



Separation of photosynthetic pigments in *Spirogyra* specis by means of thin layer Chromatography from Sola lake, Ahmedabad, Gujarat

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Abstract

The present study of three deferent sessions summer, monsoon and winter sample collaction from Sola lake, Ahmedabad surfes water this lab was to determine in *Spirogyra* specis the rate of flow values (R_f =distance of component/distance of solvent) for ceratin pigment found in chlorophylls, xanthophylls and carotene.chllorophyll, we observed the following four colours: yellow, Blue- green, Green and Carotene is red-orange the both of our experiments traveled a distance of 10.0cm. We obtained the following R_f avlues for the chlorophyll-a 0.22 to 0.32, chlorophyll-b 0.12 to0.19, xanthophyll 0.70 to 0.74 and carotene is 0.90 to 0.93 present.

Key-Words: Pigments, *Spirogyra* specis, Sola Lake, R_f

Introduction

Ahmadabad city is located at 23^o.03' N 72^o.58' E in western India at an elevation of 53 meters (174 ft). The city sits on the banks of the river Sabarmati, in north-central Gujarat. Sola Lake is located at Sola village. Its total storage capacity is 24.6crore liters and Lake Circumference is 1364 M (Fig-2). The lakes are large or considerable body of water within land (Wetzel, 1983). The average minimum is 27 °C (81 °F). From November to February, the average maximum temperature is 30 °C (86 °F), the average minimum is 15 °C (59 °F), and the climate is extremely dry. Cold northerly winds are responsible for a mild chill in January. Chlorophyll a. Chlorophyll b, carotenes and xanthophylls play a secondary role by transferring the energy they absorb to chlorophyll *a* for use in photosynthesis. In the following experiment, the pigments found in the, *Spirogyra* specis means of thin layer chromatography. *Spirogyra* is a lamentous green alga that inhabits the streams and lakes of Gujarat. Adapted to live in colder temperatures, *Spirogyra* can store large internal reserves of nutrients that can sustain maximum growth rates for several weeks. (O'Neal 1988). Nutrient enrichment in lake communities has been shown to change the original structure of the algae community (Havens 1999) and can lead to the increased growth of Lake ora such as *Spirogyra*.

Because of limitations in the availability of the nutrients nitrogen (N) and phosphorus (P) for growth, the addition of these nutrients to lake ecosystems may cause the *Spirogyra* population to increase dramatically (O'Neal 1988). This can bring changes to the species composition of algae and to the intrinsic dynamics of the lake (Havens 1999). Algal blooms, though considered by some to be a nuisance and a sign of environmental degradation, often play an important ecological role in supplying substrate and resources for epiphytic and browsing organisms (Lorenz 1991).

The present study on species of *Spirogyra* in Sola Lake water of with Qualitative Analysis Using by Thin layer chromatography (TLC) R_f velue comper for the Chlorophyll a, b, carotene and xanthophyll. The sample collection is diferent point of lake and three seasons' summer, winter and monsoon. Sola lake reveals the Chlorophyceae largest algal community.

Material and Methods

Sample collection and processing

The study was carried out diferent zone sola lake water area during January -2009 to December-2009. Algal samples were collected during 10- 11 am covering the season's summer, monsoon by plankton net (mesh size 25 μ m), Macroalgae samples collected for taxonomic identification were preserved in Lugols iodine in the field. The Magnus Binocular Microscope with camera and some microphotographs were also taken from preserved and fresh materials for identifying the specimens. Identification of algae based on the keys of

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Biswas (1949) and Randhawa (1959). The water parameters viz. pH, temperature and salinity were also studied following APHA (1975).

Spirogyra samples were collected frequently, with fewer samples of other algae collected because they were not as common. Other macroalgae were collected opportunistically. Samples collected for laboratory analysis were stored in plastic bottles and kept on ice. In the Laboratory, the macroalgae samples were rinsed thoroughly to remove macro-invertebrates, Debris and sediment, then gently squeezed out by hand to remove water, and finally placed on Blotting paper to absorb the remaining water. The wet weight of the sample was then measured, and a sub-sample taken for chlorophyll *a*, *b*, Crotenes, and Xanthophyll etc. analysis by TLC method. The remaining algae were weighed, dried at 60°C then weighed again. The dried algae were then ground to a powder using a domestic coffee grinder, and a small portion of the sample was also analysed for chlorophyll *a*, *b*, Crotenes, and Xanthophyll.

