Jurnal
Teknologi

Full Paper

QUANTIFICATION OF OIL PALM TREE LEAF PIGMENT (CHLOROPHYLL A) CONCENTRATION BASED ON THEIR AGE

Norfazira Mustafa[a*], Norsuzila Ya'acob[a], Zulkiflee Abd Latif[a], Azita Laily Yusof[b]

[a]Wireless Communication Technology (WiCoT), Faculty of Electrical Engineering, Universiti Teknologi MARA, 404500 Shah Alam Selangor, Malaysia
[b]Applied Remote Sensing & Geospatial Research Group, Faculty of Architecture, Planning and Surveying, Universiti Teknologi MARA, 404500 Shah Alam Selangor, Malaysia.

Article history
Received
4 March 2015
Received in revised form
25 March 2015
Accepted
15 March 2015

*Corresponding author
faziramustafa@yahoo.com

Graphical abstract

Abstract

The main pigments found in oil palm tree leaf are chlorophylls *a*, *b*, and *c*. Chlorophyll *a* converts light energy into chemical energy through photosynthesis process. The content of chlorophyll pigments varies by their ages. The aim of this research was to determine the concentration in chlorophyll *a* in different ages. This study is significant in oil palm fertilization for monitoring oil palm nutrient content (NPK). Chlorophyll measurement was done by extracting using methanol solvent and concentration measurement using spectrophotometric method in order to quantify chlorophyll *a* concentration based on their ages. 6 samples of oil palm tree leaves, collected from 2 different ages, were analyzed. The observed values were determined based on the absorbance at wavelength (670nm) and were calculated for concentrations value based on to the Lambert-Beer law Equation. It showed that the chlorophyll *a* concentrations at the age of mature stage had been higher than those at the old stage. The results depicted that the chlorophyll *a* concentration values at the mature stage were 0.33mg/ml, 0.32mg/ml, and 0.18mg/ml, while at the old stage were 0.22 mg/ml, 0.18mg/ml, and 0.06mg/ml.

Keywords: Oil palm tree age, chlorophyll a, chlorophyll concentration

Abstrak

Pigmen utama yang terdapat dalam daun pokok kelapa sawit adalah klorofil a, b, dan c. Klorofil a menukar tenaga cahaya kepada tenaga kimia melalui proses fotosintesis. Kandungan klorofil pigmen adalah berbeza mengikut peringkat umur mereka. Tujuan kajian ini adalah untuk menentukan kepekatan klorofil dari peringkat umur yang berbeza. Kajian ini adalah penting dalam pembajaan kelapa sawit untuk mengawal baja bagi memantau kandungan nutrien kelapa sawit (NPK). Hasil kajian menunjukkan kepekatan klorofil daun pokok kelapa sawit pada usia peringkat matang adalah lebih tinggi daripada tua. Nilai kepekatan klorofil di peringkat matang adalah 0.33mg/ml, 0.32mg/ml, dan 0.18mg/ml, manakala pada peringkat tua adalah 0.22 mg/ml, 0.18mg/ml, dan 0.06mg/ml.

Kata kunci: Umur pokok sawit, klorofil, kepekatan klorofil

1.0 INTRODUCTION

Chlorophyll plays an important role in photosynthesis for plants to manufacture foods. The energy for photosynthesis is supplied from the sunlight to be processed by chlorophyll. Chlorophyll in a chloroplast is a significant factor for plant production since chlorophyll is a pigment that gives the characteristic of green color [1]. Measurements of chlorophyll content of photosynthetic organisms are a fundamental measurement in many branches of plant biology and ecology. The amount of chlorophyll in photosynthetic organisms is an important measurement in itself, particularly for chlorophyll *a*, which is the primary photosynthetic pigment in photosynthetic organisms. The other pigments are known as accessory pigments [2]. In addition, the total chlorophyll changes of chlorophyll *a* and chlorophyll *b* can be affected by a variety of physiological stresses, leaf development and senescence, as well as related directly to the rate of primary production. Furthermore, the chlorophylls contain a large proportion of total leaf nitrogen, and therefore, measurements of chlorophyll concentration can provide an accurate indirect assessment of plant nutrient status [3]. The quantity of chlorophyll per unit area is an indication of photosynthetic capacity and productivity of a plant. Therefore, the amount of chlorophyll in the leaf tissues may be influenced by nutrient availability and environmental stresses, such as drought, salinity, as well as crude oil pollution of soil, heat, and others [4]. From the above criteria, this research study was carried out to detect the total chlorophyll *a* content in oil palm tree leaf grown in Felda Kota Gelanggi, Pahang. Since, Malaysia is one of the largest producers and exporters of palm oil in the world, which dominates 11% of the world's oil and fat production and 27% to export oil and fat [5]. The experiment was carried out at Universiti Teknologi Mara (UiTM) Shah Alam, Selangor. This research was an experimental study and was aimed at quantifying the total chlorophyll a content of two different ages for oil palm tree leaves at the age of mature and old stages. In this present study, the samples of tree leaves were extracted in Methanol. Based on previous researchers, methanol is a very good extractant for chlorophylls, particularly from recalcitrant vascular plant and algae [6] [7] [8]. Researcher [9] compared three various types of solvents to extract chlorophyll a. Better results were obtained with methanol as the extraction solvent to quantify chlorophyll a concentration. Concentration of chlorophyll *a* in the obtained extract was measured by using UV/VIS spectrophotometer at 670nm wavelength since the concentration of leaf pigment, such as chlorophyll in chloroplasts, depends on the absorption of light in the visible spectrum (400-750nm) for green leaves [10]. Then, chlorophyll concentration was calculated by using Lambert Beer Law equation. In fact, many researchers use Lambert Beer law to quantify chlorophyll concentration. The Beer-Lambert law is used to measure the absorbance of a particular sample and to analyze the concentration from the measurement. In addition, scientists can measure the concentration of a particular chemical species as it absorbs light of a particular wavelength are known [11].

