



NAAS Rating  
2012:1.3; 2013-16: 2.69  
2017-19: 3.98

Received on:  
12<sup>th</sup> October 2019

Revised on:  
8<sup>th</sup> November 2019

Accepted on:  
14<sup>th</sup> November 2019

Published on:  
1<sup>st</sup> December 2019

Volume No.  
Online & Print  
118 (2019)

Page No.  
15 to 18

*Life Sciences Leaflets is an international open access print & e journal, peer reviewed, worldwide abstract listed, published every month with ISSN, RNI Free-membership, downloads and access.*

## STUDY OF CHLOROPHYLL VARIATION “A” AND “B” FROM SELECTED AQUATIC PLANTS

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### ABSTRACT:

Chlorophyll a, b, c, d, Carotene, Xanthophylls are present in all higher plants. Then chlorophyll a and b are most important for plants. Make extract of chlorophyll in 80% acetone and estimate by spectrophotometer in Optical Density. Chlorophyll a and b are measured in amount of mg/mL. Chlorophyll is estimate of hydrophytes plants in *Ammannia baccifera* L., *Eichhornia crassipes* (Mart.) Solms, *Hydrilla verticillata* (L.f.)Royle, *Nymphaea pubescens* Willd, *Bacopa monnieri* (L.) Wettst. and *Typha angustifolia* L. The range of chlorophyll contains are 1.9826 to 6.9220 mg/mL for chlorophyll a and 2.6598 to 10.7774 mg/mL for chlorophyll b. Concentration of chlorophyll a and b was calculated using Arnon method. Chlorophyll content was higher in *Nymphaea pubescens* than other Aquatic plants.

**KEY WORDS:** Chlorophyll, Extract, Spectrophotometer, Optical Density, Patan district.

### INTRODUCTION:

Chlorophyll is the principal photoreceptor in photosynthesis, the light-driven process in which carbon dioxide is "fixed" to yield carbohydrates and oxygen. Chlorophyll measurement is an important tool to evaluate it plays an important role in plant metabolism and any reduction in chlorophyll content corresponds directly to plant growth. Seasonal variation in the chlorophyll pigments i.e. total chlorophyll, chlorophyll a and chlorophyll b in the leaves. The present study showed significant variation of pigment (total chlorophyll, chlorophyll a and chlorophyll b).

Green colour of plant is due to the present of chlorophyll. All higher plants, algae, diatoms, brown algae have contained chlorophyll a, b, c, d, Carotene, Xanthophylls and other pigments. The difference between these two chlorophylls is a methyl moiety in chlorophyll a replaced by a formyl group in chlorophyll b. The ratio of chlorophyll a to chlorophyll b in higher plants is approximately 3:1. Chlorophyll absorbs light mainly in the red (650 – 700 nm) and the blue - violet (400 – 500 nm) regions of the visible spectrum. Green light (~550 nm) is not absorbed but reflected giving chlorophyll its characteristic color. Chlorophyll a possesses a green-blue color, and chlorophyll b possesses a green-yellow colour. (Arnon, 1949).

### **MATERIALS AND METHOD:**

Collection of aquatic plants from Patan district. *Ammannia baccifera* L. *Eichhornia crassipes* (Mart.) Solms, *Hydrilla verticillata* (L.f.) Royle, *Nymphaea pubescens* Willd, *Bacopa monnieri* (L.) Wettst. and *Typha angustifolia* L. were collected from different lake in Patan district. After collection separate 1-gram fresh leaf from each species. The chlorophyll content was estimated according to the method of Arnon (1949). About 1 gr of leaf sample was cut in to small pieces and homogenized in a pre-cooled mortar and pestle using 80% (V/V) acetone. A pinch of calcium carbonate was added while grinding. The extract was centrifuged at 3000 rpm for 15 min and made up to 25 ml with 80% (V/V) acetone. The clear solution was transferred to a colorimeter tube and the optical density was measured at 645 nm and 663 nm wavelengths by Spectrophotometer. Calculate Chlorophyll after measured Optical Density using following formula.

**Table 1. Formula for Calculate Chlorophyll**

<b>Chlorophyll 'a' (<math>\mu\text{g/ml}</math>)</b>	<b>=</b>	<b>(12.7 x O.D. at 663 nm) – (2.69 x O.D. at 645 nm)</b>
<b>Chlorophyll 'b' (<math>\mu\text{g/ml}</math>)</b>	<b>=</b>	<b>(22.9 x O.D. at 645 nm) - (4.08 x O.D. at 663 nm)</b>
<b>Total chlorophyll (<math>\mu\text{g/ml}</math>)</b>	<b>=</b>	<b>(20.2 x O.D. at 645 nm) + (8.02 x O.D. at 663 nm)</b>

The chlorophyll content was expressed as mg chlorophyll per gram fresh weight of the leaf. (Estimation of chlorophyll (Arnon, 1949).

