DIVERSITY OF CYANOBACTERIA IN SUNGAI SEMADANG, KUCHING SARAWAK

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DIVERSITY OF CYANOBACTERIA IN SUNGAI SEMADANG, KUCHING SARAWAK

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<table>
<thead>
<tr>
<th>TABLE OF CONTENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENT</td>
</tr>
<tr>
<td>TABLE OF CONTENT</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
</tr>
<tr>
<td>ABSTRACT / ABSTRAK</td>
</tr>
<tr>
<td>CHAPTER 1: INTRODUCTION</td>
</tr>
<tr>
<td>1.1 Sungai Semadang, Kuching, Sarawak</td>
</tr>
<tr>
<td>1.2 Justification / Rationale</td>
</tr>
<tr>
<td>1.3 Hypothesis</td>
</tr>
<tr>
<td>1.4 Objectives</td>
</tr>
<tr>
<td>CHAPTER 2: LITERATURE REVIEW</td>
</tr>
<tr>
<td>2.1 Classification of cyanobacteria</td>
</tr>
<tr>
<td>2.2 Morphology of cyanobacteria</td>
</tr>
<tr>
<td>2.3 Symbiotic cyanobacteria</td>
</tr>
<tr>
<td>2.4 The importance of cyanobacteria</td>
</tr>
<tr>
<td>2.5 Factors that influence the occurrence of cyanobacteria</td>
</tr>
<tr>
<td>CHAPTER 3: MATERIALS AND METHODS</td>
</tr>
<tr>
<td>3.1 Sampling trip</td>
</tr>
<tr>
<td>3.2 Sampling sites</td>
</tr>
<tr>
<td>3.3 Field work</td>
</tr>
<tr>
<td>3.4 Laboratory work</td>
</tr>
<tr>
<td>3.5 Data analysis</td>
</tr>
<tr>
<td>CHAPTER 4: RESULTS AND DISCUSSIONS</td>
</tr>
<tr>
<td>4.1 Species composition of cyanobacteria</td>
</tr>
<tr>
<td>4.2 Potential cyanobacteria toxin-producers for both seasons</td>
</tr>
</tbody>
</table>
4.3 Comparison for genus of cyanobacteria between dry and wet seasons 33
4.4 Water quality features of Sungai Semadang for dry and wet seasons 35
4.5 Correlation for genus composition of cyanobacteria with water quality parameters 37
4.6 The Sorensen's Quotient of Similarity (S) for dry and wet seasons 39

CHAPTER 5: CONCLUSION AND RECOMMENDATION 41

5.1 Conclusion 41
5.2 Limitation of study 41
5.3 Recommendation for future study 42

REFERENCES 43

APPENDIX
LIST OF TABLES

Table 1. List of cyanobacteria species during dry season with two different sampling techniques (PN and VDWS) and specimen preservation methods (FS-PN and PS-PN)
Table 2. List of cyanobacteria species during wet season with two different sampling techniques (PN and VDWS) and specimen preservation methods (FS-PN and PS-PN)
Table 3. List of cyanobacteria that are Potential Toxin Producers for all four stations
Table 4. Water quality characteristics of all four stations during dry season sampling
Table 5. Water quality characteristics of all four stations during wet season sampling
Table 6. Results on analyses of Pearson correlation for genus cyanobacteria with water quality parameters for both seasons.
Table 7. Sørensen’s Quotient of Similarity (%) for four stations at Sungai Semadang during dry season
Table 8. Sørensen’s Quotient of Similarity (%) for four stations at Sungai Semadang during wet season