Separation of pigments

Procedure: Thinlayer chromatography (TLC) was employed for the separation of plant pigment like Chlorophylls, Crotenes, and Xanthophylls etc.

This technique followed several steps like ;(Stahl, 1969; Wharton and Mc Carty, 1972)

Production and activation of thin layer: 6 g silica gel G was made in to slurry with 15 ml DW. The Slurry was poured and spread on the clean, 2 mm thick and 20 cm sized glass plate with the help of spreader. Thickness of the layer was 1 mm and pore size was moderate. Thin and uniform layer of silica was prepared. The plate was must be activated in an oven at $80 \pm 2^{\circ}\text{C}$ for 30 minutes before use.

Sample Extraaction for Chlorophyll: 1g metrial was kept in a tissue ginder, 2 to 3 ml 90 % acetone was added and macerated at 500 rpm for 1 minute. It was centrifuged in closed tubes for 20 min at 500 rpm and clear supernant was collected. Extract was concentrated immediately after centrifugation used for spotting.

Sample application: Extract was dissolved in sutible solvent (90 % acetone). TLC plate was placed horizonatally on a white paper marked with a base line. Sample was applied on the line in the from of spot, drop by drop with the help of capillary with regular interval.

Choce mobile phase: Petroleum Ether, Isopropanol, and Water [100:10:0.25].

Development of chromatogram: the plate after application of sample was kept inside the chromatographic tank contained mobile solvents; in such way that the margin of the plate near to the baseline was just touched the solvent. It was then

covered with a glass plate and left for 2-4 hours at the room temperature. The separation of frations was carried out with single dimensional TLC.

Detaction of chromatogram: the plate was taken out from the tank and dried. Spots of pigment were detected with visual observation in UV cabinet in wavelength 263nm and 365nm.

Identification: Spots were identified with the help of Rf (reat of flow) value and colour.

Distance traveled by compound

Calculations: $Rf = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by solvents}}$

Distance traveled by solvents

Results and Discussion

The colours the different pigments displayed on TLC plate. We can compare the RF values to find out pigments identity, copare the results with the tyepical RF values in Table-1, we can found that all of the pigments have been identified: pigment is Chlorophyll-*a*, *b* Carotene and Xanthophyll;

The Table -1 shows the Chlorophyll *a* minimum and maximum RF value recorded at 0.22 in winter and 0.32 summer Blue/Green colour present during 2009(fig-1),the Chlorophyll *b* is recorded at RF velue 0.12 to 0.19 Green colur present in Monsoon 2009(fig-1). Some amount of algae is naturally present in all healthy lakes. By measuring chlorophyll *a*, we are determining the amount of food available to fuel the lake's food web. Too little chlorophyll *a* indicates that there may not be nough food to support an abundant biological community. On the other hand, too much chlorophyll *a* indicates that nutrient levels in the lake may be artificially high. This is a problem because algae sink to the bottom and decay, a process that depletes deeper water of oxygen. In severe cases, all of the lake's oxygen can be become depleted, resulting in fish kills. In addition, a nutrient-enriched lake with excessive algae is less appealing for recreational activities like boating and swimming.

The xanthophylls RF velue is recorded from 0.70 monsoons and 0.74 Yellow/browen colour present in winter 2009(fig-1). Xanthophylls are the typical yellow pigments of leaves. These are oxygenated carotenoids that are synthesized within the plastids. Xanthophylls do not require light for synthesis, so that xanthophylls are present in all young leaves as well as in etiolated leaves. Xanthophylls in leaves have an important function as accessory pigments, capturing certain wavelengths of sunlight not absorbed by chlorophylls, and thereby increasing overall absorptance of the visible spectrum of sunlight. The Carotene 0.90 in Monsoon and 0.93 red-orange colours present summer 2009(fig-1).Carotenes contribute to photosynthesis by transmitting the light energy they absorb from

chlorophyll. They also protect plant tissues by helping to absorb the energy from singlet oxygen, an excited form of the oxygen molecule O_2 which is formed during photosynthesis.

Table-1 The Rf values and colours for the chlorophyll pigments in this solvent mixture are as follows *Spirogyra* from sola Lake during January 2009 to December -2009.

