Faculty of Resource Science and Technology

DIVERSITY OF CYANOBACTERIA IN SUNGAI SARAWAK KIRI

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Bachelor of Science with Honours
(Aquatic Resource Science and Management)
2006

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This project is submitted in partial fulfillment of the requirements for the degree of Bachelor or Science with Honours (Aquatic Resource Science and Management)

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK (UNIMAS)
2006

For mom and dad, you guys are my inspiration to be a better person.

ACKNOWLEDGEMENT

There are not many chances that one gets to express their gratitude to those who deserve it and I would like to grab this opportunity to do so:

First of all, I want to thank my Heavenly Father for His countless blessings poured on me. Trying times were filled with hope as He gives me strength to face each day (Philippians 4:13).

A BIG Thank You is also in line for my project supervisor, Dr. Ruhana Hassan for her countless advice given during the running of my project. Madam, your guidance and support is very much appreciated.

To all the other lecturers who has taught me during the last three years, I would also want to share my gratitude to you all for your contribution in one way or another in making me who I am and teaching me what I know today.

An appreciation chain goes out to the lab assistants namely: Mr. Zulkifli, Mr. Harris and Mr. Nazri who has been helpful and patient with me whether in lab work or during field trip; course mates and fellow friends who made this challenging project fun as well as my aunties, uncles and cousins who gave me words of encouragement whenever I needed it.

Lastly and by far the least, my deepest thank you, my cherished moments and my love goes out to my family who has been part of my life for the past 21 years. Mom, Dad and Brian, THANK YOU.

Contents

Diversity of Cyanobacteria in Sungai Sarawak Kiri

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Abstract

A study was carried out to determine the distribution of cyanobacteria in Sungai Sarawak Kiri. Sampling was done at 6 stations and involved two tributaries which are Sungai Bengoh and Sungai Turn. From the study there was a total of 23 cyanobacteria genera identified. Among the prominent genera were *Lyngbya, Oscillatoria, Spirulina* and *Stigonema.* From the 23 genera identified, there were 6 which were potentially toxin producers. They are *Fischerella, Hapalosiphon, Lyngbya, Oscillatoria, Scytonema* and *Spirulina.* High correlation between cyanobacteria species composition and three water parameters was determined namely, Phosphate concentration (R^2 = 0.849758), Dissolved Oxygen (D.O.) (R^2 = 0.704859) and Biological Oxygen Demand (BOD) (R^2 = 0.743838). The relationship between phosphate concentration and cyanobacteria composition was positive while analysis showed negative relationship for BOD and DO against cyanobacteria composition.

Key words: distribution, species composition, cyanobacteria, Sungai Sarawak Kiri and correlation

Abstrak

Satu Kajian telah dijalankan untuk menentukan taburan cyanobacteria di sepanjang Sungai Sarawak Kiri. Sampel sir telah diambil dari 6 stesen dan melibatkan dua anak sungai; Sungai Bengoh dan Sungai Turn. Dari kajian ini, sebanyak 23 genera cyanobacteria telah berjaya ditentukan. Antara genera yang utama adalah Lyngbya, Oscillatoria, Spirulina dan Stigonema. Dari 23 genera yang ditentukan, terdapat 6 genera yang direkodkan berpotensi untik menghasilkan toksin iaitu Fisherella, Hapalosiphon, Lyngbya Oscillatoria, Scytonema dan Spirulina. Terdapat korelasi tinggi diantara komposisi spesis cyanobacteria dengan tiga parameter air iaitu kepekatan fosfat (R^2 = 0.849758), Oksigen terlarut (D.O.) (R^2 = 0.704859) dan BOD (R^2 = 0.743838). Terdapat hubungan positif antara kepekatan fosfat and komposisi cyanobacteria manakala analisi menunjukkan hubungan negative untuk BOD dan DO dengan komposisi cyanobacteria.

Kata kunci: taburan, komposisi spesis, cyanobacteria, Sungai Sarawak Kiri dan korelasi

1.0 INTRODUCTION

Cyanobacteria is also known as Blue Green Algae. As one of the two major groups from the Monera Kingdom, the Cyanobacteria is considered by many to be a group of chlorophyllose, photosynthetic bacteria thus accordingly called "Cyanobacteria" (Kondratyeva, 1982 cited in Bold *et al.*, 1987). Cyanobacteria also constitute one of the largest subgroups of Gram-negative photosynthetic prokaryotes (Rasmussen & Johansson, 2002).