LIST OF FIGURES

Figure 1: Map showing Sungai Semadang and its tributaries.
Figure 2: A sketch showing Sarawak, Sungai Semadang and the tributaries
Figure 3: Filamentous type: (a) Oscillatoria, and (b) Lyngbya
Figure 4: Nonfilament type: (a) Gloeocapsa, and (b) Merismopedia
Figure 5: Specialized cells in cyanobacteria: Genus Anabaena (a) akenetes and (b) heterocysts
Figure 6: A sketch showing sampling stations in Sungai Semadang
Figure 7: Genus composition of cyanobacteria for dry and wet seasons
Figure 8: Chroococcus spp.
Figure 9: Borzia spp.
Figure 10: Oscillatoria spp.
Figure 11: Lyngbya spp.
Figure 12: Pseudanabaena spp.
Figure 13: Komvophoron spp.
Figure 14: Katagynmena sp.
Figure 15: Merismopedia sp.
Figure 16: Microcystis sp.
Figure 17: Planktothrix sp.
Figure 18: Spirulina spp.
Figure 19: Heterohormogonia spp.
Figure 20: Scytonea archangelii
Figure 21: Anabaena spp.
Figure 22: Cylindropermum sp.
Figure 23: Trichormus sp.
Figure 24: Capsosira spp.
Figure 25: Stigonema sp.
Figure 26: Chloroflexus sp.
Figure 27: Synechococcus sp.
Figure 28: Synechocystis sp.
Figure 29: Raphidiopsis sp.
Figure 30: Stanieria sp.
Figure 31: Tychonema sp.
Figure 32: Isocystis sp.
Figure 33: Noctochopsis sp.
Figure 34: Tolypothrix sp.
Figure 35: Pseudophornidium sp.
Figure 36: Cylindraspermopsis sp.
Figure 37: Trichodesmium sp.
Diversity of Cyanobacteria in Sungai Semadang, Kuching Sarawak

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ABSTRACT

Blue-green algae or known as cyanobacteria is one of the microscopic algae that have wide distribution of life. A study about diversity of cyanobacteria was carried out during dry and rainy seasons to provide baseline data at the four stations started from the upstream to the downstream of Sungai Semadang. A total of 63 genera of cyanobacteria were determined for both seasons with 37 genera during dry season and 49 genera during wet season. Station 2 indicates the highest number of genera with 42 genera for dry season while Station 1 with 35 genera for wet season. Among the total genera found, there were 16 genera of potential toxin-producers namely Anabaena, Coelosphaerium, Cylindrospermopsis, Cylindrospermum, Gloeotrichia, Hapalosiphon, Lyngbya, Nodularia, Nostoc, Microcystis, Oscillatoria, Pseudanabaena, Scytonema, Synechococcus, Tolypothrix, and Trichodesmium. Correlation between genus compositions of cyanobacteria with water quality parameters had shown linear relationship with different degree of association.

Key words: Cyanobacteria, potential toxin-producer, genus composition, correlation, water quality parameters

ABSTRAK


Kata kunci: Alga bira-hijau, berpotensi menghasilkan toksin, komposisi genus, kolesasi, parameter kualiti air
CHAPTER 1: INTRODUCTION

Cyanobacteria (blue-green algae) evolved between 2.5 and 3.4 billion years ago (Campbell, 1996). They are known as blue-green algae because they are capable of making their own food through photosynthesis as their cells contain important pigment, the chlorophyll a.

Cyanobacteria occur in all aquatic environments, namely freshwater, estuaries and marine. They are cosmopolitan with most of the species exist in the water bodies all over the world (Lee, 1989). Generally, cyanobacteria seem to be more abundant in neutral or slightly alkaline habitats, although some example Chroococcus are said to occur in bog waters at pH 4 (Bold and Wynne, 1985). As for the freshwater cyanobacteria, they usually occur as phytoplankton or as attached algae in standing or running water (Lee, 1989). They are both planktonic and benthic (Bold and Wynne, 1985) which means that they can be found either at the water column as free floating organisms or attached at the sediments, rocks and other aquatic organisms.

