

Annexure – 2

**REPORT ON “THE FIELD TRIP TO
MAWPHANLUR, MEGHALAYA”**

**Submitted by the BSc 6th
semester students (Batch 2023)**

**Organised by the department of
biochemistry, Lady Keane
college, Shillong**



Objective: Experimental work on the plant (fern) in two different sites i.e in the forest area and near the road to check the chlorophyll content and also to isolate the pigments from the plant.

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Acknowledgement

In the accomplishment of completion of our report on “The field trip to Mawphanlur”. We would like to convey our special gratitude to all of our teachers of Biochemistry department and as well as our Principal, Sir Dr. D.K.B Mukhim.

Your valuable guidance and suggestions helped us in various phases of the completion of this report. We will always be thankful to you in this regard.

We are ensuring that this report was finished by us and not copied.

All VI semester students

Batch : 2020-2023

Collecting plant Specimen

Fern (*Polypodiopsida*)

**Road side area
Sample**



**Forested area
Sample**



**Collecting the
pinnules of ferns**



Weighing plant specimen



**Road side area
Sample**

**Forested area
Sample**





Preparation of 80% acetone



Crushing fern leaves

With the help of mortar and pestle



**20ml of 80%
acetone was
mixed with the
crushed fern
leaves**



The mixture was poured into a centrifuge tube

One for forested area and other for road side area



Forested sample.

Road side sample



Centrifugation

The tubes were then placed into the slots in the centrifuge machine. The speed was set to 5000 rpm for 10 minutes.



Collection of the supernatant in the volumetric flask upto 100ml



Taking absorbance

Using the spectrophotometer,
absorbance was taken at 645 nm and
663 nm to check the chlorophyll
content



READINGS

After observing in the spectrophotometer the following readings were obtained

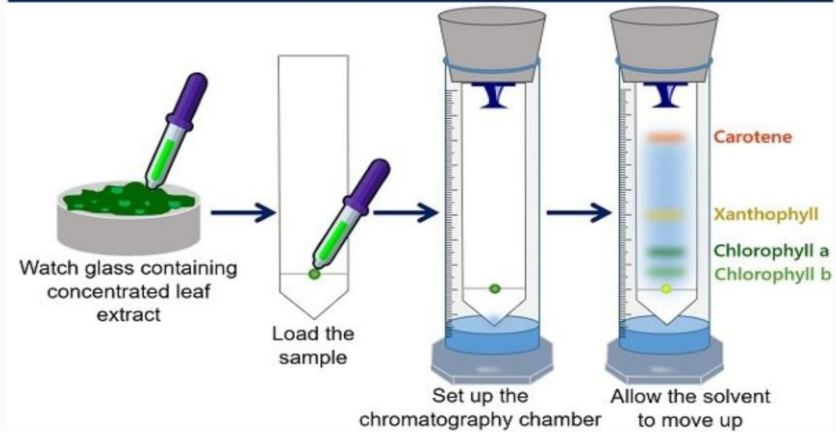
- 1) Roadside fern: at 645 nm the absorbance is 0.159
at 663 nm the absorbance is 0.478**
- 2) Forested area fern: at 645 nm the absorbance is 0.298
at 663 nm the absorbance is 0.804**

Observation

We observed that forested area ferns had more absorbance than roadside area fern.

SEPERATION OF PHOTOSYNTHETIC PIGMENTS BY PAPER CHROMATOGRAPHY

Separation of Plant Pigments through Paper Chromatography



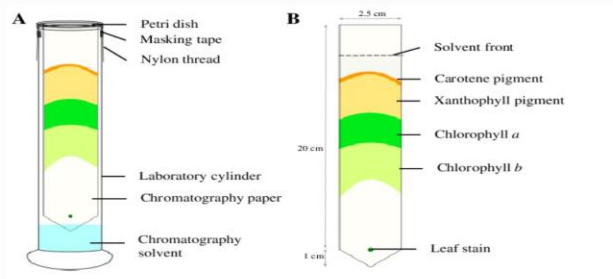
1. Tender leaves were taken and ground with the help of a mortar and pestle.