Given its own division (Division Cyanophyta), it consists of just one class; Cyanophyceae otherwise known as Schizophyceae (Bold *et al.*, 1987; Hoek *et al.*, 1995). Belonging to the Prokaryotic group of organisms, it generally possess no nuclei, no golgi apparatus, no mitochondria, no endoplasmic reticulum and plastids (Hoek *et al.*, 1995).

Cyanobacteria are highly diverse and they can be found worldwide in all ecosystems, from marine to limnic to terrestrial ecosystems (Rasmussen & Johansson, 2002). Distribution of Cyanobacteria is also very wide where it can be found from polar regions to tropical regions and also in extreme areas such as hot springs and desserts (Garcia-Pichel *et al.*, 2001; Rasmussen & Johansson, 2002; Sompong *et al.*, 2005).

1.1 Objectives

The objectives of this study are: -

i) to record and document the diversity of Cyanobacteria in Sungai Sarawak Kiri.

ii) To determine the distribution of Cyanobacteria species in Sungai Sarawak Kiri.

iii) To record physicochemical parameters of Sungai Sarawak Kiri.

iv) To determine the relationship between water quality parameters and Cyanobacteria composition.

1.2 Hypothesis

1) Strains of Cyanobacteria identified in samples collected contains various strains from toxin producing genera to non-toxic genera.
2) There will be a significant relationship between water quality parameters and Cyanobacteria composition.

1.3 Problem Statement

Cyanobacteria are important organisms in the earth's ecosystem. One of the well known importance of cyanobacteria is its ability to produce toxin. Toxic cyanobacterial blooms occur worldwide in fresh and brackish eutrophic waters (O'Brien *et al.*, 2001; Cole,1994; Horne & Goldman, 1994). Cyanobacteria are also important as nitrogen fixers of atmospheric nitrogen in the nitrogen cycle (Cole, 1994; Horne & Goldman, 1994). This special ability is due to specialized cells that are developed by the cyanobacteria called heterocysts (Sze, 1993).

From the economical aspect it has an important role in the food industry where it is believe to be a high source of protein (54% – 60%) (Cole, 1994). This amount relatively is much higher than many other plant organisms (Cole, 1994). Besides that, cyanobacteria are also useful as indicator organisms to indicate cultural euthrophication (Cole, 1994; Horne & Goldman, 1994).

Cyanobacteria are organisms that flourish at high temperatures, neutral to alkaline conditions with high nutrient concentration (O'Brien *et al.*, 2001). Even so, cyanobacteria are identified in extreme environments as well. Nitrogen, Phosphorous and Carbon and the important nutrients that often decides the dominance of cyanobacteria in the phytoplankton community (O'Brien *et al.*, 2001). Thus, to a certain level, cyanobacteria composition has relation with nutrient concentration.

Cyanoboacteria study history in Sarawak showed that there was a documentation of cyanobacteria in lentic zones in Kuching area by Ramlah (2005) and cyanobacteria in aquatic environments surrounding Kuching by Abang (2003). No study of cyanobacteria in Sungai Sarawak Kiri has ever been carried out to date, and therefore, this study is designed to document the diversity of cyanobacteria in Sungai Sarawak Kiri.

Next section of this thesis will include Materials and Methods, Results and Discussion and Conclusion.

2.0 LITERATURE REVIEW

2.1 Background of Cyanobacteria

Cyanobacteria occurs in almost all living conditions whether within the lithosphere, hydrosphere (Bold *et al.*, 1987; Hoek *et al.*, 1995) or atmosphere (Bold *et al.*, 1987) thus making it ubiquitous. There are roughly 150 genera (Bold *et al.*, 1987; Hoek *et al.*, 1995) with roughly 1500 species of cyanobacteria which has been recognized and named (Bold *et al.*, 1987). Even so, there has been speculation that some of these organisms have been inadequately described thus causing the taxonomic identification of cyanobacteria problematic and unreliable (Bold *et al.*, 1987; Rasmussen & Johansson, 2002; Schönhuber *et al.*, 1999).