The presence of cyanobacteria is also influenced by water level of the aquatic environment. During favourable conditions with high nutrient levels and warm water temperatures, cyanobacteria reproduce rapidly resulting in significant “blooms” throughout the water column (Buchanan and Zarrawell, 2005). This phenomenon commonly occurs in stagnant water or lentic zone such as in eutrophic lakes. Warm water combined with high excessive nutrients supplies especially nitrate and phosphorus will accelerated the growth of cyanobacteria.
1.1 Sungai Semadang, Kuching, Sarawak

Sungai Semadang is located at Padawan District in Kuching Division. It is a small tributary for Sungai Sarawak Kiri (Figure 1). Sungai Semadang is located at longitude N 1° 09' and latitude E 110° 16'. The water resources are from Gunung Mrobung and Gunung Ridong. There are small settlement areas for example Kampung Annah Rais which formerly known as Kampung (Annah) Negri, Kampung Padawan and Kampung Pangkalan Ampat along Sungai Semadang.

Figure 1: Map showing Sungai Semadang and its tributaries.
Figure 2: A sketch showing Sarawak, Sungai Semadang and the tributaries (Not to scale).
1.2 Justification / Rationale

Sarawak covers 124,450 square kilometers of land areas and it comprises a large segment of the western side of Borneo (Cubit and Payne, 1990). Sarawak Rivers drain all their waters into the South China Sea (Cubit and Payne, 1990). As the rivers running from the upstream to the downstream and finally to the coastal waters and seas, they discharge all their content where the influx of fresh water with its organic and inorganic content will affect other aquatic life in the marine ecosystem.

Diversity of cyanobacteria have been documented in several rivers including: (i) Sungai Sarawak and Sungai Pengkalalan Kuap, Kuching Sarawak by Abang (2002) and by Phang and Leong (1987) at rivers and tributaries in Ulu Endau Area, Johore (which cover Lubuk Udang, Sungai Jasir, Sungai Anak Jasir, Sungai Sempahong, Sungai Marong, Sungai Bong, Sungai Zain, Sungai Jasinet, Sungai Jasir Waterfalls, Sungai Tapir, Sungai Bunut, Sungai Pelawar, Sungai Pantai Burung, Janing Barat, Stream on South Plateau and on bank along road from Lubuk Udang to Sungai Anak Jasir).

However, there is no data for Sungai Semadang, Kuching Sarawak. This study is designed to document diversity of cyanobacteria that exist in Sungai Semadang including those which are potential toxin-producer. The information will be useful in awareness campaign dedicated to the community living along Sungai Semadang (who is dependent on the river for their water resource and food).
1.3 Hypothesis

1. Cyanobacteria exist in Sungai Semadang including some genera which are potential toxin-producer.

2. There will be linear relationships between water quality of Sungai Semadang and cyanobacteria composition.

1.4 Objectives

1. To identify and document the diversity of cyanobacteria in Sungai Semadang which included list of cyanobacteria that are potential toxin-producer for dry and wet seasons.

2. To study on the relationship between water quality parameters (pH, temperature (°C), dissolve oxygen (DO), biological oxygen demand (BOD₅), chemical oxygen demand (COD), total suspended solids (TSS), and three nutrient concentration of ammoniacal-nitrogen (NH₄-N), nitrate-nitrogen and orthophosphate with genus composition of cyanobacteria.

3. To calculate the Sorensen’s Quotient of Similarity (S) for diversity of cyanobacteria in both seasons.

4. To record the water quality of Sungai Semadang for both seasons.
CHAPTER 2: LITERATURE REVIEW

Cyanobacteria display a remarkable diversity of forms and they differ in morphology, structure and function, and in their mode of response to the environmental stimuli (Skulberg et al., 1993).

2.1 Classification of cyanobacteria

Cyanobacteria belong to the Kingdom Monera and contain just one class called Cyanophyceae (Hoek et al., 1995). About 150 genera and 2000 species of Cyanobacteria had been documented and identified (Fott, 1971). According to Anagnostidis and Komarek (1988; 1989; 1991), the classifications of these prokaryotic algae are consisting of four orders. See details of cyanobacteria classification in Appendix 1.

