

NILAMANI MAHAVIDYALAYA



RUPSA, BALASORE

A

**PROJECT REPORT OF
"DEPARTMENT OF BOTANY"**

6th Semester +3 Examination 2022

Guided by :

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Lect . in Botany**

Submitted by :

**Group 1 Students
+3rd Year Botany (H)**

[Title of the Project-Effect of CO₂ in Photosynthesis of Different Aquatic Plant and their Chlorophyll- 'A' estimation]

A project report submitted in partial fulfillment of the degree of bachelor of Science by

The Group -1 Students.

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Under the Guidance of

(Dr. Ardhendu Kumar Dash)

Nilamani Mahavidyalaya , Rupsa ,Balasore

DECLARATION

We Group-1 Students do hereby Declare that the project report entitled "Effect of CO₂ in photosynthesis of different plants and their chlorophyll 'A' estimation" being submitted to Nilamani Mahavidyalaya, Rupsa, Balasore, Odisha for the award of bachelor of science is an original piece of work done by us and the same has not been submitted elsewhere for any other academic degree Or diploma to this college or any other college/university.

Group -1 Students

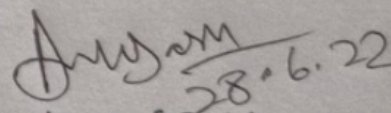
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CERTIFICATE

This is to certify that the project report entitled "Effect of CO₂ in photosynthesis of different plants and chlorophyll 'A' estimation" submitted by group-1 student . for the a ward of the degree of bachelor of Science form Nilamani Mahavidyalaya Rupsa, Balasore, Odisha, India is a benefit record of work carried out by them under my guidance. Neither this project report nor any part of it has been submitted for any degree of academic award elsewhere.

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ABSTRACT

Form the result of chapter-1, chapter-2, chapter-3,chapter-4 we concluded that the photosynthesis occurs in different aquatic plant that is hydrilia contain 10.4, marsilea contains 5,limnophylla contain6,potamogeton contain 7 and ceratophyllum contain 3 units of bubbles and the heighest number of bubbles occurs in hydrilla plant is 9 unit bubbles and the lowest bubbles accurs in ceratophyllum plant that is 3 unit of bubbles.

When the CO_2 added in the photosynthesis the plant bubbles are increase in hydrilla plant is 9 Marsilia contain 6, potamogeton contain7, limmophila contain 7 & ceratophyllum plant contains 8 No of bubbles so in the plant of hydrilla large no of bubbles are evoived and in the ceratophyllum plant the smail no of bubbles are evolved.

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Photosynthesis on different aquatic Plant

The process by which green plants and some other organism use sun light to synthesize nutrients from carbon dioxide and water. Photosynthesis in plants generally involves the green pigments chlorophyll and generates oxygen as a oil product.

Photosynthesis in aquatic plants ability to perform photosynthesis is the main distinguishingly feature between green plants and other organisms on earth in this chemical process, carbon dioxide and water are combined in presence of light energy to produce carbohydrate and other by product, most plants and many bacteria get their food by photosynthesis , aquatic plants get water and carbon dioxide from their aquatic environment and like the/land plants, light energy from the sun. Even through the plant is under water, it still gets its energy from the sun because sunlight can pass through water. The leaves on the surface of water gather light and carbon dioxide for photosynthesis while the many cutiele prevents excess water absorption from the surrounding environment. So, absorpthion of carbon dioxide and light is not an issue for aquatic plants having floating leaves.

Photosynthesis involves the same molecules and chemical reactions in land plants and aquatic plants, floating plants photosynthesize much like plant that grow on land. However the process presents more of a challenge for aquatic plants if they are fully submerged below the surface of the water, measuring photosynthesis via the production of oxygen. Oxygen can be measured by counting bubbles evolved from pond weed or by using the audus apparatus to measure the amount of gas evolved over a period of time.

