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Chlorophyll Extraction of Few Mangrove Species (*Avecenia officinalis*, *Sonneratia apetala*, *Sonneratia caseolaris* L.)

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Dedicated

To

My beloved Family.....

Abstract

Chlorophyll is a very important photosynthetic green pigments which helps trees to produce their food. This pigments quantity varies species to species and depend different types of factors. This pigment controls the photosynthesis process and their quantity also influence the tress growth and other development. The measurement of chlorophyll content effectively helps to indentify trees problems.The main issues needed to recognize the value of chlorophylls and their derivatives in food technology, including aspects related to the structures naturally present in foods, arising from biosynthetic and catabolic processes, food processing, and cooking operations. In addition, common analytical procedures to isolate chlorophyll standards and available methods to identify and characterize the chlorophyll profile of food products and extracts will be presented. The intricate structural arrangements of the basic tetrapyrrole structure responsible for a wide range of chlorophyll-related derivatives and their susceptibility to undergo oxidation processes when out of their genuine cellular organelles are challenges for the development of analytical methodologies and industrial applications as coloring matter. A review of the existing chlorophyll formulations produced to give or reinforce green hues in foods is issued with special emphasis on their composition and current legal regulations

Dimethylsalfoxide(DMSO) is a widely used solvent for chlorophyll extraction

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DECLARATION

I, Banasri Sarker, declare that this thesis is my own work and that, to the best of my knowledge and belief, it reproduces no material previously published or written, nor material that has been accepted for the award of any other degree, except where due acknowledgement had been made in the text.

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APPROVAL

This project thesis submitted to the Forestry and Wood Technology Discipline, Khulna University, Khulna, Bangladesh, in partial fulfillment of the requirements for the BSc.degree in Forestry. I have approved the style and format of the project thesis.

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Chapter-1

1. Introduction

1. 1 Background of the study

Mangroves are a group of highly salt-tolerant woody plants. The high water use efficiency of mangroves under saline conditions suggests that regulation of water transport is a crucial component of their salinity tolerance. Mangroves are inherently plastic and can change their structure at the root, leaf and stand levels in response to salinity in order to exclude salt from the xylem stream, maintain leaf hydraulic conductance, avoid cavitation and regulate water loss (e.g. suberization of roots and alterations of leaf size, succulence and angle, hydraulic anatomy and biomass partitioning). Mangrove plants in the tropical belt are well known for their productivity and faster carbon sequestration than any other tropical forests. Chlorophyll is a principal pigment in plants. In converting light energy to chemical energy, it allows photosynthesis, i.e., light-induced carbon fixation (primary production) to take place. As a biomass indicator of aquatic micro-algae that support food webs in the sea, it is probably the most frequently measured biochemical parameter in oceanography (Jeffrey and Mantoura,1997). Although mangrove species are generally prefer exposed habitats,there are considerable differences in light requirement among species during the course of forest succession(putz and chan 1986, Tanouchi et al 2000).Chlorophyll content varies within a species and among the mangrove species coupled with other environmental factors. The mangrove plants of the Sundarbans are least explored. Laboratory extraction of chlorophyll from mangrove plants is not available which could otherwise been helpful to identify productive efficiency of a particular species and subsequently the ecosystem quantitatively. In this study, three pioneer species (*Avecenia officinalis, Sonneratia apetala, Sonneratia caseolaris*) of the Sundarbans mangrove forest were selected to extract leaf chlorophyll in the laboratory.

1.2 Objective of the study

A. To review the all available literature on chlorophyll extraction.
B. To determine the chlorophyll of *Avecenia officinalis, Sonneratia apetala, Sonneratia caseolaris* species.

Chapter-2

2.1 Chlorophyll: general description

The most important common pigment in photosynthesis is chlorophyll. Chlorophyll is a green photosynthetic molecule. It is mainly found in plants, algae, cyanobacteria. Chlorophyll is a cyclic tetrapyrolle, which is similar in structure to that of hemoglobin with the exception that the central metal is magnesium versus iron.

2.2 Types of chlorophyll

There are two main forms of chlorophyll found in nature: chlorophyll-a and chlorophyll-b. There is a small difference between the two types, basically that each absorbs light from the sun at slightly different wavelengths. In natural plants containing chlorophyll, there is a ratio of 3:1 chlorophyll-*a* (a bluish-black solid) to chlorophyll-*b* (a dark green solid), which both work together to reflect the dark green pigment that's visible to the human eye.

