CHLOROPHYLL *A* CONCENTRATION OF FRESH WATER PHYTOPLANKTON ANALYSED BY ALGORITHMIC BASED SPECTROSCOPY

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by

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LIST OF SYMBOLS AND ABBREVIATIONS

BBM	Bold's Basal Medium
Chl *a*	Chlorophyll *a*
NIR	Near infrared
VIS	Visible
R	Reflectance spectrum
Rs	Reflectance of water surface
Rrs	Reflectance for remote sensing
R_L	Light reflectance
R	Coefficient
C_a	Concentration of chlorophyll *a*
µg/L	microgram per litres
mg/L	milligram per litres
λ	Wavelength

KEPEKATAN KLOROFIL *A* FITOPLANKTON AIR TAWAR DIANALISIS DENGAN SPEKTROSKOPI BERASASKAN ALGORITMA

ABSTRAK

Fitoplankton ialah tumbuhan microskopik sel tunggal yang memainkan peranan penting di dalam ekosistem sebagai pengeluar primari utama melalui aktiviti fotosintesis. Objektif utama kajian ini ialah untuk mengkaji pencirian melalui hubungan pantulan dan kepekatan klorofil *a* fitoplankton di dalam air tawar. Melalui, kajian ini, pantulan fitoplankton diambil dengan menggunakan spektroradiometer untuk melihat hubungan antara pantulan dan panjang gelombang fitoplankton di dalam air tawar. Satu lokasi tasik dalam Universiti Sains Malaysia yang dikenali sebagai Tasik Harapan dipilih untuk kajian ini. Sebanyak 20 liter sampel air diambil dan ditapis dengan menggunakan jaring fitoplankton. Sampel air yang diambil dianalisis di dalam makmal untuk menentukan pantulan dan kandungan kepekatan klorofil-a fitoplankton. Dua jenis spektrometer digunakan di dalam kajian ini pertama, spektroradiometer digunakan untuk mengukur pantulan fitoplankton dan kedua, spektrofotometer digunakan untuk mengukur kepekatan klorofi *a*. Sampel air yang diambil dikultur terlebih dahulu dengan memasukkan dalam medium yang dikenali sebagai Bold's Basal sebelum nilai pantulan diambil agar pengukuran pantulan tepat ke atas fitoplankton. Algoritma-algoritma yang menghubungkan kepekatan klorofil *a* dengan pantulan fitoplankton digunakan. Keputusan yang baik diperolehi di dalam kajian ini yang dibuktikan oleh nilai R^2 yang baik di dalam

analisis keputusan melalui algoritma yang dibangunkan. Tiga kawasan jalur yang difokuskan di dalam kajian ini ialah jalur merah, jalur hijau dan jalur biru. Jalur-jalur ini sesuai digunakan untuk menganalisis fitoplankton. Nilai pantulan bagi setiap jalur ditentukan merujuk kepada kepekatan klorofil *a* untuk kalibrasi algoritma. Selain itu, pelbagai nilai panjang gelombang diuji dan nilai R^2 dibandingkan. Akhirnya, tiga panjang gelombang dipilih iaitu 438 nm, 550 nm dan 675 nm. Pemilihan tiga jarak gelombang ini adalah kerana gelombang-gelombang sepadan dengan fitoplankton dan klorofil *a*. Lagipun nilai R^2 yang diperolehi juga sesuai dengan menggunakan algoritma yang dibangunkan di dalam kajian ini. Pengesahan penggunaaan algoritma juga dilakukan di tasik yang sama tetapi tarikh pengambilan sampel air tidak sama. Keputusan pengesahan ini memberi keputusan yang sangat baik dengan nilai R^2 yang tinggi. Hasil kajian daripada keputusan tasik ini, menunjukkan algoritma yang dibangunkan untuk mengukur kepekatan klorofil *a* fitoplankton berjaya. Selain itu, beberapa cadangan juga diberikan untuk membaiki kajian ini pada masa akan datang.

