Chlorophyll \( a \) Concentration of Fresh Water Phytoplankton Analysed by Algorithmic based Spectroscopy

Fairuz Binti Johan\(^1, * \), Mohd Zubir Bin Mat Jafri\(^1,b \), Lim Hwee San\(^1,c \), Wan Mazznah Wan Omar\(^2,d \) and Tan Chun Ho\(^1,e \)

\(^1\)School of Physics, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia
\(^2\)School of Biological Science, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia

\(*\)fairozejohan@yahoo.com.my, \(^b\)mjafri@usm.my, \(^c\)hlim@usm.my, \(^d\)wmazznah@usm.my, \(^e\)chunho86@hotmail.com

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 is to investigate properties of reflection and chlorophyll \( a \) concentration of phytoplankton in fresh water. The reflection of phytoplankton is taken using spectroradiometer to observe the relationship between the reflectance and the wavelengths of phytoplankton. Tasik Harapan is selected as a study area. This lake is located in Universiti Sains Malaysia (USM), Penang, Malaysia. In this study, the water samples collected are filtered using phytoplankton net and analyzed in the laboratory to determine the reflectance and chlorophyll \( a \) concentration of phytoplankton. The water samples taken were prior to culture in the medium known as Bold's Basal Medium (BBM) before the reflections are taken so that accurate reflection measurements of phytoplankton can be acquired. Two spectrometers are 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 algorithms that related between chlorophyll \( a \) concentration and reflectance of phytoplankton are used. In this study, three regions also are focused, which are the red band, green band and blue band. These bands are related in analyzing phytoplankton. Reflectance of each band specified referred to the concentration of chlorophyll \( a \) for calibration algorithm. 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 water sampling for validation was also taken from the same lake and was analyzed using the same algorithm. The best results are obtained in this study, which are evidenced by the good correction coefficient, \( R^2 \) in the analysis results using the developed algorithm.

1. Introduction

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. Fresh water is a natural resource that is vital to the ecosystem life, whereas good water quality is required for many ecosystems [1] and rich with a variety of flora and fauna, including phytoplankton as a life resource. Everyone knows, phytoplankton give more advantages in today’s technology, such as the measurement of phytoplankton biomass is important in aquatic ecology studies. Among the commonly discussed
factors that influenced biomass loss are light, temperature, nutrient and control algal growth. 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 light availability and sedimentation [2]. Consequently, the study on phytoplankton communities and water quality become popular since the last decades [3]. Phytoplankton are microscopic single-celled plants and plays a major role as dominant primary producer of plankton community through photosynthetic activities in aquatic ecosystems. Phytoplankton have a few groups, such as green algae, cyanobacteria, red algae, diatom, dinoflagellates and yellow-green algae.

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 fields of science and technology including in phytoplankton study. However, spectroscopic techniques are very sensitive and must be set up carefully to study to get an accurate result [4]. The spectral reflectance in certain wavelength range corresponding to the chlorophyll $a$ concentration is identified according to the optical properties of phytoplankton [5]. Spectral reflectance is measured when imaging is done under different conditions including sediment, pigment, chlorophyll of water, depth, distribution and optical properties of the medium [6].

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 a spectrum. The different regions of 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 to 400 nm and in the VIS region, the ranges of wavelength between 380 until 750 nm, whereas in the NIR region, the wavelength ranges from 750 to 2500 nm. In phytoplankton, many previous study are focused on the visible (VIS) and near infrared (NIR) regions, which could be used in vegetation studies [7].

The existing of the chlorophyll $a$ concentration in phytoplankton and the phytoplankton spectra reflectance is related to the optical activity of the pigment, the composition and appearance of the algal cells. Chlorophyll, in various forms, is bound within the living cells of phytoplankton found in surface water. Chlorophyll is an important pigment in marine and freshwater algal species and has always been used as an indicator for phytoplankton biomass as well as bio-production calculation in water bodies [8]. The measurement of algal biomass is important in aquatic studies and is commonly estimate the concentration of chlorophyll $a$ in fresh water with 90% acetone method using spectrophotometer [9]. This is because spectrophotometry is the classical method of determining the quantity of chlorophyll in surface water. The main objective of this study is to investigate properties of reflection and chlorophyll $a$ concentration of phytoplankton in fresh water.

2. Methodology

2.1 Sample data collected

In this study, the 40 samples were taken in the morning about 10 a.m. These samples were taken using the phytoplankton net in the filtrate 20 litres of lake water for each sample. The samples were taken randomly but still around the entire study area. The water sample was filled into plastic bottles of 500 ml and stored in an ice box containing ice packs to ensure the conditions of phytoplankton remained good and would not die. After the samples were taken, the samples were brought to the plankton lab for analysis.