Chlorophyll a

The green pigment which is responsible for the absorption of light, providing energy for oxygenic photosynthesis is called chlorophyll a. It is found in all plants, green algae, and cyanobacteria. In chlorophyll a, the most effectively absorbing wavelengths of the spectrum are 429 nm and 659 nm, which are responsible for violet-blue and orange-red colors, respectively. Chlorophyll a reflects blue-green color, which is responsible for the green color of most of the land plants. Chlorophyll a is the most important pigment in photosynthesis, which serves as the primary electron donor in the electron transport chain of photosynthesis. On the other hand, it transfers the light energy trapped in the antenna complex into the photosystems P680 and P700, where the specific chlorophylls are present in the thylakoid membrane of the chloroplast. Chlorophyll a consists of a chlorin ring, where four nitrogen atoms surround a magnesium ion. Several side chains and hydrocarbon tails are also attached to

the chlorin ring. The C-7 position of the chlorin ring is attached to a methyl group in chlorophyll a.

Chlorophyll b

The green pigment which is responsible for collecting light energy and passing into chlorophyll a during photosynthesis is Chlorophyll b. It is found in plants and green algae. In chlorophyll b most effectively absorbing wavelengths of spectrum are 455nm and 642nm which are responsible for violate and red colors respectively. Chlorophyll b reflects a yellow green color. In land plants most of chlorophyll b is found light trapping antenna in photo system p-680.The structure of chlorophyll b is mostly similar to Chlorophyll a.

2.3 Functions of chlorophyll

Chlorophylls are ubiquitous pigments in the plant kingdom that play a key role in photosynthesis, a vital function for life on Earth. The main activity of chlorophyll is producing glucose which helps to sustain trees life in our environment. In this process , NADP and ATP also produced and these are transferring electrons to molecules and also holding energy in protein bonds. Chlorophyll does not produce oxygen directly for these reasons these electrons are driven this process and helps water molecules to be splited. Through this process oxygen produce and released in our environment.Chlorophyll stimulates tissue growth,it prevents the advancement of bacteria and speeds up the wound healing process. Chlorophyll is used as a coloring agents due to its selective absorbance of light of certain wavelengths and consequents green colours. It plays a light harvesting roles in photosynthesis process.

2.4 Susceptibility of chlorophyll

Susceptibility of chlorophyll mainly depends on temperature, PH, oxygen, metals and different enzymes. In different conditions molecular structure of chlorophyll causes discolorations .It's mainly show the lower quantity of chlorophyll.

The photosynthetic capacity, chlorophyll fluorescence, chloroplast ultrastructure , and antioxidant activities in the leaves were evaluated. Exposure to 40 and 45°C for 6 h resulted in a significant decrease in the photosynthetic rate (P_n), carboxylation efficiency , the maximal photochemical efficiency of photosystem II, and the light-saturated photosynthetic rate, which were related to the reduction of CO_2 assimilation, inactivation of photosystem II and photosynthetic electron transport.
Effect of pH on the chlorophyll degradation and visual green colour loss in blanched green peas were studied at 70, 80, 90 and 100 °C in buffered solutions of pH 5.5, 6.5 and 7.5. The degradation of chlorophylls *a* and *b* followed a first-order reaction and the temperature-dependence of these reactions was modelled by the Arrhenius equation.
(Koca, 2007)

2.5 Chlorophyll and carbon sequestration relationship

Carbon sequestration is the process involved in carbon capture and the long-term storage of atmospheric carbon dioxide. Carbon, in the form of carbon dioxide can be removed from the atmosphere by chemical processes, and stored in stable carbonate mineral forms. This process is known as 'carbon sequestration by mineral carbonation or mineral sequestration. The process involves reacting carbon dioxide with abundantly available metal oxides either MgO or CaO to form stable carbonates.

Chapter-3

3. Description of methods used for chlorophyll determination

3.1 Description of methods

There are different method which are used for the extraction and determination of chlorophyll from leaf. Some of them are described bellow

Method No: 1

Using Acetone

Samples should be collected on the day of lab, kept out of bright light, and refrigerated until just before coming to lab. Filtered samples for chlorophyll analysis may be stored up to a few weeks if they are kept in a desiccator and frozen. The following procedures should be conducted in dim light.

After shaking the sample, measure out 50 ml in a graduated cylinder and place in a small plastic bottle. Preserve with Lugol's solution. Pour the sample into one of the settling chambers provided. This sample can be used later for identifying algal species.

