division process (cytokinesis), the cell covering, and the nuclear division process (mitosis)²⁶.

Cyanobacteria

Cyanobacteria (blue-green algae) are common members of freshwater algae, the plankton of marine, and brackish throughout the world. It mostly found on rocks, soils, and in symbioses with plants and fungi. Cyanobacteria have a simple structure at the subcellular level and lack a nucleus, also as prokaryotes a characteristic feature defining them along with bacteria²⁷. Research found that cyanobacteria have a photosynthetic appearance that supports them to perform photosynthesis as in algae and higher plants, but they lack chloroplasts in which these reactions occur in the latter organisms. Unicellular and filamentous forms are commonly found among cyanobacteria. Both morphotypes are capable of producing structures visible to the naked eye, such as pinhead or larger, spherical, or irregular colonies (e.g., of *Microcystis*), and bundles of filaments (e.g., of *Aphanizomenon*) like sawdust in shape and size. Further differentiation among cyanobacteria includes the ability of certain filamentous genera, such as *Anabaena*, *Aphanizomenon*, *Gloeotrichia*, *Nostoc*, and *Nodularia* to fix

atmospheric nitrogen enzymically in specialized cells termed heterocysts. Several of the filamentous genera also produce other differentiated cells termed akinetes (spore stages), which permit them to survive periods of adverse conditions such as cold and drought. In addition, research found that cyanobacteria produce high mass populations in natural and controlled water resources, which lead to the common cyanobacteria blooms, scums, and mats, but not invariable consequences of eutrophication with rich nutrients for waters and plant²⁸. Cyanobacteria large growths and accumulations are often considered undesirable because of its effect on water colour, quality, and odours. It also cause some turbidity in recreational and amenity facilities, and have potentially synthesize over many low molecular weight compounds that cause taste and odour problems. These substances often result in complaints regarding recreational and amenity water bodies as well as the quality of raw and treated drinking water. Low molecular weight compounds such as cyanobacteria toxins or cyanotoxins produced by cyanobacteria are highly concern because of their high toxicity to vertebrates including mammals. These compounds are ignored during associated the problems with taste and odour compounds because they are colourless and odourless^{29,30}. Cyanobacteria have higher toxicity if compared with other biological toxins such as plant, fungal, and shellfish toxins. Saxitoxins produced by cyanobacteria are considered as chemical weapons by the international chemical weapons convention³¹. Also, Saxitoxin and microcystin are listed in the core list of toxins prevents to export and control by the Australia Group³². Among the cyanobacterial genera that include toxin-forming species, the ones of particular concern when mass populations occur include *Microcystis*, *Anabaena*, *Planktothrix* (formerly known as *Oscillatoria*), *Aphanizomenon*, *Cylindrospermopsis*, *Phormidium*, *Nostoc*, *Anabaenopsis*, and *Nodularia*). Together, these genera can produce a wide range of cyanobacterial toxins of varying structures, toxicities, and modes of action.

Cyanobacteria are prokaryotes have a simple cell structure with a real nucleus Prokaryotic. Their body is made from a single cell, often clustered cells as colonies of different shapes. The optimum temperatures for the growth of blue-green

algae, ranging from 35-40 C. Cyanobacteria are typically much larger than bacteria in size, it contain many types of pigments such as carotenoids and phycocyanin. A characteristic of water soluble pigment in cyanobacteria gives the group of cyanobacteria their blue green coloration. In addition, cyanobacteria living in individuals places in fresh and salt water, and some other types live in moist soil. The water distinctive bluish colour is results for cyanobacteria blooms when it dies³³.

Cyanobacterial blooms

The blue-green algae include many groups, widespread, and lives in fresh and salt water and most of them live submerged in fresh water. Most marine species living on the beach, whereas some species live in soil and on the rocks, and when there are favourable conditions can be seen and clear the murky waters became Growth with a very green, blue, greenish, or reddish brown, within a few days only. Many types of organization of buoyancy float have the surface to form a thin scum of green and blue colours. Also, maintain these cyanobacteria population does not abnormally consider as high for a long time, and will die quickly within one to two weeks. If conditions remain favourable, we can replace the last bloom quickly than its predecessor. Interfere successive blooms are making the flowers look like continuing growth for several months³³. Phenomenon of fast growth for cyanobacteria called bloom which effects water colour to make lake water appears like pea soup. Cyanobacteria are classified as algae commonly and called blue-green algae which a distinct group of bacteria capable of photosynthesis. The sunlight and nutrients are converted by cyanobacteria into energy for growth and reproduction³⁴.