Conclusion

The results obtained in this paper are very good performance obtained by analysis of pigments of algal *Spirogyra* with different types of kisses season variation in chlorophyll a, b, xanthophyll and caroten content. An extract of pigments yielded, on chromatographic separation, no fewer than a dozen pigments, including chlorophyll b is decrease in winter and summer and carotene several newly described xanthophylls increased in summer. The Chlorophyll content concentration like Chlorophyll-b(Green)>chlorophyll-a(Blue green)>xanthophyll(YellowBrown)>and>carotene(Red-Orange) were studied comparatively during January-2009 to December-2009(Fig-1).

Acknowledgement

We gratefully acknowledge The Sir P.T. Science college P.G. Center of Biology Department and spatial planning for Support and Laboratory facility this study.

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Fig. 1: Separation of spirogyra specis photosynthetic pigments on silica-gel thin layer chromatography strips using as the developing solvent

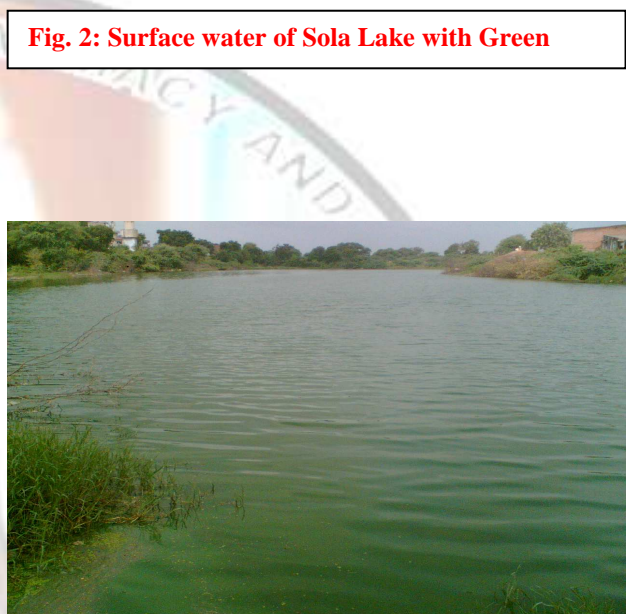
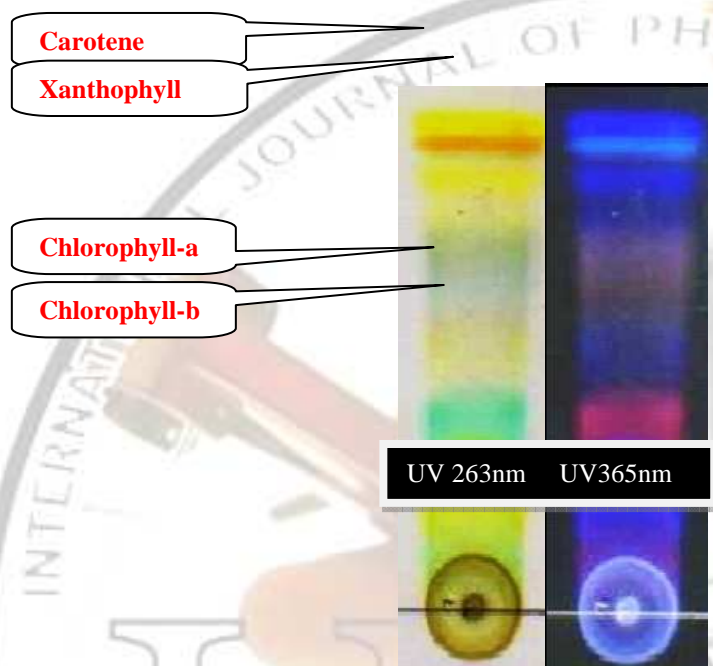


Fig. 2: Surface water of Sola Lake with Green

Table 1: The Rf values and colours for the chlorophyll pigments in this solvent mixture are as follows *Spirogyra* from sola Lake during January 2009 to December -2009

Season	Chlorophyll -a RFvalue	Chlorophyll -b RFvalue	Xanthophylls RFvalue	Carotene RFvalue
	blue/green	green	yellow/ brown	Red- orange
Summer	0.32	0.12	0.71	0.93
Monsoon	0.25	0.19	0.70	0.90
Winter	0.22	0.12	0.74	0.92