CHLOROPHYLL *A* CONCENTRATION OF FRESH WATER PHYTOPLANKTON ANALYSED BY ALGORITHMIC BASED SPECTROSCOPY

ABSTRACT

Phytoplankton are microscopic single-celled plants that play an important role in the ecosystem as a major primary producers through photosynthesis. The main objective of this study was to investigate properties through reflection and chlorophyll *a* concentrations of phytoplankton in freshwater. In this study, reflection phytoplankton were taken using spectroradiometer to observe the relationship between the reflectance and the wavelengths of phytoplankton in freshwater. The lake selected for this study is known as Tasik Harapan located in Universiti Sains Malaysia. In this study, 20 litres water samples undergone the filtering using phytoplankton net. The water samples collected were analyzed in the laboratory to determine the reflectance and chlorophyll *a* concentration of phytoplankton. Two spectrometers were used in this study, firstly, spectroradiometer which was used to measure the reflectance of phytoplankton and secondly, spectrophotometer which was used to measure the concentration of chlorophyll *a*. The water samples taken were prior to culture in the medium known as Bold's Basal Medium before the reflections were taken so that accurate reflection measurements on phytoplankton can be acquired. The algorithms that related between chlorophyll *a* concentration and reflectance of phytoplankton were used. The good results are obtained in this study

which are evidenced by the good correlation in the analysis results using the developed algorithm. Three regions were focused which were the red, green and blue. These bands have been identified to be appropriated in analyzing phytoplankton. Reflectance of each band specified referred to the concentration of chlorophyll *a* for calibration algorithm. The wavelength range was tested and the R^2 were compared. Finally, three wavelengths of 438 nm, 550 nm and 675 nm were selected. The selection of these three wavelengths were found to be strongly correlated to phytoplankton and chlorophyll *a*. The value of R^2 obtained also in accordance with the developed algorithm in this study. The water sampling for validation was also taken from the same lake but not on the same date and was analysed using the same algorithm. The validation results showed very good results with a high R^2 value was obtained. The results from this lake proved that the algorithm developed was successful in measuring the concentration of chlorophyll *a* in phytoplankton. In addition, some suggestions are given to repair this study in the future.

CHAPTER 1

INTRODUCTION

1.1 Background

Fresh water is natural occurring water on the surface of the Earth, like lake, pond and river. Fresh water ecosystem is rich with variety of organism including phytoplankton species. Tasik Harapan is one of the fresh water ecosystem that can be found in Universiti Sains Malaysia (USM) Penang, Malaysia. Fresh water has characteristics of low concentrations of soluble salts and other dissolved solids. Fresh water term does not have the same meaning with potable water. Many surface fresh water and ground water are not suitable for drinking. Besides, fresh water is a natural resource that is vital to the ecosystem life whereas good water quality is required for many ecosystems service (Meybeck & Helmer 1996). From Carpenter et al. (2011), in most parts of the world have suffered fresh water ecosystems degradation seriously. This degradation is strongly related to surrounding land use types reflecting undergoing activities (Perry & Vanderklein, 1996). Consequently, the study on aquatic communities (phytoplankton) and water quality become popular since the last decades (Stomp et al., 2011). Understanding functionality of relationship between ecological lake and watershed changes is a very important step for the effective, long-term conservation or management strategies in selection and application (Silva et al., 2011). Hence, the scientists have used biological indicator for detecting potential changes of biotic communities structure caused by spatial and temporal scale (Burns & Galbraith, 2007; Van Egeren et al., 2011).

About 70% of the Earth is constituted by water, in which phytoplankton is prevailing organism which is representing the set of autotrophic photosynthetic organisms present in plankton. Phytoplankton is one of the aquatic organisms in fresh water ecosystem and primary biotic community indicating changes in ecological (Padisák et al., 2006). Phytoplankton is also a microscopic plant that is basis of food chain for organisms in the ecosystem (Nyananyo et al 2006). Besides, it is also important as implication in the regulation of marine ecosystems, for examples ecotones between terrestrial, fresh water, and marine habitats. Other functions of phytoplankton concern the global climatic changes control by the fixing of carbon dioxide excess and regulating the biogenic emissions of sulfur on worldwide scale. Phytoplankton need to stay on the water surface to absorb sunlight for photosynthesis activity. Phytoplankton are influenced by several physical parameter such as temperature, nutrient, water current, pH, conductivity, dissolve oxygen and others (Shamsudin, 1991). These physical parameters are important in phytoplankton distribution determining. In addition, the distribution of phytoplankton are also dependent on spatial and seasonal characteristic in the area (Wong and Wong, 2004). Phytoplankton are also beneficial as ecological indicator for assessment of water quality and ecosystem health (Sagert et al 2008, Webber et al 2005).