1.1 Oil Palm Age Classification

Scientifically, oil palm has both male and female flowers on the same tree. Mature trees are single-stemmed, and grow to a height of 20 to 30 meters. The leaves are pinnate, and reach between 3 and 5 meters long. A young tree produces about 30 leaves a year, while customary trees over 10 years produce about 20 leaves a year. Palm fruit takes five to six months to mature from pollination to maturity. After eight months in the nursery, healthy plants grow 0.8 to 1.0 meter in height and 5 to 8 leaves display function [12]. Based on an article from the MPOC (2008), oil palm yields vary according to age and management. Table 1 shows the palm age stratified into three age categories.

Table 1 Oil Palm Age Categories

Type of Oil Palm	Age
Young	0 years to 3 years
Mature	4 years to 25 years
Old	above 25 years

1.2 Visible Spectrum of Vegetation

In the visible pattern of spectrum (0.4-0.6μm), healthy vegetation usually exhibits a valley in the (0.45-0.67 μm) range of spectrum due to the strong absorption of this energy and reflects the green spectrum (0.55μm). Figure 1 shows that the absorption in the visible had been due to chlorophyll, which was used by plants in the process of photosynthesis. The radiation absorption by vegetation in this region were divided into two main absorptions, which were blue (0.45 μm) and red (0.67 μm) that absorbed most of chlorophylls a and b [13].

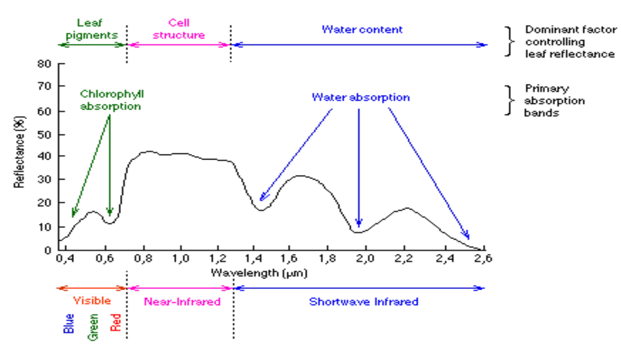


Figure 1 Typical Spectral Response Characteristic of Green Vegetation

1.3 Chlorophyll Classification

There are two main types of chlorophylls; chlorophyll *a* and chlorophyll *b*. The difference between these two chlorophylls is a methyl moiety in chlorophyll *a* replaced by a formyl group in chlorophyll *b* [14]. The ratio of chlorophyll *a* to chlorophyll *b* in higher plants is approximately 3:1. Figure 2 shows the absorption spectra of chlorophylls *a* and *b*. Besides, the amount of chlorophyll in photosynthetic organisms is an important measurement in itself, particularly for chlorophyll *a* (Chl *a*), which is the primary photosynthetic pigment in nearly all known oxygenic photosynthetic organisms [15].

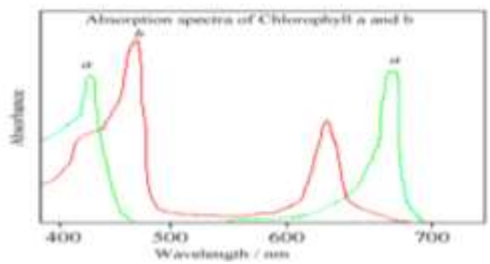


Figure 2 Absorption Spectra of Chlorophylls *a* and *b*.