The four orders are Order Chroococcales consist of seven families, Order Oscillatoriales consist of six families, Order Nostocales consist of four families, and Order Syngonematales consist of eight families. Summaries based on the classification, the cyanophycean has total of 193 genera had been documented and identified.
2.2 **Morphology of cyanobacteria**

The simplest morphology of the cyanobacteria is unicell, free living or enclosed within a mucilaginous envelope (Lee, 1989). The cell walls of the Cyanophyceae are composed of a number of layers but only the inner two layers are the same (Lee, 1989). The major component of the cell wall is peptidoglycan and is similar to the gram-negative bacteria (Sze, 1998).

Cyanobacteria can be divided into two types namely (i) non-filamentous, and (ii) filamentous. For filamentous cyanobacteria, the presence and absence of specialized cells called heterocysts and akinetes further subdivides the species (Sze, 1998). Among the four orders of cyanobacteria, Order Chroococcales is nonfilamentous while Order Oscillatoriales, Order Nostocales, and Order Stigonematales are filamentous.

General definitions of the four orders are defined as the following:

(i) Order Chroococcales – have solitary cells, colonies, pseudoparenchymatic colonies, pseudofilaments, without trichomes, heterocysts and akinetes lacking and obligatory simple cell division (mainly fission resulting in non-motile monocytes or motile planocytes) (Anagnostidis and Komarek, 1986).

(ii) Order Oscillatoriales – have typical trichomes, motile or immotile; cross division of cells; sheaths, false branching and gas vesicles lacking or facultatively present, without true branching; heterocysts and akinetes lacking; reproduction by homaogonia and hormocytes and rarely by planococci (Anagnostidis and Komarek, 1988).
(iii) Order Nostocales – they are filamentous cyanophytes with true trichomes; trichomes isopolar or heteropolar, with false branching or unbranched; lack of true branching; presents of heterocysts and/or akinetes, akinetes facultatively; cells divide always perpendicularly to the trichome axis, only in one direction in succeeding generations; planocytes lacking; monocysts known in one genus (*Coleodesmium)*; and reproduction mainly by hormogonia or hormocytes (*Anagnostidis and Komarek, 1989*).

(iv) Order Stigonematales – they are filamentous cyanophytes with true trichomes, sometimes combined with pseudofilaments; true branching always presents which at least in a part of a life cycle, false branching facultatively; thallus heteropolar or isopolar; heterocytes occur facultatively in several genera, akinetes rarely; cells divide perpendicularly to the trichome axis and/or irregularly in more directions; reproduction by hormogonia hormocytes, hormocysts, akinetes, nanocytes, planocytes and monocysts (*Anagnostidis and Komarek, 1991*).

Below are some figures of cyanobacteria taken from Sze (1998).

(a) ![Image](image1.png)

(b) ![Image](image2.png)

**Figure 3**: Filamentous type: (a) *Oscillatoria*, and (b) *Lyngbya*
2.3 Symbiotic cyanobacteria

Some cyanobacteria live as symbionts with or even within other plants for example, in many lichens which is in the roots of Cycas and in the unicellular fungus of Geosiphon, Nostoc involved as symbiont while Anabaena is symbiont in the leaf of the water fern Azolla (Hock et al., 1995).
2.4 The importance of cyanobacteria

There are two important contributions of cyanobacteria in biological communities namely: (i) they are photosynthetic producers of organic material and oxygen, and (ii) they are capable to fix nitrogen from the atmosphere. Specialized cell known as heterocysts play a role to separate the reactions of nitrogen fixation from exposure to oxygen when oxygen is present (Sze, 1998). During the absence of oxygen, some of the nonheterocystous cyanobacteria show nitrogenase activity in ordinary vegetative cells (Sze, 1998). This nitrogen-fixing capacity of cyanobacteria has been made use in the cultivation of rice where their growth in rice paddies is encouraged (Bold and Wynne, 1985).