2.2 Study Area

Tasik Harapan is chosen as the study area because it was suitable for studying phytoplankton chlorophyll concentration in term of size, condition and colour of the lake. This lake is located in Universiti Sains Malaysia (USM). Furthermore, the location is also near to make observations. The proposed algorithm has been tested on the lake. This lake has an apparent distribution of phytoplankton chlorophyll concentration and is suitable to be studied. Tasik Harapan is one of the lakes that exists in this university. The area of Tasik Harapan is about 8000 m$^2$. 
2.3 Cultured Method
In this study, culture methods were also needed so that when the reflection reading of phytoplankton were taken, reflectance was shot exactly on phytoplankton rather than the other solid. The medium used in culturing is called Bold’s Basal Medium (BBM). This medium was rotated in an autoclave to sterilize equipment and supplies. After preparing the samples were done, these samples were stored in cultured room.

2.4 Optical Instrument and Software
Reflectance of phytoplankton was taken using ASD spectroradiometer (handheld2) with height from sample to spectroradiometer of 30 cm and 1 degree FOV. The reflectance radiation wavelength regions to be recorded range from 325 to 1075 nm and was supported by tripod stand. Each sample was taken ten readings. An average of ten measurements was recorded for each sample. All the results were analyzed by using ViewSpec Pro software. After reflectance was taken, the sample was taken rapidly for analysis to measure the concentration of chlorophyll $a$.

2.5 Extract and Measurement Chlorophyll $a$ Concentration
In this study, the samples were extracted by using a solution of 90% acetone. After extracting chlorophyll $a$, the concentration of chlorophyll $a$ is measured using spectrophotometry method using model HITACHI U-1900 UV-Visible spectrophotometer with selected wavelengths at 750 nm, 664 nm, 647 nm and 630 nm and 90% acetone was used as a blank in the spectrophotometer [10]. The concentration of chlorophyll $a$ was calculated using formula by Jeffrey and Humphrey, 1975 [11]. The absorbance at 750 nm was subtracted from those three wavelengths to give the turbidity-corrected value.

2.6 Regression Algorithm
Equations for different wavelength such as (1) and (2) are needed to assess the content of chlorophyll concentration.

$$C_a = a_0 + a_1R_1 + a_2R_2 + a_3R_1R_2 + a_4R_1^2 + a_5R_2^2 + a_6R_1R_3 + a_7R_1^2 + a_8R_2^2 + a_9R_3^2$$  \quad (1)

$$C_a = c_0 + c_1R_1 + c_2R_2 + c_3R_3 + c_4R_1R_2 + c_5R_1R_3 + c_6R_2R_3 + c_7R_1^2 + c_8R_2^2 + c_9R_3^2$$  \quad (2)

Equation (1) is an algorithm model for two wavelengths. The algorithm for three wavelengths such as equation (2) can produce with enlarged equation (1), where, $j = 0,1,2,...$ is the constant of the equation. It can be empirically determined using regression analysis.

3. Result and Discussion
3.1 Reflectance spectra of phytoplankton
Figure 1 shows the six spectral reflectance graphs of randomly picked samples using spectroradiometer for the study area at Tasik Harapan, Universiti Sains Malaysia (USM), Penang. In general, the graph shown by the six samples during the study has similar patterns. This could probably because many factors such as distribution, chlorophyll, composition, species, pigment, condition, and measurement instrument while taking the reading. For graph of reflectance against wavelength, there are two obvious peaks in the green (500-600 nm) and NIR (700 nm) regions due to several factors, and high absorption in the blue (400-500 nm) and red regions. Besides, a broad reflectance in this region (500-600 nm) is because of minimum absorption by phytoplankton pigment. Phytoplankton pigments absorb blue and red light, but have minimum absorbance in green light. In the red region, wavelength near 675–677 nm has the lowest reflectance and highest absorbance. In this region the spectra reflectance of phytoplankton was the lowest which also corresponded to high absorption by chlorophyll.
3.2 Correlation between Absorbance and Chlorophyll a Concentration