Assemble the filtering apparatus using a Whatman GF/C filter. Shake raw water sample and quickly pour at least 50ml into a graduated cylinder. Note volume, then pour into the filtering apparatus. Filter at low suction pressure. More sample can be added as long as the final volume filtered is recorded (the entire sample should be filtered if the filter paper does not clog first). While there is still about 5 ml of water left in the filter tower, add 10 ml of $MgCO_3$ suspension. Make sure that cover the filter evenly and that shake the suspension immediately before use.

If sample is to be analzyed later, carefully fold filter in half and place in pre-cut foil labeled with: date, lake, depth, volume filtered. Place foil in container filled with desiccant in freezer.

Acetone should be used only under a fume hood.

Remove the filter containing the algae sample and place in a cold tissue grinder tube.

Add 4-5 ml of 90% alkaline acetone (keep acetone on ice).

Grind the sample filter vigorously for approximately 30 seconds while keeping the tube on ice. Then rearrange the filter if it has been compressed to the bottom of the tube. Grind sample for another 30 seconds.

Dump contents of grinding tube into a 15ml graduated centrifuge tube.

Rinse pestle and grinding tube with 90% acetone into the graduated centrifuge tube.

Centrifuge tube for 15 minutes at medium speed.

Record volume of extract in the centrifuge tube.

Fill cuvette with extract and read absorbance at 750 (turbidity blank), 665,663, 645, and 630 nm on a spectrophotometer. Use acetone as a reference.

Add 25 µl of 2N HCl to the extract, mix thoroughly and wait at least 1 minute.

Reread absorbance at the previous wavelengths.

Rinse out cuvette with acetone and shake dry prior to use with the next sample.

Method No: 2

Using Ethanol

After shaking the sample, measure out 50 ml in a graduated cylinder and place in a small plastic bottle. Preserve with Lugol's solution. Pour the sample into one of the settling chambers provided. This sample can be used later for identifying algal species.

Assemble the filtering apparatus using a Whatman GF/C filter. Shake raw water sample and quickly pour at least 50ml into a graduated cylinder. Note volume, then pour into the filtering apparatus. Filter at low suction pressure. More

sample can be added as long as the final volume filtered is recorded (the entire sample should be filtered if the filter paper does not clog first). While there is still about 5 ml of water left in the filter tower, add 10 ml of $MgCO_3$ suspension. Make sure that cover the filter evenly and that shake the suspension immediately before use

If sample is to be analzyed later, carefully fold filter in half and place in pre-cut foil labeled with: date, lake, depth, volume filtered. Place foil in container filled with desiccant in freezer.

Remove the filter and place it in a test tube with 15 ml 90% ethanol.

Carefully heat the tube in a water bath (a beaker on a hot plate) to boiling (78 degree C). Allow to boil for 1 - 2 minutes.

Place a rubber stopper lid into the test tube and thoroughly mix the contents of the tube on a vortex mixer.

Remove the filter and centrifuge the sample at half speed for 5 minutes and transfer about 10 ml of the supernatant to the spectrophotometer cuvette.

Zero the spectrophotometer using 90% EaOH at 665 nm, then read the transmittance of the sample at 665 nm (Eb665) and 750 nm (Eb750). The measurement at 750 nm is a correction for turbidity.

Acidify the sample by adding 0.1 ml of 2N HCl (or 0.01 ml of 4N HCl per ml of extract) directly to the cuvette.

Mix well, and after 5 minutes read the transmittance at 665 nm (Ea665) and 750 nm (Ea750). Using the same procedure, acidify a cuvette of 90% EtOH to see if the blank must be corrected.

Method No: 3

Using Dimethylformamaid (DMF)

DMF is toxic and messy. We should wear rubber gloves and work under a fume hood.

After shaking the sample, measure out 50 ml in a graduated cylinder and place in a small plastic bottle. Preserve with Lugol's solution. Pour the sample into one of the settling chambers provided. This sample can be used later for identifying algal species.

Assemble the filtering apparatus using a Whatman GF/C filter. Shake raw water sample and quickly pour at least 50ml into a graduated cylinder. Note volume, then pour into the filtering apparatus. Filter at low suction pressure. More sample can be added as long as the final volume filtered is recorded (the entire sample should be filtered if the filter paper does not clog first). While there is still about 5 ml of water left in the filter tower, add 10 ml of $MgCO_3$ suspension. Make sure that cover the filter evenly and that shake the suspension immediately before use.
If sample is to be analzyed later, carefully fold filter in half and place in pre-cut foil labeled with: date, lake, depth, volume filtered. Place foil in container filled with desiccant in freezer.