Three kinds of cyanobacteria that are able to fix nitrogen are: (i) filamentous heterocystous species (Tiwari, 1977); (ii) certain unicellular (nonheterocystous) species (Wyatt and Silvey, 1969; Rippka et al., 1971); and (iii) certain nonheterocystous filamentous species (e.g. *Plectonema boryanum*) (Stewart and Lex, 1970), although, only under microaerophilic conditions (Bold and Wynne, 1985).

Some cyanobacteria species also served as human and animal food such as the Chinese consider *Nostoc commune* as a delicacy whereas *Aphanathece sacrum, Nostoc verrucosum, Nostoc commune,* and *Brachytrichia* as side dishes in Japan (Watanabe, 1970). The filamentous cyanobacterium *Spirulina* is common in lakes with high soda content and high pH are abundance in some lakes of the African Rift Ralley makes it a major food foie large flamingo populations
(Sze, 1998). Besides, native peoples near Lake Chad in Africa and Lake Texcoco in Mexico have used *Spirulina* as food because *Spirulina* has high protein content which is up to 70 percent of its dry weight, vitamins and trace metals (Sze, 1998).

Some planktonic cyanobacteria produce toxins (Gorham and Carmicheal, 1988; Skulberg *et al.*, 1993; Carmicheal, 1994). (Appendix 2). In freshwater, they include bloom-producing species of *Anabaena*, *Aphanizomenon*, *Microcystis*, *Oscillatoria* and *Nodularia* (Sze, 1998). The toxins (neurotoxins that affect neurons and hepatotoxins that affect liver cells) released when the animal ingest the algal cells and they can be fatal to mammals, birds, and fishes (Sze, 1998). Low doses of cyanobacteria toxins usually cause intestinal and liver dysfunction in animals, while higher doses may result in severe gastrointestinal illness, liver damage and possibly death (Buchanan and Zurawell, 2005). According to Moikena and Cha (1971), blooms of *Lynghye mascula* at coast of Hawaii had caused severe dermatitis on the skin of swimmers. The symptoms included inflammation and swelling of the mucous membranes of the eyes and nose, redness and pus on the skin.

In Sarawak, two studies have been carried out related to diversity of cyanobacteria namely: (i) Abang (2002) has carried out survey and identified eleven genera - *Anabaena*, *Aphanizomenon*, *Aphanocapsa*, *Aphanatheca*, *Borzia*, *Chroococccus*, *Merismopedia*, *Microcystis*, *Oscillatoria*, *Planktolyngbya* and *Spirulina* and, (ii) Ramlah (2005) has identified nine genera in the lentic zones - *Anabaena*, *Anacystis*, *Calothrix*, *Chamaesiphonales*, *Gleoeotrichia*, *Lynghya*, *Microcystis*, *Oscillatoria*, and *Spirulina*. Out of twenty genera identified by Abang (2002) and
Ramlah (2005), there are seven potential toxin-producer species namely *Anabaena*, *Aphanizomenon*, *Gloeotrichia*, *Lyngbya*, *Microcystis*, *Oscillatoria* and *Spirulina* (Aishah, 1996).

2.5 *Factors that influence the occurrence of cyanobacteria*

Phosphorus (P) is an essential nutrient for growth and development of algae and other aquatic plants but it can cause water pollution if high concentration (25 to 100 µg total P L⁻¹, eutrophic condition) present in the water (Eghball and Pote, 2003). Although phosphorus is not needed for growth in such large amounts as carbon, oxygen, nitrogen and hydrogen, but it is most common growth limiting element in freshwater (Horne and Goldman, 1994). The most interest form is soluble phosphate (PO₄) because it can be used directly by algae including cyanobacteria. PO₄ can be measured using spectrophotometer resulting blue color PO₄ with acid molybdate solution (Horne and Goldman, 1994).