Photosynthesis involves the same molecules and chemical reactions in land plants and aquatic plants, floating plants photosynthesize much like plants that grow on land. However the process presents more of a challenge for aquatic plants if they are fully submerged below the surface of the water.

Aquatic plants have waxy leaves because the aquatic plants remain submerged in the water most of the time therefore for gathering more sunlight their leaves are broad and because they are inside water therefore to reduce sap loss due to osmosis their leaves adopted a waxy covering.

In sugar cane, which is one of the most efficient plants, about 8% of the light absorbed by the plant is preserved as chemical energy given that photosynthesis is the direct or indirect source of all human food, thus kind of slaking is clearly just not good enough oxygen dissolves into water from two sources i.e. the atmosphere and from plants in the water. The primary source of oxygen for a pond is from microscopic algae or submerged plants, in the presence of sunlight, these produce oxygen through photosynthesis and release this oxygen into the pond water, aquatic plants require special adaptations for living submerged in water or at the water's surface. The most common adaptation is aerenchyma, but floating leaves and finely dissected leaves are also common aquatic plants. Aquatic plants can only grow on water or in soil that is permanently saturated with water. They absorb carbon dioxide from atmospheric air through their stomatal openings (present in upper and lower side of leaves) water from the soil through their root system and last but not the least, radiant energy from sunlight, hence land plants undergo photosynthesis naturally without any special adaptations.

The stem of the aquatic plants is flexible because it helps the leaves to float, they bend with the flow of water so the plants do not get damaged by strong current, the stems of such plants may have hollow, air filled pockets which help the plants to remain at the sunlight surface. Underwater plants provide oxygen, food and shelter. The health of submerged aquatic vegetation is an important environmental indicator of overall ocean and estuary health, sea grasses in bays and lagoons for instance are vital to the success of small invertebrates and fish plants such as duck weed, mosquito fern, water hyacinth and water meal and free floating submerged floating-leaved - these plants are anchored by roots to the bottom of the pond but their leaves and flowers grow to and float on the water surface.

(1) Photosynthesis in Hydrilla plants

The point is that Hydrilla and few other submerged Hydrophytes are a working model to demonstrate the effect of environmental factors on photosynthesis. Hydrilla has a stem with a system of air cavities in the inverted plants all oxygen evolved in photosynthesis is evolved as bubbles from the cut end.



Hydrilla causes significant environmental and economic problems in Florida and elsewhere, its long stems often grow to the surface to form large, dense infestations that crowd out and replace native aquatic plants, its stems are slender, branched and up to 25 feet long. Hydrilla is a genus of aquatic plant usually treated as containing just one species. *Hydrilla verticillata* through some botanists divide it into several species.

Species - $\textcircled{11}$ *Verticillata*

Family - Hydrocharitaceae

Kingdom - Plantae

Genus - *Hydrilla* Rich

Marsilea is a genus of approximately 65 species of aquatic ferns of the family. These small plants are of unusual appearance and do not resemble common ferns. Marsilea quadrifolia, an amphibious fern has the ability to develop photochemistry of photosynthesis, in this study we found that the four leaflets of the amphibious fern *Marsilea quadrifolia* are capable of adjusting their leaflet angle. An efficient micropropagating protocol has been developed for *Marsilea quadrifolia* through direct organogenesis, *Marsilea* is heterosporous i.e. it produces two types of spores – microspores and megaspores. These spores are produced in microsporangia and megasporangia respectively. These sporangia are borne in special type of spore producing organ called sporocarp.

Divisions of Marsilea

Kingdom – Plantae

Family – Marsileaceae

Class – Polypodiopsida

Order – Salviniiales

(3) Photosynthesis in *Potamogeton crispus*

Botanical name – *Potamogeton crispus* *

Common name – pond weed

Potamogeton crispus is a submerged aquatic perennial that can reach 30-80 cm (1-2.5 feet) in length. The parts are spindle shaped measure 1.5-3 cm (0.5-1 in) and can be located terminal or axillary. The stems of this plant are flattened, there are two ranks of sessile leaves that are arranged spirally, the leaves are linear or long in shape, measuring 3-8 cm (1-3 in) long and 5-12 mm (0.2-0.5 mm) wide. The leaf margins are undulate and the apex of the leaf is obtuse.