Fold filter and place in a small amber vial with 7 ml of DMF. Make sure filter is submerged in solvent.

Place in freezer for at least 12 hours

Heat vials in degree C water bath for 15 minutes. Allow vials to cool before handling.

Gently shake samples for for 15-20 seconds. Pour contents of vial into a graduated centrifuge tube. Rinse filter inside vial and inside vial walls with DMF. Shake for 5 seconds. Pour into centrifuge tube. Repeat rinse.

Record centrifuge tube volume and then centrifuge for 10 minutes at medium speed.

Pour contents of tube into a cuvette and read on spectrophotometer or fluorometer.

Add one drop of 10% HCl to the cuvette, mix and read again.

Dispose of DMF waste in a labeled container.

Calculations

Convert all transmittance to absorbances, to be used in the following equations:

- kChla µg/l = 29.6[(Eb665 - Eb750) - (Ea665 - Ea750)] * ev/(V * P)
- Phaeophytin = (20.8 * Ea665 * ev/V) - Chla

where,

- Ea= absorbance, acidified
- Eb = absorbance, base
- ev = volume of alcohol used in extraction, in ml
- V = volume of filtered sample, in liters
- P = path length (Note that this test assumes use of a 1 cm path length)

Method No 4:

Using DMSO

DMSO is a hazardous substance. Before handling, ensure nitrile gloves, lab coat and safety glasses are worn. All work with DMSO must take place under a fume hood, as DMSO is an irritant if inhaled. DMSO is a C1 combustible liquid. It should not be used or stored near any source of ignition and should be stored well away from oxidising agents. DMSO is not a dangerous good.

Add 1.0 mL DMSO to each Eppendorf containing macerated leaf.

Place eppendorf tube into matrix mill and mix.extract at 30 hz for 2 minutes.

Centrifuge and remove supernatant.

Add 1.0 mL of DMSO to pellet and re-extract.

Centrifuge, remove supernatant and add to other 1.0 mL

Determination by using spectrophotometer

Calibrate at zero absorptance using a blank of pure DMSO.

Measure absorbance of blank and samples at 645 and 663 nm no longer than 20 minutes after extraction procedure completed.

A blank of pure DMSO will be included in each run. The absorbance of this blank will be subtracted from the absorbance readings of each sample before any calculations have been made.

Calculations

There are a lot of different equations for calculating amounts of chlorophyll. Among them Arnon's (1949) equations for calculation of chlorophyll extracted in 90% acetone were proven by Hiscox & Israelstam (1979) to be virtually identical to chlorophyll extracted in DMSO.

Arnon's (1949) equations are as follows.

Chla (g l-1) = 0.0127 A663 – 0.00269 A645

Chlb (g l-1) = 0.0029 A663 – 0.00468 A645

Total Chl (g l-1) = 0.0202 A663 + 0.00802 A645

Chapter-4

4 Materials

4.1 Sample Collection

Collecting tree branch of Baen (*Avecenia officinalis*),Ora (*Sonneratia caseolaries* L.) , Keora(*Sonneratia apetala*) was collected from the Sundarbans mangrove forest .

4.2 Sample Preparation

After collecting, the samples were covered in polybags and these branches also put into bottles those are filled with sugar water. Then quickly reached in campus and prepared as early as possible for experiments.

4.3 Extraction

The next step involves delivering DMSO to a vial. DMSO is a hazardous substance. Before handling, ensure nitrile gloves, lab coat and safety glasses are worn. All work with DMSO must take place under a fume hood, as DMSO is an irritant if inhaled. DMSO is a C1 combustible liquid. It should not be used or stored near any source of ignition and should be stored well away from oxidising agents. DMSO is not a dangerous good. Add 1.0 mL DMSO to each Eppendorf containing macerated leaf .Place eppendorf tube into matrix mill and mix. Extract at 30 hz for 2 minutes. Centrifuge and remove supernatant. Add 1.0 mL of DMSO to pellet and re-extract. Centrifuge, remove supernatant and add to other 1.0 mL .

4.4 Spectrophotometer

Calibrate at zero absorptance using a blank of pure DMSO. Measure absorbance of blank and samples at 645 and 663 nm no longer than 20 minutes after extraction procedure completed. A blank of pure DMSO will be included in each run. The absorbance of this blank will be subtracted from the absorbance readings of each sample before any calculations have been made.