Figure 2(a), 2(b) and 2(c) show the scatter plot of the absorbance and concentration of chlorophyll a value for phytoplankton. The absorbance values increased with concentration of chlorophyll a. The increasing value was experienced by all the wavelengths. Figure 2 (a), 2(b) and 2(c) also show that these three wavelengths which were 630, 647 and 664 nm produced the higher R^2. The values of R^2 for absorbance for these three wavelengths were 0.9196, 0.8895 and 0.9984. The correlation of these three wavelengths are also quite high and all these three wavelengths are located in the red region. The concentration of chlorophyll a for these 40 samples were produced from by using spectrophotometry method. This instrument was chosen to measure the actual values for chlorophyll a because spectrophotometry is the classical method of determining the quantity of chlorophyll in surface water. From Figure 2(a) and 2(b), the data were not as precise as desired on the points obtained for the trend line analysis compared to Figure 2(c) the all almost closed to the line and the value of R^2 was near to 1. It was assumed that the inconsistencies in the data were due to errors in the dilution of the stock solution when making the phytoplankton samples. By the way, these results indicate still can be accepted because the values of R^2 more than 80%.
Figure 2(a), (b) and (c): Absorbance versus chlorophyll $a$ concentration for 630 nm, 647 nm and 664 nm.
3.3 Correlation between Reflectance and Chlorophyll a Concentration

Figure 3(a), 3(b) and 3(c) shows the scatter plot between chlorophyll a concentration and reflectance of phytoplankton at wavelength 438, 550 and 675 nm. The graph patterns for these two variable relationships are polynomial pattern. The scattering response of chlorophyll a concentration is decreased with increased value of reflectance spectra. At the beginning, the graph is steeper and gradually approaches horizontal line. This result indicates that the values of reflectance change with different concentration of chlorophyll a for phytoplankton. From the result, the graph pattern between these two variables in this study is quite similar with the previous research, Babin, 2003 [12]. The chlorophyll a concentration content of phytoplankton ranges from 0.1 to 2.7 mg/L. The points were not closed to the line because of this the correlation coefficient, R for two variables is low and cannot show a relationship between these two variables is perfect. This is due to dependent parameter like instrument setting, chlorophyll a content and water samples. Chlorophyll a concentration could be influenced by a few factors such as light, nutrient, total phosphorus (TP), total nitrogen (TN), pH, water flow, water temperature and others.

![Graph showing correlation between reflectance and chlorophyll a concentration](image-url)
3.4 Validation The Concentration Of Chlorophyll a from The Algorithms Developed.

Figure 4(a) and 4(b) show the measurements of predicted and actual concentration of chlorophyll a for two and three wavelengths involving three selected wavelengths which are 438, 550 and 675 nm. The algorithm that used to measure the chlorophyll a concentration of phytoplankton for two wavelengths and three wavelengths as shown in Equation (4.1) and (4.2) with the correction coefficient, $R^2$ of 0.753 and 0.879. Meanwhile, the value of $R^2$ for validation are 0.768 and 0.856. The $R^2$ values are quite high. The algorithms are efficient in measuring concentration of chlorophyll a for phytoplankton. Compared to two wavelengths, three wavelengths are more efficient for measuring concentration of chlorophyll a based on $R^2$ value.

Concentration of chl a (mg/L) = $1.67 + 299 R_{438} - 33.1 R_{675}$  
- $7217 R_{438} R_{675} - 14022 R_{438}^2 - 973 R_{675}^2 + 373702 R_{438}^2 R_{675}$  
+ $112440 R_{438} R_{675}^2 - 3317051 R_{438}^2 R_{675}^2 + 112440 R_{438} R_{675}^2$  
$R^2 = 0.753$

Validation 
$R^2 = 0.768$

Concentration of chl a (mg/L) = $3.45 + 66.2 R_{438} - 100 R_{550}$  
- $3.9 R_{675} + 5349 R_{438} R_{550} - 16643 R_{550} R_{675} - 12682 R_{438} R_{675}$  
+ $3077 R_{438}^2 + 5209 R_{550}^2 + 15992 R_{675}^2$  
$R^2 = 0.879$

Validation 
$R^2 = 0.856$

Figure 3(a), (b) and (c): Concentration of chlorophyll a versus reflectance for 438 nm, 550 nm and 675 nm
4. Conclusion
In this research, the concentration of chlorophyll $a$ was measured using a spectrophotometry technique. This method was adopted because it is the classic method and high accuracy, but the costs incurred are quite expensive and taken a long time to measure the chlorophyll $a$ concentration. The result shown the graph between the concentration of chlorophyll $a$ and observance of phytoplankton have straight line with high value of $R^2$. Besides, the results also shown the graph between the concentration of chlorophyll $a$ and reflectance of phytoplankton for three wavelengths 438 nm, 550 nm and 675 nm. The concentration value of chlorophyll $a$ is decreased with reflectance, but the correlation coefficient, $R$ for two variables are lower. This is because the result depends on instrument setting, chlorophyll $a$ content and water samples. Chlorophyll content is influenced by a few factors, such as nutrient, phosphorus, pH, water flow, water temperature and others.