There are several reasons why phytoplankton community can be used in assessing water quality as described below (Reynolds et al., 1993; Whitaker et al., 2003; Ekwu et al., 2006; Soininen et al., 2007):

a) The incident of specific of phytoplankton could be limited primary by abiotic conditions such as lake depth and biotic recent. Abiotic and biotic factors that influenced the abundant and distribution of phytoplankton in fresh water.

b) The biogeographical studies have showed an increase in the issue of distance decay patterns in microorganism community composition in recent evidence.

Phytoplankton species are proposed to reflect trophic conditions (Rajo et al., 2000). Trophic is means nutrition or growth. For example, in eutrophic fresh waters, the relative importance of chrysophtes to biomass or biodiversity (community structure) decrease but cynobacteria increase (Watson et al., 1997). The phytoplankton size is the most important single characteristic affecting the ecology. When the phytoplankton become bigger, their volume increase their radii while their surface area arise in proportion to only the square of radius. Figure 1.1 shows the size and types of phytoplankton, meanwhile in **Appendix A** shows the volumes, surface area and surface area per volume ratio of phytoplankton species (Reynolds 2006).

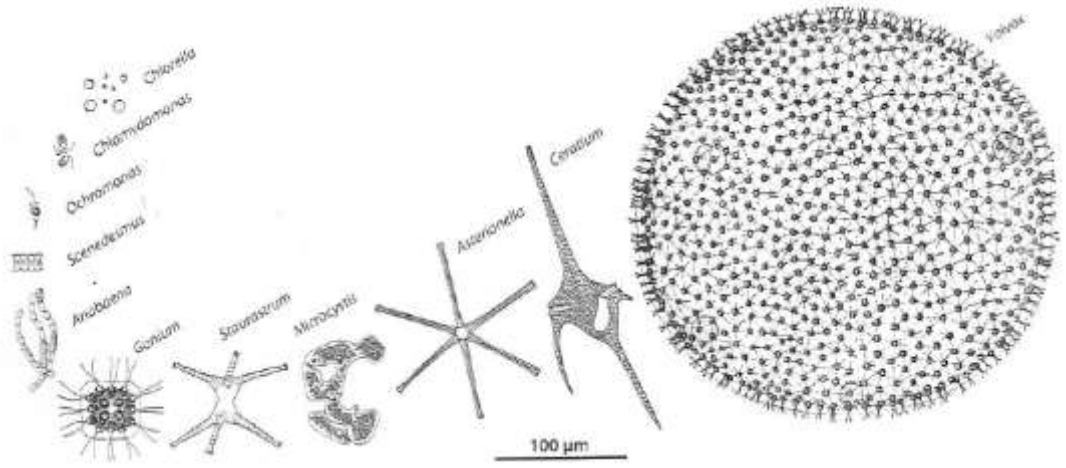

Figure 1.1 : Size and types of phytoplankton, (G.M, 1950, Freshwater Algae of the United State)

1.2 Spectroscopy

Spectroscopy is used in studying of absorption, emission and other radiation (ie. scattering) by matter which is related to the processes on the wavelength of the radiation. In addition, spectroscopic techniques are widely used in almost all technical field of science and technology including in phytoplankton study. However, spectroscopic techniques are very sensitive and must be set up carefully in study to get an accurate result (Stoner, 2014).

Spectroscopy has a various type and each type shows pictures and characteristics spectrum of matter differently. The variation in intensity of the radiation as a function of the wavelength or frequency showing in a graph is called spectrum (Fusino, 2009). The different regions in the electromagnetic spectrum such as ultraviolet (UV), visible (VIS) and infrared (NIR) are dependent on the matter characteristics. In the UV region, the wavelength ranges from 200 nm to 400 nm and in the VIS region, the ranges of wavelength between 380 nm until 750 nm whereas in the NIR region, the wavelength ranges from 750 nm to 2500 nm. Figure 1.2 shows the electromagnetic spectrum ranges from radiowaves to gamma ray.