1.4 Accuracy of Spectroscopic Measurement

In order to have an exact spectroscopic measurement of absorbance, one must consider the absorbance range in which readings are made. Absorbance should be measured between 0.3 and 0.85. Leaf extracts with an absorbance <0.3 in the red region do not yield correct pigment values due to several interfering factors, such as a base line that is not fully zeroed. Thus, values <0.3, whether read by the researcher or given as digital values by the instrument, are not acceptable. Meanwhile, absorbance values >0.9 might indicate problems with the accuracy of the detector. Since the detector system examines the transmitted light of the cuvette, the absorbance is calculated from this value. When transferring the linear transmission unit to the logarithmic absorbance unit, the accuracy is exponentially reduced with rising values [16].

2.0 EXPERIMENTAL

Based on figure 3 below, the research methodology in this study began with identifying the research area, which was for collecting the samples. After all samples were collected, lab procedure was carried out to quantify chlorophyll *a* concentration. The process started from extracting the chlorophyll using methanol, then separating chlorophyll *a* from the solvent using distilled water and filter paper, and followed by measuring the absorbance using UV/VIS spectrophotometer. Lastly, the total of chlorophyll *a* concentration was calculated with Lambert Beer Law equation.

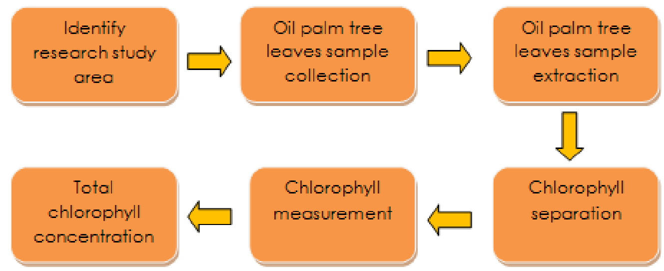


Figure 3 Flow Work of Research Study

2.1 Study Area

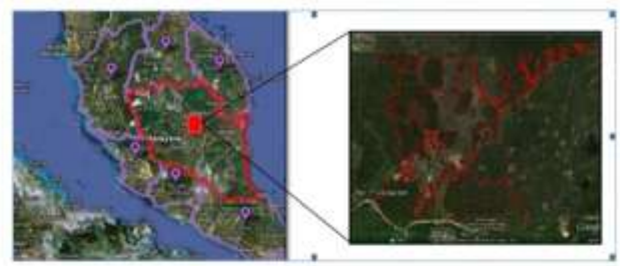


Figure 4 Location of Study Area in Felda Tekam

Felda Tekam is one of the ground plans of the Federal Land Development Authority (FELDA), located in the district of Bera , Pahang Darul Makmur. The farms here are run by the second generation of Felda settlers, and some have left it to the management of Felda plantation. Among other major activities are beef cattle farming, agriculture, and industrial in small scale.

2.2 Oil Palm Tree Leaves Sample Collection

The research was carried out on a sunny day with a total of 6 samples of oil palm leaves, which were collected at Felda Tekam from two blocks at two different development stages (matured and old). Table 2 below portrays the code sample for mature and old leaves. The leaves were collected from the uppermost tree and called as leaf number 9, as shown in figure 5 below.

Table 2 Codes for Oil Palm Leaf Samples

Code sample	Development stage
1R27	Mature
1R6	Mature
1R63	Mature
123R80	Old
123R101	Old
123R90	Old

Figure 5 Oil Palm Tree Leaf number 9

2.3 Oil Palm Leaf Sample Extraction

Extraction of chlorophyll was executed by soaking 500mg of 6 sample leaves into1.5ml methanol, as shown in figure 6. The sample leaves were torn into small pieces and were weighed approximate to 500mg before soaking. Then, the sample was incubated at room temperature in a dark place for 72 hours.

Figure 6 500mg of oil palm leaves were soaked in 1.5ml methanol.

2.4 Separation of Chlorophyll by using Distilled Water

Many methods can be utilized to separate pigment from solvent extractant. For instance, researcher [17] used ¼ volume of distilled water to separate spinach leaf pigment from acetone solvent. In this study, the sample leaves that had been soaked in methanol were heated in the oven at a temperature of 50°C-55°C for 24 hours to let the samples dry. Then, 25ml of distilled water was added into the dried sample leaves for chlorophyll separation. Filter paper was used to separate the chlorophyll.