Potamogeton has an apical growth form leading to increasing age of the tissue with depth in the canopy. The light absorbance was higher and independent of areal chlorophyll concentration for floating leaves, but lower and strongly chlorophyll

(2) Photosynthesis in Marsilea plant

Botanical name – Marsilea

Common name – water fern

Marsilea, commonly known as water fern, is represented by about 53 living and fossil species. The living species occur on all parts of the world but are more common in warmer parts of the world, such as tropical Africa and Australia. They are aquatic or amphibious, the aquatic species usually grow in shallow ponds but fruiting bodies are formed only in the terrestrial habitats. The amphibious species grow in water logged soil, partly submerged Marsilea an Australian species.



dependent for submerged leaves. Photosynthesis of submerged leaf forms declined two fold when transferred to water - saturated air, owing to a highly increased respiration. Emphasis was on plants from north temperate salt water and hard water lakes to explore both possibilities of CO₂ limitations.

Genus - Potamogeton

Family - Potamogetonaceae

Order - Alismatales



(4) Photosynthesis in Limnophyllaheterophylla

Botanical name - Limnophyllaheterophylla

Common name - Beremi

An aquatic herb mainly submerged but with shoots that often emerge above the water surface, rooting at nodes, leaves are arranged in whorls of four to ten sessile 2-3 cm long. Below water they are finely twice pinnatifid. Above water they are undivided but shallowly toothed, flowers occur singly,

sessile in the axils of the upper ; leaves about water limnophila indica both non-native species present in the U.S, are frequently cultured as aquarium plants.

Family – Plantaginaceae

Genus – Limnophila



(5) Photosynthesis in ceratophyllum demersum

Botanical name – Ceratophyllum demersum

Common name – fern wort

An aquatic plant, ceratophyllum demersum has stems that reach lengths of 1-3 mts (3-10 feet) with numerous side shoots making a single specimen appear as a large, bushy mass, the leaves are produced in whorls of six to twelve, each leaf 8-40 mm long simple or parted into two the eight thread like segments edged with spiny teeth. They are stiff and brittle, it is monoecious with separate male and female flowers produced in the same plant. The flowers are small 2mm long with eight or more greenish-brown

petals, they are produced in the leaf. The fruit is a small nut 4-5 mm long, usually with two spines, two basal and one apical 1-2 mm long, plants with the two basal and spines very short are sometimes distinguished as *Ceratophyllum demersum* and those with no basal spines sometimes distinguished as *Ceratophyllum demersum*. The photosynthetic carbon fixation rate of *Ceratophyllum demersum* plants was measured in several stages of post-summery photosynthesis in *Ceratophyllum demersum*, carbon fixation rates in relation to the plants physiological stage.



Species - *C. demersum*
Family - Ceratophyllaceae
Order - Ceratophyllales

RESULTS

Tabulations which above discuss of different type of aquatic plants in photosynthesis:-

Sl No	Aquatic plant name	Normal light	No of Bubbles Evolved				No of Bubbles Unit Time
			1 st 5 mnt	2 nd 5 mnt	3 rd 5 mnt	Mean bubbles	
1	Hydrilla	Sunlight	42	45	48	45	9
2	Marsilea	Sunlight	22	25	19	25	5
3	Limnophylla – Heterophylla	Sunlight	37	30	31	30	6
4	Potamogeton-crispüs	Sunlight	30	35	40	35	7
5	Ceratophyllum	Sunlight	25	11	29	11	3

Photosynthesis

Light-dependent reactions

Light-dependent reactions occur in the thylakoid membranes of chloroplasts. They convert light energy into chemical energy in the form of ATP and NADPH. This process involves the absorption of light by photosynthetic pigments, the splitting of water, and the transfer of electrons to various carriers.