### **RESULTS AND DISCUSSION:**

Total chlorophyll found in descending manner like *Nymphaea pubescens*, *Hydrilla verticillata*, *Bacopa monnieri*, *Ammannia baccifera*, *Eichhornia crassipes* and *Typha angustifolia*. *Nymphaea pubescens* is floating hydrophytes and direct contact of sun light. So it has more amount of chlorophyll. *Hydrilla verticillata* (l.f.) Royal (Bam) is submerged hydrophytes plants then it has

less amount of chlorophyll than *Nymphaea pubescens*. *Bacopa monnieri* (L.)Wettst leaves are reddish colour. Then it has less amount of chlorophyll. *Eichornia crassipes* (mart) Solms leaves succulence's yet it has less chlorophyll than *Eichornia crassipes*(mart)Solms. *Typha angustifolia* L. and *Eichornia crassipes*(mart)Solms leaves are succulence's but *Typha angustifolia* L. has less chlorophyll than *Eichornia crassipes*(mart)Solms and it has another pigments.

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Table 2. Optical Density taken by spectrophotometer:

Wavelength	<i>Ammannia baccifera</i> (OD)	<i>Bacopa monnieri</i> (OD)	<i>Eichornia crassipes</i> (OD)	<i>Hydrilla verticillata</i> (OD)	<i>Nymphaea pubescens</i> (OD)	<i>Typha angustifolia</i> (OD)
645nm	0.33	0.32	0.16	0.46	0.59	0.15
663nm	0.38	0.54	0.19	0.57	0.67	0.19

Table 3. Amount of chlorophyll “a” and “b” in mg/mL:

SN	Species	Chlorophyll “a” (mg/mL)	Chlorophyll “b” (mg/mL)	Total chlorophyll (mg/mL)
1	<i>Ammannia baccifera</i>	3.9383	6.0066	9.7136
2	<i>Bacopa monnieri</i>	5.9972	5.1248	10.7948
3	<i>Eichornia crassipes</i>	1.9826	2.8888	4.7558
4	<i>Hydrilla verticillata</i>	6.0016	8.2088	13.8634
5	<i>Nymphaea pubescens</i>	6.9220	10.7774	17.2914
6	<i>Typha angustifolia</i>	2.0095	2.6598	4.5538

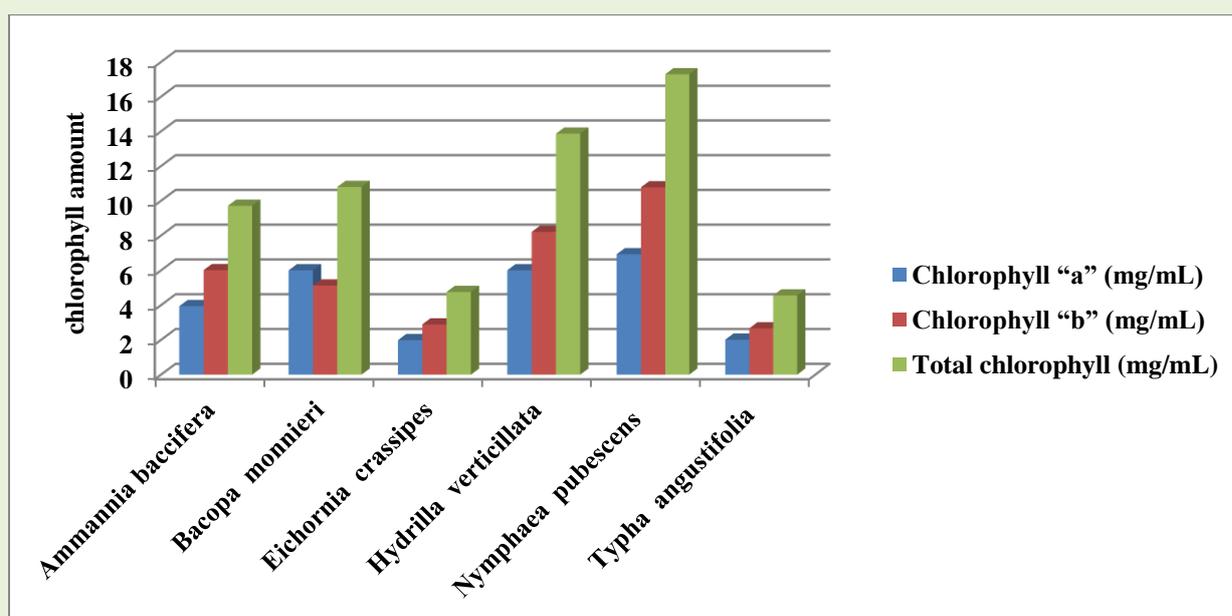


Fig: 1: Indicates Chlorophyll amount in studied aquatic plants