For the lithosphere, cyanobacteria can usually be seen to form extensive strata on moist, shaded, bare soil and also may appear as gelatinous incrustation on moist rocks, other inanimate objects or plants (Bold *et al.*, 1987). Aquatic species on the other hand inhibit both the marine environment and fresh water environment. However, most cyanobacteria are found in freshwater environment (Bold *et al.*, 1987; Hoek *et al.*, 1995).

Basically there are two types of groups in the aquatic environment, where the cyanobacteria are either attached to submerged objects or are planktonic (Bold *et al.*, 1987). The planktonic groups are usually found in still or slow flowing freshwater (Hoek *et al.*, 1995). As cyanobacteria represent a major portion of the plankton community, it thus serves as important food source for more complex aquatic animals (Bold *et al.*, 1987).

Cyanobacteria are also notoriously known as toxin producers as well as cause unpleasant tastes and odours in drinking water (Lanciotti *et al.*, 2003). A number of these toxin producers form toxic blooms which directly threatens water quality (Baker *et al.*, 2000). There are at least 19 genera comprising of 41 species which has already been identified to have toxic properties (Ressom *et al.*, 1994).

2.2 Distribution of Cyanobacteria

Different strains of cyanobacteria are widely varied from one another. Within water blooms, cyanobacteria strains such as *Mycrocystis* and *Anabaena* appear to be frequently dominant (Bold *et al.*, 1987). As for symbiotic relations with hosts, the *Nostoc* can be found within plant bodies of liverworts while *Anabaena* can be found within water ferns (*Azolla*) (Bold *et al.*, 1987; Rasmussen & Johansson, 2002). Cyanobacteria can also act as epiphytes on algae or other aquatic plants and serve as components of lichens (Bold *et al.*, 1987; Rasmussen & Johansson, 2002).

Although commonly found within certain species of hosts such as water ferns, bryophytes and lichens, cyanobacteria has great adaptive flexibility and thus have low host specificity (Rasmussen & Johansson, 2002) (Appendix 1).

Besides being found in numerous hosts, there is also the existence of autotrophic species of cyanobacteria (Bold *et al.*, 1987). These organisms are able to grow and reproduce in culture media containing only inorganic compounds and sufficient light (Bold *et al.*, 1987). As for strains such as *Nostoc commune* and *Nostoc muscorum*, growth is in heterotrophic state (Bold *et al.*, 1987) with the ability to grow in the absence of combined nitrogen and use atmospheric nitrogen as replacement in their metabolism (Bold *et al.*, 1987; Hoek *et al.*, 1995). This ability is among the well known characteristics of cyanobacteria which is known as nitrogen fixation (Bold *et al.*, 1987; Hoek *et al.*, 1995).

2.3 Morphological characteristics of Cyanobacteria

In the Cyanophyta, there are generally three types of cellular organization; unicellular, colonial and filamentous (Bold *et al.*, 1987; Mur *et al.*, 1999). Simple parenchymatous organization do exist within cyanobacteria groups but flagella never occur at any stage in the life cycle (Hoek *et al.*, 1995). All three main organization are interconnected to one another.

2.3.1 Unicellular organization

For unicellular species, organisms are single cell, either solitary or attached and occur singly when daughter cells separate after reproduction by binary fission (Bold *et al.*, 1987; Mur *et al.*, 1999). Cyanobacteria that are usually found in unicellular form are the *Chroococcus* (Figure 2 – 1), *Gloeocapsa* (Figure 2 – 3) and *Chamaesiphon* (Figure 2 – 2) (Bold *et al.*, 1987). *Chroococcus* and *Gloeocapsa* when sampled are difficult to find in actual isolated single cells due to the abundance of cell division (Bold *et al.*, 1987). *Chamaesiphon* are epiphytic organism, frequently found on surfaces of algae or aquatic flowering plants and are attached to their hosts by holdfasts (Bold *et al.*, 1987).