2. To this a small quantity of 100% acetone was added.

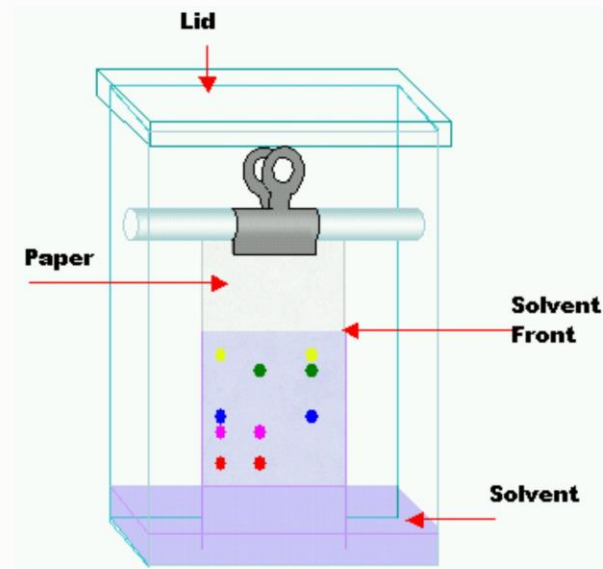


3. On the piece of chromatography paper, a very light line 1.0 cm from the edge of the paper was drawn.

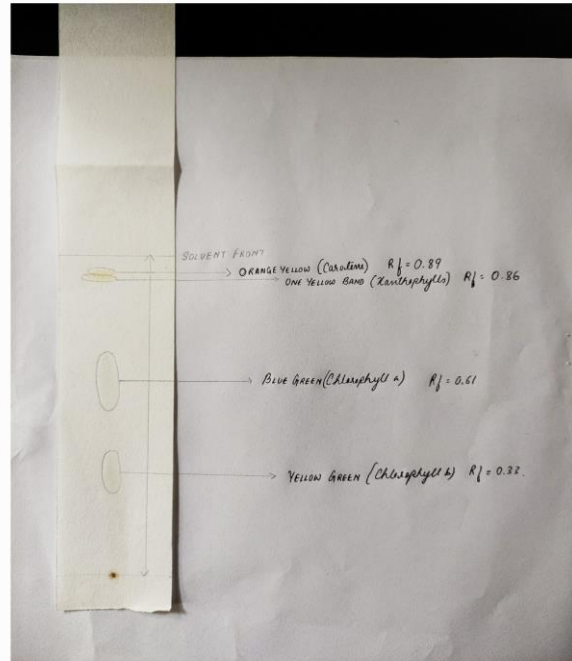


4. Then with a capillary tube, the extract was spotted on the paper. The first coating of the extract was allowed to dry and this procedure was repeated twice more till the spot was dark green.

5. Then the paper was dipped in the chromatography glass jar containing the solvent [Petroleum ether:Acetone (9:1)] and an ascending chromatography mode was performed for approximately 1 hour to separate the photosynthetic pigments.



6. After performing the chromatographic run, the paper was taken out of jar and the solvent front was marked. Also the pigment spots on the paper were marked and the paper was dried using dryer.



RESULTS

- Carotenes : Orange yellow ; $R_f = 0.89$
- Xanthophylls : one or two yellow bands ; $R_f = 0.86$
- Chlorophyll-a : Blue green ; $R_f = 0.61$
- Chlorophyll-b : yellow green ; $R_f = 0.33$

CONCLUSION

Based on the experiment comparing the chlorophyll content from two different sites, one being a forested area and the other a roadside location, as well as isolating the pigments from the plants and separating the photosynthetic pigments using paper chromatography, we can draw the following conclusions:

1. **Chlorophyll Content:** The chlorophyll content in plants from the forested area is expected to be higher compared to plants from the roadside location. This is because plants in forested areas typically receive more sunlight and have access to a richer supply of nutrients, leading to increased photosynthetic activity and higher chlorophyll production.
2. **Pigment Isolation:** The isolation of pigments from plants involves extracting the pigments from the leaves using appropriate solvents. By grinding the leaves and treating them with solvents like acetone or ethanol, the pigments can be released. The resulting solution can then be used for further analysis.
3. **Paper Chromatography:** Paper chromatography is a technique that separates different pigments based on their relative solubility and affinity for the paper. The pigments in the extracted solution can be applied to a chromatography paper strip and placed in a suitable solvent (e.g., a mixture of petroleum ether and acetone). As the solvent moves up the paper strip by capillary action, it carries the pigments along. Different pigments will travel at different rates based on their solubility and interaction with the paper, resulting in distinct bands or spots.
4. **Pigment Separation:** By analyzing the chromatography paper strip after the solvent has reached a suitable height, the separated pigments can be observed. Typically, chlorophyll a, chlorophyll b, and carotenoids (such as beta-carotene) are expected to be visible. Chlorophyll a is the primary photosynthetic pigment, while chlorophyll b and carotenoids act as accessory pigments, broadening the range of light wavelengths that can

be captured for photosynthesis.

In summary, the experiment comparing chlorophyll content between a forested area and a roadside location, along with the isolation of pigments from plants and separation of photosynthetic pigments using paper chromatography, would likely reveal higher chlorophyll content in plants from the forested area. The paper chromatography results would show distinct bands or spots corresponding to different pigments, including chlorophyll a, chlorophyll b, and carotenoids.



THANK YOU.

Expenditure incurred on the programme = Rs 11,000.00