The light-dependent reactions produce ATP and NADPH, which are used in the Calvin cycle. The rate of these reactions is directly proportional to the intensity of light.

Chlorophyll *a* is the primary photosynthetic pigment, but other pigments like chlorophyll *b* and carotenoids also play a role. These pigments absorb light energy and transfer it to chlorophyll *a*.

During photosynthesis, plants take in carbon dioxide (which is present throughout the day) and water and turn it into sugar, among other compounds. Plants also produce oxygen during photosynthesis.

Photosynthesis requires light energy and the energy that they "capture" from sunlight to make their own type of chemical energy (ATP). Then they use the ATP to make a type of "sugar" called glucose. They also produce oxygen (O₂) from carbon dioxide and water. When they use the ATP to drive the production of the sugars from the carbon dioxide and water, oxygen gets released. More than 10 billion tons of carbon are "fixed" by plants on earth every year. This means that carbon molecules are converted from being part of a simple gas (carbon dioxide) into more complex molecules (carbohydrates) making carbon available as food for non-photosynthesizers like humans. They also produce oxygen at the same time. Take a look at the tree next to the house. That tree was made by this process of pulling the carbon out of the air and making it into sugar form. Amazing sunlight provides energy in the form of electromagnetic radiation. Sunlight is actually made up of photons, which are very small particles that carry electro-magnetic force in the first step of

photosynthesis, the plants capture a photon and harness its energy in order to start photosynthesis. During photosynthesis, the plants take carbon dioxide (which is present throughout the air) and water which is also on the air and dirt and turn it into sugar, among other compounds, plants also produce oxygen during photosynthesis.

Photosynthesis takes place in two stages, the first of which captures the energy and the second which makes sugars, the first one requires water, because the plant uses the hydrogen atoms in water to collect the energy. The second requires both : sugar contains carbon, oxygen and hydrogen of which the first two come from carbon dioxide and the last comes from water, the excess oxygen is released as oxygen gas.

How does CO₂ affect the growth of a plant?

Increased carbon dioxide levels in air restrict plants ability to absorb nutrients. The rapidly rising levels of carbon dioxide in the atmosphere affect plants absorption of nitrogen, which is the nutrient that restricts crop growth in most terrestrial ecosystems, effects of increasing carbon dioxide levels and climate change on plant growth. Evapotranspiration and water resources the atmospheric carbon dioxide concentration has risen from about 270 parts per million before 1700 to about 355 PPM today.

CO₂ effects on plants increase global warming CA- Trees and other plants help keep the planet cool but rising levels of carbon dioxide in the atmosphere are turning down this global air conditioner.

The increase in photosynthesis caused by e[CO₂] results is an increase in carbohydrate production which alters the plants carbon and nitrogen metabolism. Apart from this direct effect on photosynthesis many physiological process are regulated indirectly particularly via sugar sensing and signaling pathways.

As light intensity increases the rate of photosynthesis will increase as long as other factor are in adequate supply, if carbon dioxide and light levels are high but temperature is low, increasing temperature will have the greatest effect on reaching a higher rate of photosynthesis. The increasing concentration of CO₂ and other "green-

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same molecules and chemical reactions in land plants and aquatic plants. Unlike plants photosynthesize, most like plants that grow on land, however, the process presents more of a challenge for aquatic plants if they are fully submerged below the surface of the water.

Leaves are the main site for photosynthesis, leaves contain chloroplasts, which are the organelles in plant cells where photosynthesis occurs. Chlorophyll that absorbs visible light mainly in red and blue wave lengths, only a few molecules of chlorophyll absorb green wave lengths.

The only difference between photosynthesis in aquatic and land plants is where in their environment they get these nutrients. Land plants get water from the ground through their extensive root system, carbon dioxide from the air through their stomata and energy from the sun.