Nitrogen (N) is important and most abundant element in living cell after carbon, hydrogen and oxygen. In aquatic ecosystem, nitrogen present as gas N₂ and play role as limiting factor because they are not always present in adequate form in natural water. Cyanobacteria as living organism react with the content of nitrogen where some of them forming heterocysts to fix the nitrogen when oxygen presents. For organic nitrogen, it could be estimated using the acid Kjeldahl digestion procedure or with an automatic analyzer (which simultaneously analyzed carbon and hydrogen) (Horne and Goldman, 1994). Organic nitrogen produce by wastewater in two forms namely: (i) ammonia (NH₃) which is toxic to aquatic life and, (ii) ammonium (NH₄⁺) which is
non toxic substance. Ammonia content has positive correlation with temperature and pH (NREB, 2004).

Maximum growth rates are attained by most cyanobacteria at temperature above 25°C (Robarts and Zohary, 1987). These optimum temperatures are higher than for green algae and diatoms and that is the reason in temperate and boreal water bodies most cyanobacteria bloom during summer (Mar et al., 1999).

Cyanobacteria bloom phenomenon commonly occurs in stagnant water with an influx of point source such as at the upper column of lakes and shoreline area. It can also happen in water bodies which the water are slow moving or not flowing where excess nutrients accumulate and consequently provide the favourable condition for cyanobacteria growth.
CHAPTER 3: MATERIALS AND METHODS

This section consists of five parts which will explain about sampling trip, location of the sampling sites, what have been done during field and laboratory work, and how the data is being analysed.

3.1 Sampling trip

Two sampling trips were conducted namely: (i) the dry season on September 7th and 8th 2005, and (ii) wet season on February, 6th 2006. Four sites were sampled as stations along Sungai Semadang started from the upstream until the end of the downstream river (Figure 6). Global Positioning System (GPS) device was used to record the location for each station. Based on observation during wet season, the water level was slightly increased compared to the dry season conditions.

3.2 Sampling sites

Two days field trips were carried out for the first sampling. The results obtained during these field trips represented data for dry season. For the second sampling which was during wet season, one day field trip had been carried out. There were four sampling stations started from the upstream of Sungai Semadang until the downstream of the river that flow to Sungai Abang, Padawan (Figure 6).
3.2.1 Station 1

Station 1 was located at N01° 07' 19.4", E110° 16' 11.4" which was at the upstream of Sungai Semadang with rocky, sandy at the river bank, fast flowing and clear water. Samples were taken at area that has exposed to direct sunlight with large rock to prevent strong current water flow. During the first and second sampling, the weather was a bit rainy. Based on observation, there were not much physical differences at the station for both sampling except the water look a bit clearer than previous sampling.

3.2.2 Station 2

Station 2 was located at N01° 08' 18.6", E110° 15' 44.7" which was situated at the meeting point of two small rivers combined to form another river. The water was clear, shallow with fast moving, strong current, and small rocks and sandy river bank. Samples were taken at areas that had directly exposed to the sunlight and slow flowing water at the subsurface. The weather was warmer and sunny for both sampling period.

3.2.3 Station 3

Station 3 was located at N01° 11' 10.7", E110° 14' 52.4" under the Jambatan Apong near the Kampung Penigkalan Amput. The weather was sunny for both sampling. The river was wider than the previous two stations with fast flowing, shallow and clear water during the first sampling. The river bank was covered with sand and there were rocks at the river. Samples were taken at locations with direct exposure of sunlight and slow flowing water. During the second
sampling, the water level was increase and it was turning into milky coloration due to bridge construction at the upstream of the river near to the Borneo Heights Island, Padawan.

3.2.4 Station 4

Station 4 was located at N01° 14' 41.5", E110° 14' 59.7". The river was wider and the water looked turbid compared to the other stations for both sampling. It was fast flowing and had muddy river bank. This station was located at the end of Sungai Semadang which was the downstream that flows to Sungai Abang (which is another tributary of Sungai Sarawak Kiri). Samples were obtained at the river bank where there was slow moving water and accessible.

In general, the sampling sites are categorized as riverine forests with some bamboo trees found near to the river bank and they are located in the secondary forest.