Generally, the spectral reflectance of phytoplankton in this study showed similar pattern. The result also was shown two obvious peaks which were in green and NIR regions. The spectra composed of high absorbance in the blue region near to 440 nm and low absorbance with high reflectance in the
green region part of spectrum (550–570 nm). There was a high absorbance (low reflectance) in the red region (~675 nm). Meanwhile, in the NIR region the reflectance increased and decreased slowly.

A simple method to detect and calculate the chlorophyll $a$ concentration of phytoplankton in the fresh water was introduced. It based on the spectroradiometer in the calculation of the concentration of chlorophyll $a$. The developed algorithms in this study were created based on the concept of water reflectance properties and can be measured the concentration of chlorophyll $a$ in the study area with acceptable accuracy by the value of $R^2$. This is evidenced in the concentration of chlorophyll $a$ measurement and also used as validation in the Tasik Harapan. The methods of chlorophyll $a$ concentration measurement are introduced in this study was used to determine the distribution of phytoplankton in fresh water with more ease, simpler saving a cost and time. The results of studies performed in this research achieve the objectives.

Reference

[1] Meybeck, M. & R. Helmer, (1996) An introduction to water quality. In Chapman, D. (ed), Water quality assessments, 2nd ed. Taylor & Francis, New york: 1-22.

[2] Richardson, K., & Jorgensen, B. B. (2013). Eutrophication: Definition, History and Effect Eutrophication in Coastal Marine Ecosystems (pp. 1-9): American Geophysical Union.

[3] Stomp, M., Huisman, J., Mittelbach, G. G., Litchman, E., & Klausmeier, C. A. (2011). Large-scale biodiversity patterns in freshwater phytoplankton. Ecology, 92(11), 2096-2107. doi: 10.1890/10-1023.1

[4] Stoner,J.O.(2015)"Spectroscopy"http://global.britannica.com/EBchecked/topic/558901/spectroscopy

[5] Johan, F., Jafri, M.Z., Lim, & Wan Maznah W.O. (2015, 24-25 Nov 2015). Estimating the Chlorophyll a Concentration of Phytoplankton from an Empirical Analysis in Smart Instrumentation Measurement and Application (ICSIMA), IEEE 3rd International Conference on 3015, doi: 10.1109/ICSIMA.2015.7559027.

[6] Peddle, D. R., Peter White, H., Soffer, R. J., Miller, J. R., & LeDrew, E.F. (2001). Reflectance processing of remote sensing spectroradiometer data. Computers & Geosciences, 27(2), 203-213. doi: 10.1016/S0098-3004(00)00096-0.

[7] Johan, F., Jafri, M.Z., Lim, H.S., & Sim, C. K. (2013, 1-3 July 2013). Preliminary study: Spectral reflectance properties of microalgae in freshwater in Space Science and Communication (IconSpace), IEEE Internation Conference on 2013, pp. 337-340.

[8] Duan, H., Ma, R., Zhang, Y., Loiselle, S. A. Xu, J., Zhao, C., Shang, L. (2010). A new three-band algorithm for estimating chlorophyll concentration in turbid inland lakes. Environmental Research Letters, 5, 044009.

[9] Johan, F., Jafri, M.Z., Lim, H.S., & Wan Maznah, W.O. (2014, 9-12 Dec. 2014). Laboratory measurement: Chlorophyll-$a$ concentration measurement with acetone method using spectrophotometer in Industrial Engineering and Engineering Management (IEEM), IEEE International Conference on 2014, pp. 744-748.

[10] Jeffrey, S. W. (1975). Preparation and some properties of crystalline chlorophyll c1 and c2 from marine algae. Biochim. biophys. Acta 279, 15-33.

[11] Jeffrey, S.W., and G.F. Humphrey. (1975). New spectrophotometric equations for determining chlorophylls a,b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pflanzen. 167: 191-194.

[12] Babin, M., Stramski, D., Ferrari, G.M., Claustre, H., Briceaud, A., Obolensky, G., & Hoepffner, N. (2003). Variations in the light absorption coefficients of phytoplankton, nonalgal particles. And dissolved organic matter in coastal waters around Europe. Journal of Geophysical Research: Oceans, 108 (C7), n/a-n/a. doi 10.1029/2001jc000882.