Cyanobacteria have different types of adaptations which allow them for persistence, optimal growth, and support them by the ability to outcompete algae during good conditions. In general, many species produce hidden cells or components that remain inactive until the condition is become suitable then they arise again. Some species have specific cells which able to convert nitrogen gas into nitrogen fixation forms, dissimilar to algae which produce photosynthesis from carbon dioxide gas. Most cyanobacteria types can able to utilize different carbon sources³⁵.

Toxicity of cyanobacteria blooms

It is not truth that all cyanobacteria blooms are toxic, even some blooms caused by identified toxin producers aren't produce toxins or may produce toxins at low levels³⁶. When bloom occurs in specific area, warning should be given to the peoples using their water source, even if the toxins detected in relatively small amounts. It is still ambiguous until now what is the triggers that cause to produce toxin by cyanobacteria, at least scientist have not been reported the main reasons about that.

Few years ago, researchers found that only about 10% of all blooms types are considered toxins producer. Moreover, later studies in developed countries such as USA, and Canada reported that probability of producing toxins for individual bloom including *Anabaena*, *Microcystis*, and *Aphanizomenon* is between 45%-75%, which is greater than previous expectation³⁷⁻⁴⁰.

Many evident showed that cyanobacteria bloom is toxic and can cause dead to large number of animals within or around water such as fish, waterfowl, and terrestrial animals. Researcher found many symptoms from sub-lethal poisonings with different kind of animals, quantity of toxin consumed, and nature of toxin itself. Mostly, any unexpected animal illness, and unexplained death existing near water, it should be suspected that water containing toxic bloom³³. Moreover, numerous accidents existing which cases of animal poisonings over the world. Some reports showed that also human illness and death have been documented in many countries lastly. Numerous bloom forming common species reported they produce potent toxins, and cyanobacteria is become known well for long time it produce toxic. Research showed that cyanobacteria produce different types of toxins such as different type of nerve toxins, common liver toxins called microcystins, and shellfish poison named saxitoxin which considered the less common types of toxins⁴¹⁻⁴³.

Furthermore, to treat cyanobacteria in water, chemical compound must be used. Using chemical in natural lakes is not allowed because it can cause other different problems. Thus, using chemicals can effect organisms and human too because it is also toxic to other form of life. In near future, research may be found suitable solution by

reducing the nutrients amount lakes. User activities and entertaining of lakes help to reduce the nutrient amount input into lakes through individual and group action^{44,45}.

Concluding remarks

The production of potent toxins by bloom-, scum- and mat-forming cyanobacteria, in fresh-, brackish and marine waters, appears to be a global phenomenon. The harmful cyanobacteria blooms are being reported worldwide due to several factors, primarily eutrophication, climate change and more scientific monitoring. All but cyanobacteria toxins (cyanotoxins) are mainly a marine occurrence. Cyanotoxins occur in fresh (lakes, ponds, rivers and reservoirs) and brackish (seas, estuaries, and lakes) waters throughout the world. Organisms responsible include around 40 genera but the major ones are *Aphanizomenon*, *Anabaena*, *Microcystis*, *Cylindrospermopsis*, *Nostoc*, *Lyngbya*, and *Oscillatoria*. Cyanobacteria toxins include cytotoxins and biotoxins with biotoxins being responsible for acute lethal, acute, chronic and sub-chronic poisonings of wild or domestic animals and humans. The biotoxins include the neurotoxins and saxitoxins as well as the hepatotoxins; microcystins, nodularins and cylindrospermopsins. Water quality management to decrease toxic cyanobacterial mass developments, and plans to attenuate the possible effects of cyanobacterial toxins, require an understanding of the occurrence and features of the toxins and of the exposure pathways through which the toxins present risks to health. More studies on the biogenesis of toxins, combined with the arising interest in using molecular methods to differentiate toxic and non-toxic strains will increase our understanding of toxin production in the near future.

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