Plants including aquatic plants produce oxygen and they also use oxygen. Here's how these processes work, during a sunny day dissolved oxygen in water is generally plentiful because photosynthesizing algae and aquatic plants are constantly releasing it into the water, the fizzing is due to the trapped carbon dioxide, just like your soda, carbon dioxide is dissolved in water. Unfortunately, for aquatic plants, gasses diffuse slower in water versus the air this is one reason terrestrial plants cannot survive under water for long periods of time. Aquatic plants depend on carbon dioxide for life and growth, just as fish depend on oxygen. Plants use carbon dioxide during the process of photosynthesis. Carbon dioxide also called CO_2 is found in water as a dissolved gas it can dissolve in water 200 times more easily than oxygen. Aquatic plants depend on carbon dioxide for life and growth just as fish depend on oxygen, plants use carbon dioxide during the process of photosynthesis.

Aqueous carbon dioxide $\text{CO}_2(\text{aq})$ reacts with water forming carbonic acid $\text{H}_2\text{CO}_3(\text{aq})$. Carbonic acid may lose protons to form bicarbonate HCO_3^- and carbonate.

Aim of the Experiment

Determination the rate of photosynthesis under different carbon dioxide concentration.

Requirements

- (1) Wilmutt's bubbler
- (2) Hydrilla plant
- (3) Sodium bicarbonate
- (4) Water
- (5) Vaseline
- (6) Graph paper
- (7) Weight Box

Description of the apparatus

Wilmutt's bubbler consists of a wide mouth glass bottle with a rubber stopper on mouth. The rubber stopper bears a large stemmed reservoir containing an internal jet.