Figure 2 – 1: *Chroococcus sp.*
(adapted from Treffkorn, 1999)

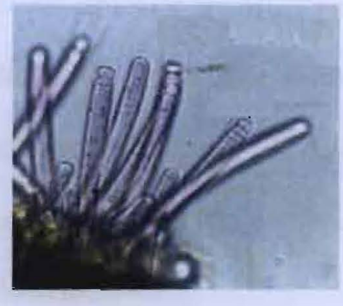

Figure 2 – 2: *Chamaesiphon sp.*
(adapted from OU Image Index, 2002)

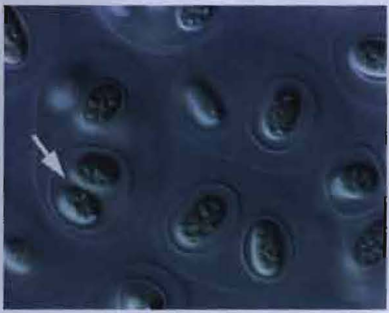

Figure 2 – 3: *Gloeocapsa sp.* (adapted from Conneticut College, 1998)

2.3.2 Colonial organization

Colonial grouping can be subdivided into two types which are the incipient colonial form and the permanent colonial form (Bold *et al.*, 1987). Incipient colonial grouping occurs from temporary coherence of several generations of recently divided cells and is usually of the unicellular cyanobacteria. Therefore principal mode of replication is by a series of successive binary fissions converting a single mother cell into many minute daughter cells (Mur *et al.*, 1999). Permanent colonial grouping are made of a certain number of cells that grow within a common sheath (Bold *et al.*, 1987).

Two groups that illustrate permanent colonial structure are *Microcystis* (Figure 2 – 4) and *Merismopedia* (Figure 2 – 5) (Bold *et al.*, 1987). The main difference between these two groups is the shape of the colony (Bold *et al.*, 1987). *Microcystis* has dense cellular colonies in spherical or irregular shape while *Merismopedia*'s colony is flattened or a slightly curved plate (Bold *et al.*, 1987).

Figure 2 – 4: *Microcystis sp.*
(adapted from PlantBio, 2004)

Figure 2 – 5: *Merismopedia sp.*
(adapted from Egmond, 2005)

2.3.3 Filamentous organisms

Filamentous cyanobacteria morphology is the result of repeated cell divisions occurring in a single plane at right angles to the main axis of the filament and come in three forms which are unbranched filaments, branched or falsely branched (Bold *et al.*, 1987; Mur *et al.*, 1999). Groups that are associated with this form are: -

- **Unbranched:** *Oscillatoria, Lngbya, Nostoc* (Figure 2 – 6) and *Anabaena* (Figure 2 – 7)
- **Falsely branched:** *Scytonema*, *Rivularia* and *Gloeotrichia*
- **Truly branched:** *Calothrix*, and *Hapalosiphon*

The filamentous cyanobacteria can also be divided into two based on specialized cells. The two groups are filamentous cyanobacteria without specialized cells and filamentous cyanobacteria with specialized cells.

2.3.3.1 Filamentous Cynobacteria without specialized cells

Filamentous cynobacteria basically have disc-shaped cells and are arranged in a uniseriately (Sze, 1993). Variation between strains are determined by looking at the sheath and difference of filaments; whether straight or spiraled (Sze, 1993).

Figure 2 – 6: *Nostoc sp.* (adapted from Taton *et al.*, 2003)

2.3.3.2 Filamentous Cyanobacteria with specialized cells

The specialized cells that differentiate both filamentous groups are heterocysts and the akinetes (Sze, 1993). Heterocysts are cells that are formed by cyanobacteria in which nitrogen fixation occur and is translucent with a thick wall (Sze, 1993; Bold & Wynne, 1985). Akinetes on the other hand are cells that resistant, non-motile and are formed by the transformation of a vegetative cell into a thick-walled dormant cell (Sze, 1993; Bold & Wynne, 1985).

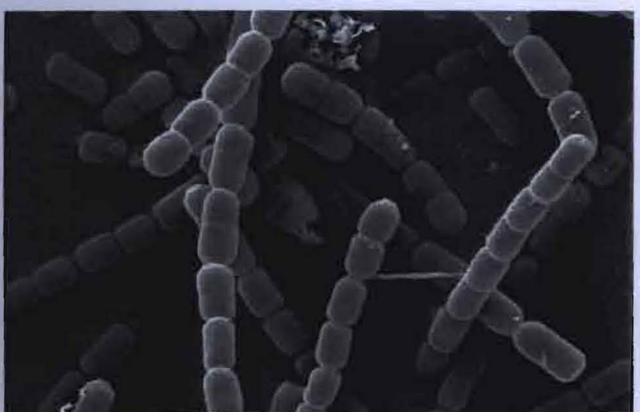

Figure 2 – 7: *Anabaena sp.* (adapted from Mount Allison University, 2005)

2.4 Morphological Identification of cyanobacteria

There is no system that can offer proper identification of cyanobacteria through its morphological features (Anagnostidis and Komarek, 1985). For a comprehensive classification of cyanobacteria, it would take a combination of discipline such as biology, biochemistry, cytology and even understanding ecological demands (Anagnostidis and Komarek, 1985).