4.5 Color identification

After determining the color and it may ready to put spectrophotometer to know the wavelength.

4.6 Wavelength analysis

Various absorbances in different wavelength was measured by using spectrophotometer.

4.7 Methods:

Firstly, I was preheating the water in water bath at 65 degree Celsius. Then I took 7ml DMSO in a glass vials and put into the preheated water of the water bath for 5 minutes. I took three disks (each 3.038cm2; approx. 100mg fresh leaves wt. total) from each leaf sample into the glass vials. Time is a critical factor during chlorophyll extraction and it may vary with species. It is necessary to give trail of different time for extraction. After the extractions samples removed from the water bath and topped to 10ml.The spectrophotometer calibrated at zero absorbance using a blank of pure DMSO. Absorbance of blank and samples at 645 and 663 nm wave lengths . The elapsed time between removal from the water bath and completion spectrophotometer measurements was in order of 20 minutes.

Arnon's (1949) equations are as follows.

Chla (g l-1) = 0.0127 A663 – 0.00269 A645

Chlb (g l-1) = 0.0029 A663 – 0.00468 A645

Total Chl (g l-1) = 0.0202 A663 + 0.00802 A645

Chapter-5

5. Results and Discussion

Total Chlorophyll

Total chlorophyll for *A. officinalis*, *S. apetala* and *S. caseolaris* were found as 0.005513 gl^{-1}, 0.019514 gl^{-1}, 0.011782 gl^{-1} respectively (Table-1). Among these three species, *S. apetala* has the highest amount of Total chlorophyll and the lowest amount was found for *A. officinalis*. there was significant variation of total Chlorophyll among the species ($p<0.05$) (Table 2 and Table 3, Fig 1).

Table-1: Chlorophyll content in the leaves of *A. officinalis*, *S. apetala* and *S. caseolaris*

Species	Chla(gl-1)	Chlb(gl-1)	Total Chl(gl-1)
A. officinalis	0.004071	0.001443	0.005513
S. apetala	0.002789	0.016733	0.019514
S. caseolaris	0.00934	0.002444	0.011782

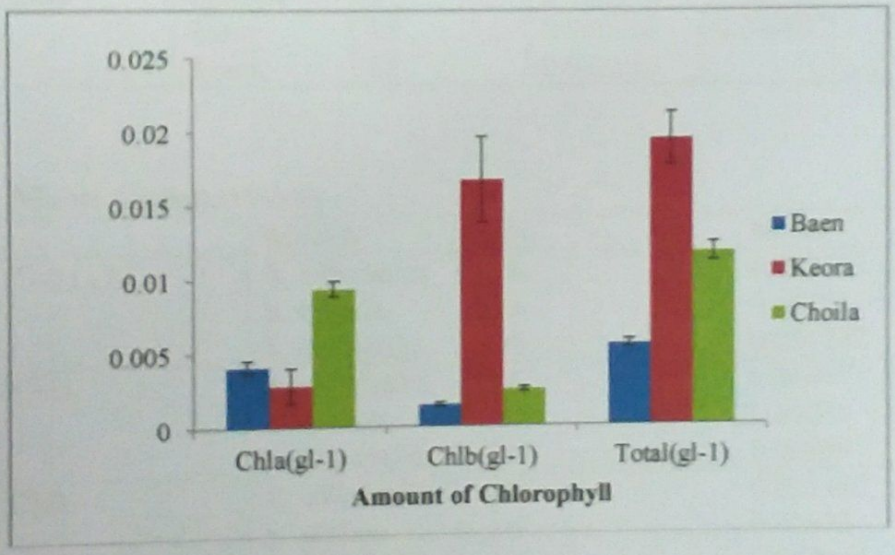

Fig 1: Chl a, chlb and Total chlorophyll of *A. officinalis*, *S. apetala* and *S. caseolaris*

Chlorophyll *a* and Chlorophyll *b*

Chlorophyll *a* was found to be the highest for *S. caseolaris* (0.00934 gl^{-1}) and lowest for *S. apetala* (0.002789 gl^{-1}). Whereas, chl *b* was found highest for *S. apetala* (0.016733 gl^{-1}) and lowest in *S. caseolaris* (0.002444 gl^{-1}). One way analysis of variance for chl b was found to be significantly different ($p<0.05$) (Table 2). The amount of Chl *a* in *S. caseolaris* was significantly ($p<0.05$) different fom *S. apetala* and *A. officinalis* (Fig. 1,Table 3).