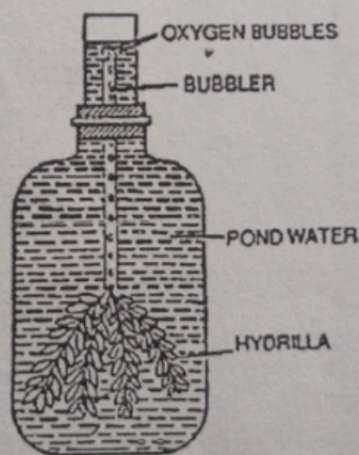


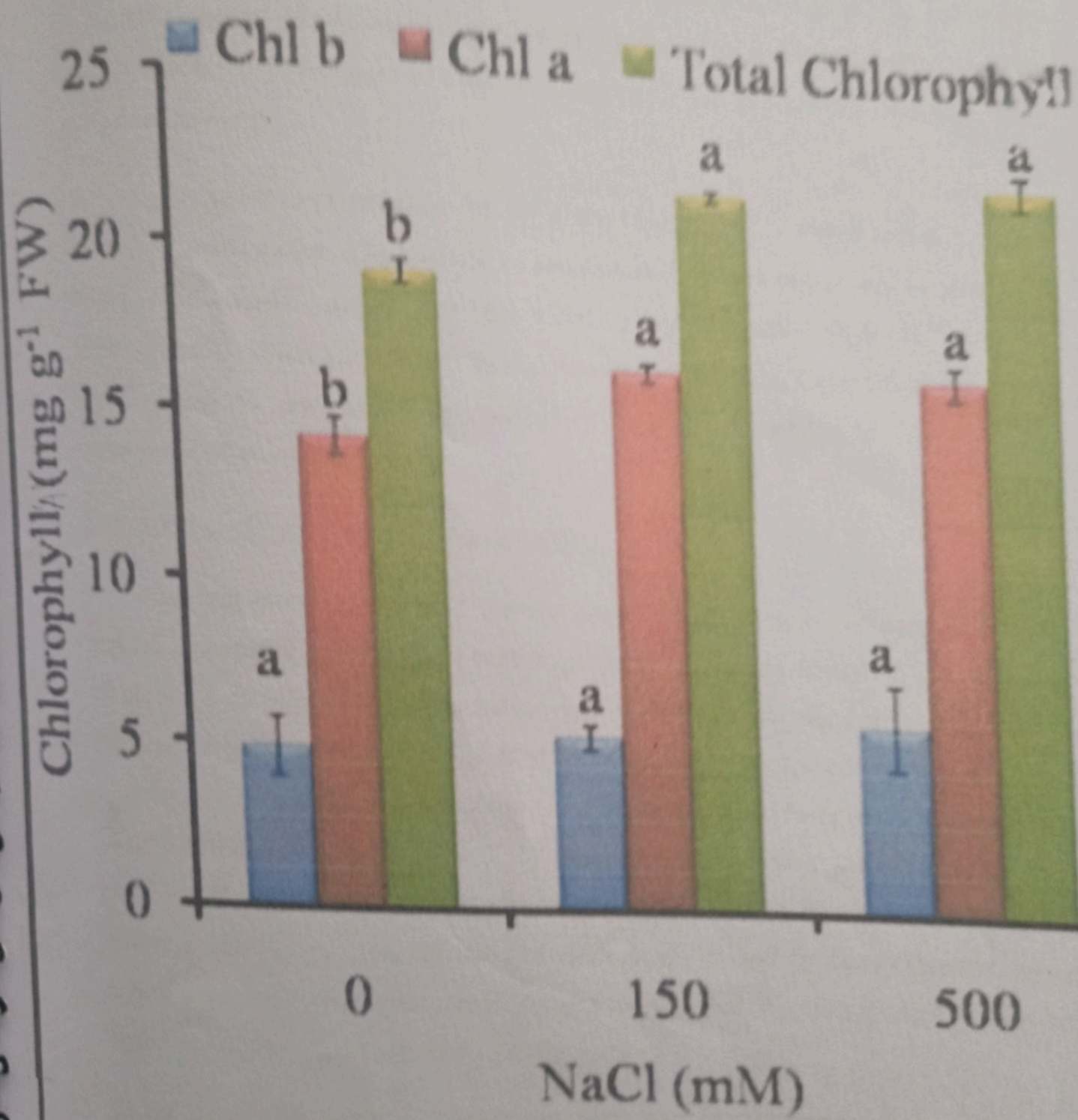
Fig. 5.23. Wilmutt's bubbler.

Clean thoroughly the NaHCO_3 bottle and fill it with good water. Take a healthy hydrilla, give an oblique cut in the stem and insert the stem into the lower end of the bottle which remains inside the bottle, make the apparatus air tight, weigh and adjust the apparatus in the fractions of 0.1 gm, 0.2 gm, 0.3 gm, 0.4 gm. Keep the apparatus in light and add 10 gm of NaHCO_3 in the bottle, stir gently and replace the cover. After some time note the number of bubbles per minute at this rate and different doses of NaHCO_3 and record the number of bubbles evolved per minute. When using a particular dose prior to it (decolor) the water and use fresh water for each dose of NaHCO_3 , record three observations.

Result

Table

Plant Name	No. of Obs.	Conc. of NaHCO_3	Time in Min.	No. of Bubbles Evol.	Mean
Hydrilla	1	0.1 gm	5	3	8
	2			6	
	3			4	
	1	0.2 gm	5	6	
	2			8	
	3			8	
1	0.3 gm	5	6	8	
2			8		
3			6		
1	0.4 gm	5	4		4
2			8		
3			6		
1	0.5 gm	5	12	11	
2			13		
3			12		



Green substance in producers that traps light energy from the sun. Which is photosynthesis chlorophyll is vital for photosynthesis which helps plants get energy from light, amount of chlorophyll present in a plant or in a green solution, not one method which have been used to estimate chlorophyll. No attempt with distilled water was added to make.

Chlorophyll is responsible for the green color of many plants and algae, seen through a microscope. Chlorophyll is concentrated within organelles in structures called chloroplasts, plants are perceived as green chlorophyll is vital for photosynthesis which allow plants to absorb energy from light. Chlorophyll molecules are arranged in and around photosystems that are embedded in the thylakoid membranes of chloroplast.