2.5 Chlorophyll Measurement

Concentration of chlorophyll *a* was measured at the wavelength of 670nm using UV/VIS spectrophotometer, while Lambert Beer Law was performed to quantify chlorophyll a concentration. Lambert Beer Law is the linear relationship between absorbance and concentration of an absorbing species. The total concentration of chlorophyll *a* can be calculated by using this Lambert Beer Law formula [18]:

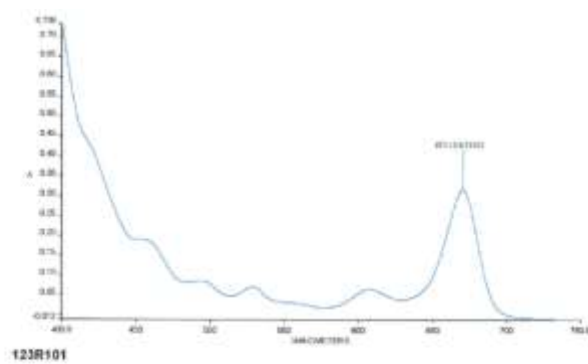
$$c = \frac{\mathrm{A}}{\varepsilon\, \ell} \qquad (1)$$

Where:
C= Concentration (mg/ml)
A= Absorbance
ε= Molar Extinction Coefficient
ℓ= Path length (cm)

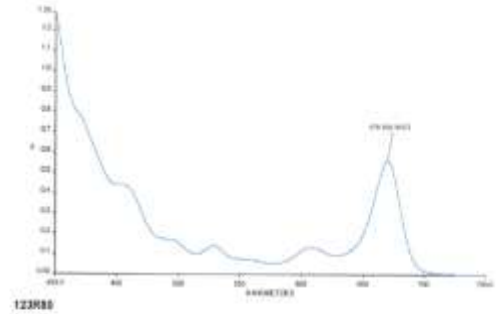
3.0 RESULTS AND DISCUSSION

3.1 Absorbance Measurement

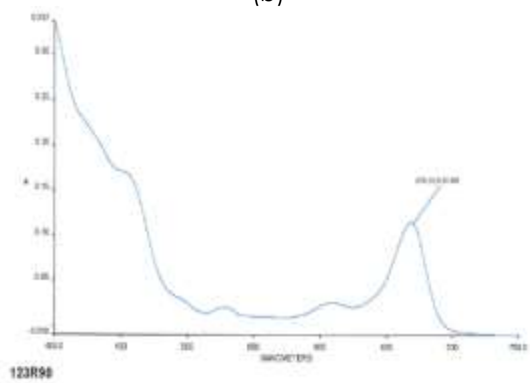
Absorbance measurement was carried out in order to identify the absorbance spectrum of isolated chlorophyll *a* in methanol. Moreover, absorbance value was used to quantify the concentration of chlorophyll *a* by using Lambert Beer Law equation. The maximum absorption of extracted pigment strongly depends on the type of solvent and the type of spectrophotometer. Figures 7 and 8 below show the results of absorbance spectrum for chlorophyll *a* in oil palm leaf samples, which were extracted in methanol at a wavelength of 670nm. Figure 7 shows the result for the absorbance measurement for oil palm leaf samples at old stage, while figure 8 explains the sample at mature stage. From the measurement, the absorbance spectrum for all the samples had been under the accuracy tolerance, whereby the absorbance should be measured between the range of 0.3 and 0.85 [19]. The value of absorbance spectrum can be seen in table 3.



(a)

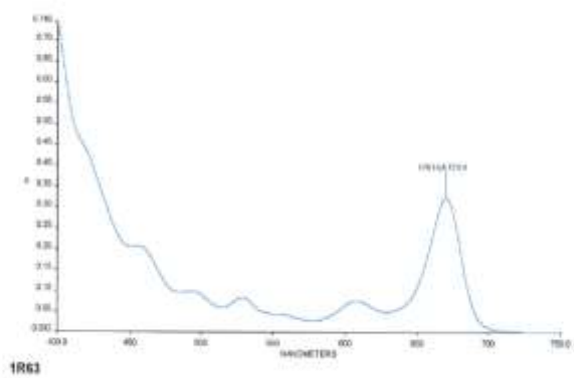


(b)

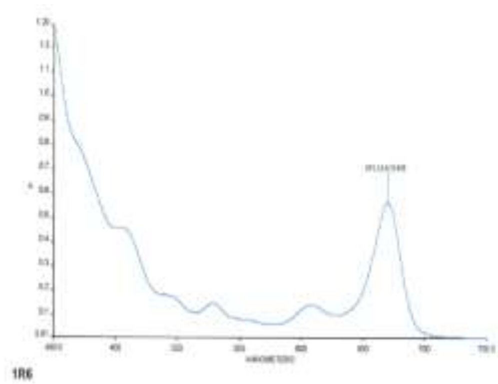


(c)