Variance Comparisons

	Source	DF	AdjSS	AdjMS	F-Value	P-value
Total Chl	Species	2	0.00059	0.000295	40.18	0
	Error	15	0.00011	0.000007		
	Total	17	0.0007	0.000072		
Chla	Species	2	0.000145	0.000072	19.38	0
	Error	15	0.000056	0.000004		
	Total	17	0.000201			
Chlb	Species	2	0.000878	0.000439	25.84	0
	Error	15	0.000255	0.000017		
	Total	17	0.001133			

Mean Comparisons

	Species	N	Mean	StDev
Total Chl	A. officinalis	6	0.005513	0.00073
	S. apetala	6	0.01951	0.00435
	S. caseolaris	6	0.011782	0.001613
Chla	A. officinalis	6	0.004071	0.001093
	S. apetala	6	0.00279	0.00289
	S. caseolaris	6	0.00934	0.001284
Chlb	A. officinalis	6	0.001443	0.000378
	S. apetala	6	0.01673	0.00712
	S. caseolaris	6	0.002444	0.000357

Tukey Pairwise Comparisons

	Species	N	Mean	Grouping
Total Chl	S. apetala	6	0.01951	A
	S. caseolaris	6	0.011782	B
	A. officinalis	6	0.005513	C
Chl a	S. caseolaris	6	0.00934	A
	A. officinalis	6	0.004071	B
	S. apetala	6	0.00279	B
Chl b	S. apetala	6	0.01673	A
	S. caseolaris	6	0.002444	B
	A. officinalis	6	0.001443	B

Chapter-6

6. Conclusion

Sonneratia apetala has the highest amount of total chlorophyll in comparison to other two species, *A. officinalis* and *S. caseolaris.*

Chapter-7

7. Recommendation

There is a great opportunity of extracting chlorophyll of mangrove species. However, the following issues need to be investigated –

a. All region of mangrove forest species are selected for these experiments.
b. More experiments are needed with available methods of mangrove species.
c. Major mangrove species should be covered with these experiments.

Chapter-8

8. References

A. E. Solovchenko, O. B. (2001). A Spectrophotometric Analysis of Pigments in Apples . *RUSSIAN JOURNAL OF PLANT PHYSIOLOGY vol48 No5* , 693-700.

Aris Hosikian, S. H. (2010). Chlorophyll Extraction from Microalgae:A Review on the Process Engineering Aspects. *International Journal of Chemical Engineering* , 1-11.

Asish Parida, A. B. (2002). Stress Causes Changes in Photosynthetic Pigments, Proteins, and Other Metabolic Components in the Leaves of a True Mangrove, Bruguiera parviflora, in Hydroponic Cultures . *of Plant Biology 45 (1)* , 28-36.

Biber, P. D. (2007). Evaluating a Chlorophyll Content Meter on Three Coastal Wetland Plant Species. *A Journal on Agricultural, Food and Enviornmental Sciences vol 1* , 1-11.

E□ectofdi□erentdryingmethods onchlorophyll, a. c. (2015). SandopuSravanKumar,PrabhakaranManoj,NandiniPShettyand ParvatamGiridhar. *J Sci Food Agric* , 1812-1820.

Koca, N. (2007). *Effect of pH on chlorophyll degradation and colour loss in blanched green peas* , 609-615.

Linda A. Lawton, C. E. (1994). Extraction and High-performance Liquid Chromatographic Method for the Determination of Microcystins in Raw and Treated Waters . *The Analyst vol 119* , 1525-1528.

LORENZE, C. J. (1966). A method for the continuous measurement of in vivo chlorophyll concentration. *Deep-Sea Research, Vol. 13* , 223-227.

MOTS, j. F. (1965). SPECTROPHOTOMETRIC CHARACTERISTICS OF CHLOROPHYLLS a AND b AND THEIR PHEOPHYTINS IN ETHANOL . *BIOCHIMICA ET BIOPHIYSICA ACTA 109* , 448-453.

Porra, R. J. (2002). The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. *Photosynthesis Research 73* , 149-156.

S.Jeffery, F. V. (2011). A quantitative review of the effects of biochar application to soils on crop productivity using meta- analysis. *Agriculture, Ecosystems and Enviornment 144* , 175-187.

SEELY, L. P. (1966). *The Chhrophylls.* New York and London: ACADEMIC PRESS.

SESTAK, Z. (1959). A Method of Storage of Leaf Samples for Chlorophyll Analysis . *BIOLOGIA PLANTARUM (PRAHA) 1(4)* , 287-294.