Estimation of chlorophyll by Arnon's method

Several methods are used for this purpose but a simple and easy incubation method dispensing, grinding and centrifugation procedures is described. The recovery of chlorophyll pigments by incubation method in which tender leaf tissue in 80% buffered acetone at 4°C give higher yield of pigments compared to other methods, estimated by Arnon's (1949) method. The results total chlorophyll (mg / gm) = $OD_{652} \times 1000$, $34.5 \times V/1000 \times W$. In the present study, the chlorophyll was extracted from the leaves from nine medicinal plants and characterized by UV - visible spectroscopy, concentration of chlorophyll-'a' and 'b' was calculated using Arnon's method and chlorophyll content was higher in *innaspodica* than other medicinal plants which are used in study.

Extraction of chlorophyll in DMSO method:-

The extraction of chlorophyll by grinding and centrifugation gives incomplete recovery of chlorophyll, when acetone incubation method was tested against DMSO

Pigment Concentration of the leaves (chlorophyll/carotenoids)

Leaf	Chlorophyll <i>a</i> [$\mu\text{g cm}^{-2}$]	Chlorophyll <i>b</i> [$\mu\text{g cm}^{-2}$]	Carotenoids [$\mu\text{g cm}^{-2}$]
<i>Prunus avium</i>	544.1	165.7	163.4
	534.3	168.7	154.0
	513.4	163.6	144.5
	203.0	61.9	34.4
	181.2	78.2	62.1
	181.2	78.2	62.1
	174.0	67.6	61.9
	174.0	66.7	56.5
	16.08	60.9	61.6
	14.11	58.8	62.2
	115.2	39.7	37.1
	69.7	23.1	51.6
	24.2	22.2	38.6
	18.9	8.7	53.9
7.3	5.5	26.1	
<i>Beta vulgaris</i>	424.1	122.2	105.6
	375.9	98.8	97.0
	335.0	87.0	75.5
	300.1	82.0	85.1
	245.0	59.3	72.4
<i>Fragaria vesca</i>	359.1	97.3	87.8
	128.5	37.0	35.5
<i>Acer negundo</i>	114.0	40.4	8.2
<i>Larix laricina</i>	188.9	42.0	33.1
	178.6	48.5	35.1
	142.2	20.3	21.0
	38.4	8.5	14.4
	5.7	3.9	24.9
<i>Thuja occidentalis</i>	261.4	83.1	58.5
	251.3	90.1	56.7
<i>Glycine max</i>	137.9	86.3	33.1
	20.7	17.2	15.9
	11.2	4.2	20.9
<i>Lycopersicon esculentum</i>	82.8	54.7	14.8
	16.0	7.3	10.4
<i>Helianthus annuus</i>	138.2	49.1	37.9

Pigment analysis: The pigment concentrations were measured right after completion of reflectance measurement. Chl *a* and total Cars were extracted in 80 % acetone. The concentration was determined according to Lichtenthaler (1987). Relative importance of Cars increased with decreasing concentrations of Chl *a* (Fig. 1).

Parameter fitting: The following power and exponential functions were used to describe the relationship between Car/Chl_a and the three pigment indices (PI) shown

REFERENCES

1. [Illegible text]

2. [Illegible text]

3. [Illegible text]

4. [Illegible text]

5. [Illegible text]

CONCLUSION

from the result of chapter-1 chapter-2 chapter-3 we concluded that the photosynthesis occurs in different aquatic plant, that is hydrilla contain 10.4, marsilea contains 5, limnophylla contains, potamogeton contain 7 and ceratophyllum contain 3 units of bubbles and the highest number of bubbles occurs in hydrilla plant is 9 unit bubbles and the lowest bubbles occurs in ceratophyllum plant that is 3 unit of bubbles.

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