Based on *Microcystis* study carried out by Welker *et al.* (2004), cyanobacteria are morphologically distinguished using characteristics such as cell diameter, border and consistency of the mucilage as well as number of cell division planes.

Another study conducted by Thacker and Paul (2004) also mentions that traditional taxonomy of cyanobacteria is largely based on morphological measurements among which are cell length and cell width.

Colour of cyanobacteria on the other hand, is insufficient to distinguish between organisms of the algae group or the cyano-group due to varied genera and species of cyanobacteria (Bold *et al.*, 1987). Generally, cyanobacteria cells are usually blue-green to violet but when in a large mass, the colour emitted by the group may be blue-green, black, dark purple, brown or even red (Bold *et al.*, 1987; Hoek *et al.*, 1995).

2.5 Classification of Cyanobacteria

The following is one of the many taxonomy for morphological classification for the cyanobacteria which was documented by Bold *et al.* (1987); Later documentation of cyanobacteria taxonomy focuses on genetic, ribosomic and protein classification (Welker *et al.*, 2004; Taton *et al.*, 2003; Schönhuber *et al.*, 1999).

Division: Cyanophyta

Class: Cyanophyceae

Order: Chroococcales

Family: Chroococcaceae

Genera: *Chroococcus, Gloeocapsa, Microcystis, Merismopedia*

Order: Chamaesiphonales

Family: Chamaesiphonaceae

Genus: *Chamaesiphon*

Order: Oscillatoriales

Suborder: Oscillatorineae

Family: Oscillatoriaceae

Genera: *Oscillatoria, Lyngbya*

Suborder: Nostochineae

Family: Nostocaceae

Genera: *Anabaena, Nostoc*

Family: Scytonemataceae

Genera: *Scytonema*

Family: Stigonemataceae

Genus: *Hapalosiphon*

Family: Rivulariaceae

Genera: *Rivularia, Gloeotricia, Calothrix*

2.6 Previous Studies

There are three previous studies of cyanobacteria that has been done in Malaysia. Two of the studies are done in Sarawak while one is done in Johor.

Abang (2003) has carried out a project related to cyanobacteria diversity in Kuching area. Samples were collected in 15 areas among which were aquaculture pond, river, estuary and seawater. From this study, 11 genera of Cyanobacteria were identified of which 4 are potentially cyanobacterial toxin producers. The 11 genera identified were *Anabaena sp.*, *Aphanizmenon sp.*, *Aphanocapsa sp.*, *Borzia sp.*, *Chroococcus sp.*, *Merismopedia sp.*, *Microcystis sp.*, *Oscillatoria sp.*, *Planktolyngbya sp.*, and *Spirulina sp.*.

Ramlah (2004) has documented the diversity of cyanobacteria in four locations around the Kuching district. The four location for study were the; Institute of Research Center and Fish Production (Tarat), shrimp aquaculture pond in Lembaga Kemahuan Ikan Malaysia (LKIM), Tasik Semada (Balai Ringin), and Tasik Biru (Bau). In comparison to the previous study done by Abang (2003), this study identified only 9 genera of cyanobacteria which were *Anabaena sp.*, *Anacystis sp.*, *Calothrix sp.*, *Chamaesiphonales sp.*, *Lyngbya sp.*, *Microcystis sp.*, *Oscillatoria sp.*, *and Spirulina sp.*. From the 9 genera identified, 3 were potentially toxin produers.

Phang and Leong (1987) documented a study on freshwater algae in the Ulu Endau area, located in the state of Johore. 16 sites with 22 collection localities were used for the study of which all were main rivers or tributaries of the main river in that area. From the samples collected, there were 12 genera of Cyanophyta identified which belonged to 5 families. The families of Cyanophyta that were identified were; Nostocaceae, Osillatoriaceae, Rivulariaceae, Stigonemataceae and Scytonemataceae.