The benefits of spectroscopy technique that used in this study are fast and easy. The different ranges of wavelength are very important to know because the comprehensive information can be found by these specific ranges for instant, the characteristics of the biochemical composition of a sample. Usually, the spectroscopy analysis is used for reflectance, absorption and transmission measurement of matter. These instruments measure the amount of energy reflected from the ground or object over different wavelengths (Milton, 1987). These

measurement can be converted to spectral radiance value if suitable equipment calibration factors are valid. The magnitude of spectral is depended on radiance solar incoming amount.

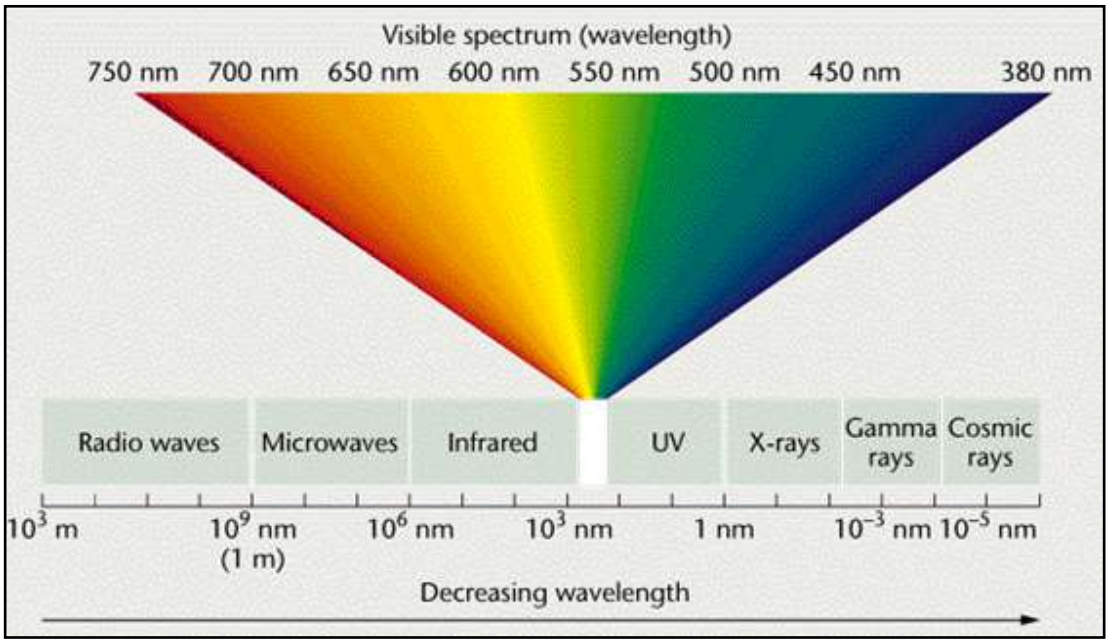

Figure 1.2 : Spectrum of electromagnet (klug & Cummings, 1997)

1.3 Problem Statement

Measuring chlorophyll *a* concentration is a step in the process of monitoring for nuisance phytoplankton blooms that may influence the taste and odor of drinking water sources. These blooms may actually create conditions that are toxic to human, fish. wildlife and livestock. Bodies of water used as drinking water source are also monitored for phytoplankton concentration for the early detection of phytoplankton blooms to minimize filtration system clogs. However, measuring chlorophyll *a* concentration is not simple and time consuming due to the need of sample preparation and may require complicated preparation.

1.4 Research Objectives

The objectives of this study are

1. To investigate the optical properties of phytoplankton.
2. To find the correlation between reflectance and chlorophyll *a* concentration of phytoplankton.
3. To develop a new algorithm for calculating chlorophyll *a* concentration of phytoplankton in fresh water.
4. To estimate the concentration of phytoplankton from the algorithm developed.

1.5 Research Scope

This research is mainly focus on optical sensor algorithms to measure concentration chlorophyll *a* of phytoplankton in fresh water. Images and data of phytoplankton will be analysed using the theoretical and statistical methods. Some software also used in analysis.