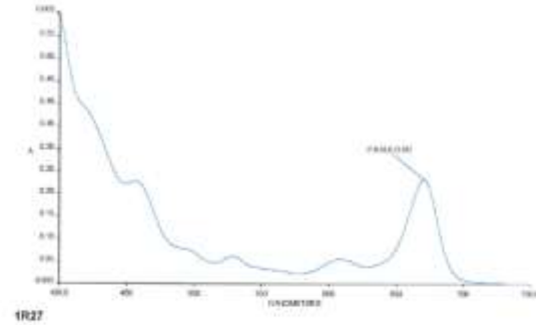
Figure 7 Absorption Spectrum of Chlorophyll *a* in Methanol for Old Stage shown in (a), (b), and (c)



(d)



(e)



(f)

Figure 8 Absorption Spectrum of Chlorophyll *a* in Methanol for Mature Stage shown in (d), (e), and (f)

Table 3 Absorbance Value for Chlorophyll *a* measured using UV/VIS Spectrophotometer

Sample	Absorbance Value
1R27	0.5841
1R6	0.5666
1R63	0.3253
123R80	0.3645
123R101	0.3612
123R90	0.3140

3.2 Determination of Chlorophyll *a* Concentration

The aim of this study was to quantify the concentration of chlorophyll *a* in oil palm leaves. Lambert Beer Law Equation was utilized to calculate pigment concentration. Chlorophyll *a* concentration was calculated at the absorbance wavelength of 670nm. To exactly determine the concentration, molar extinction coefficient also must be identified, whereby in this study, the value was constant = 1.78mg/ml^{-1} with the path length of cuvette 1cm. Table 4 shows the results of chlorophyll *a* concentration for oil palm leaf samples for two different ages (matured and old).

Table 4 Total Concentration of Chlorophyll *a* using Lambert Beer Law Equation

Leaf Sample	Total Chl *a* Concentration (mg/ml)	
	Mature	Old
1R27	0.33	
1R6	0.32	
1R63	0.18	
123R80		0.22
123R101		0.18
123R90		0.06

From the results depicted in figure 9, it can be concluded that the concentration of chlorophyll *a* in oil palm tree leaves at the matured stage had been higher than those at the old stage. So, more attention must be given to oil palm trees at the mature stage in order to produce more yields in the future.

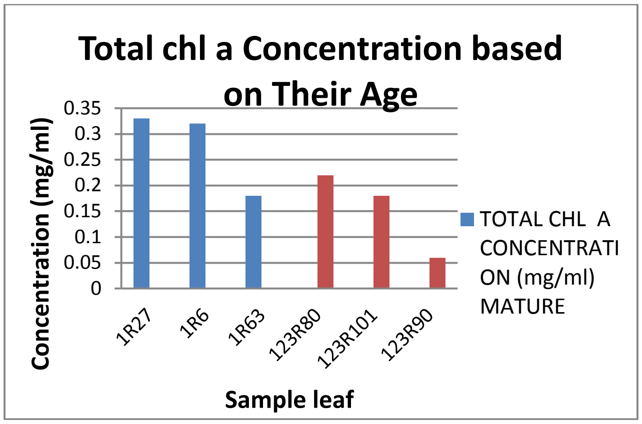


Figure 9 Total Concentration of Chlorophyll *a* Based on Their Age

4.0 CONCLUSION

In this study, 6 samples of oil palm leaves were collected at two different ages; mature and old ages. The results showed that the concentration of chlorophyll *a in* oil palm tree leaves at the age of mature stage had been higher than the old stage, whereby the values of chlorophyll *a* concentration at the mature stage were 0.33mg/ml, 0.32mg/ml, and 0.18mg/ml, while the old stage provided 0.22 mg/ml, 0.18mg/ml, and 0.06mg/ml. Good fertilizer management is the key to high productivity and efficiency in most oil palm plantations. However, it is beneficial to go beyond maintaining healthy palm and yields. Theoretically, the relationship between leaf analysis and plant productivity is generally evident for most crops, and an assessment of fertilizer needs to be based on such analysis.

Acknowledgement

The authors would like to thank the Faculty of Electrical Engineering for helping to complete the research study and also to the Federal Land Authority (FELDA) for granting the permission to access the study site for data collection.

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