1.6 Research Location

In this research, 40 water samples were taken at Tasik Harapan, Universiti Sains Malaysia (USM) Penang around 10 o'clock morning. These water samples were collected to measure the chlorophyll *a* concentration of phytoplankton in this lake area by using spectroradiometer method. The coordinates of study area is 05° 35' N, 100° 29' E with width approximately about 8000 m^2. This location was chosen because it was very near and therefore easier to make observation frequently.

1.7 Novelty of Study

Studying the spectrum of phytoplankton in fresh water provides the new algorithms of measuring concentration of chlorophyll *a* in suitable wavelengths for phytoplankton. The concentration of the sample can be calculated easily within minutes using these algorithms.

1.8 Structure of the Thesis

Overall, this research is the study of chlorophyll *a* concentrations of phytoplankton using developed algorithms. Chapter 1 gives a small introduction to this study. This chapter also recounts the objectives and scope of the study. Chapter 2 describes the background of fresh water, phytoplankton, reflectance and chlorophyll *a* in details. Besides, in this chapter also provides graphs, images and tables on phytoplankton. Chapter 3 provides the publication of the theory of algorithms for measuring the concentration of chlorophyll *a* in the phytoplankton. This chapter also shows steps to develop the algorithms. In addition, techniques and measures are also given in this chapter. Chapter 4 provides research methods and tools that were used when the data were collected in this study. All measures carried out in this study were described in this chapter. Chapter 5 gives the data and also provides discussion of the study. This chapter also describes tests conducted on-site studies and other locations. Finally chapter 6 provides the overall conclusions of the study and provide recommendations for future research.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Human is influenced strongly with aquatic ecosystem, especially phytoplankton. Everyone knows, phytoplankton gives more advantages in today's technology such as the measurement of phytoplankton biomass is important in aquatic ecology studies. It is frequently estimated from chlorophyll *a* concentration determination. The physical, chemical and biological factors (Platt & Denham, 1980; Smayda, 1980; Carrick et al, 1993; Cloern, 1996; Lucas et al, 1999a,b) are factors that influenced phytoplankton abundance in fresh water. Among the commonly discussed factors that influenced biomass loss are nutrient availability, control algal growth, light and temperature. The impacts of one factor are dependent on other the factors, for example, the nutrient loading effect on phytoplankton abundance in ecosystems is dependent on other factors, including effect biomass gains and losses, light availability (Hitchcock & Smayda, 1977; Cole & Cloern, 1984; Bledsoe & Phlips, 2000), and sedimentation (Richardson & Jorgensen, 2013). The process of eutrophication can be accelerated (Smayda, 2013) in the area to significant human development. The frequency and intensity of algal bloom increasing can due to significant changes in the function and structure of the ecosystem effect, for example the water colour change of fresh water or ocean change from green to brown or from blue to red. Hence, in this chapter, definition and background of phytoplankton, reflectance and concentration of chlorophyll are given. Besides, the best method of chlorophyll *a* concentration measurement of phytoplankton is also discussed in this chapter.

2.2 Definition and Background of Phytoplankton

Fresh water is a fundamental resource for human life, and the services provided by surface fresh water ecosystems underpin global water security, food security and economic productivity Hanjra & Qureshi, 2010). Fresh water is defined as water having a low salt concentration which is usually less than 1%. Fresh water is vitally important to flora and fauna, including phytoplankton as a life resources. A good water quality of this system is required for many ecosystem services. Biological characteristics of fresh water, the development of flora and fauna in surface is governed by various species of animals and plants as well as physical performance of individual organism. The primary production of organic matter such as phytoplankton and macrophytes is extremely intensive in lakes and also reservoir but usually limited in rivers. Lakes have features low in average current velocity on surface value of 0.001 to 0.01 ms^{-1} (Meybeck & Helmer, 1996).

Phytoplankton is also known as algae that heterogeneous assemblage of organisms. The first use of the term 'plankton' was by Viktor Hensen (Reynold 2006; Hutchinson, 1967; Schwartz 1968), German biologist in the latter half of the nineteenth century. He began a series of expeditions to gauge the abundance, composition and distribution of microscopic organism in the open ocean. However, the existence of such organism had been demonstrated by another investigator, Johannes Müller some years earlier. Table 2.1 shows the type of plankton based on different habitats.