IV. METHODS

BIOLOGICAL:

Plankton collection and enumeration:

Monthly collections of surface samples of the phytoplanktons were made at fixed places by towing a fine bolting silk net. Bulk water samples were also collected from the same locations for a detailed assessment of the quantity of phytoplankton. Known volumes of lake water were concentrated by centrifugation and the phytoplanktons were quantitatively estimated by using a haemocytometer, taking the mean of five countings of 400 squares.

Collection and estimation of epiphytic diatoms:

Quantitative estimations of epiphytic diatoms were carried out on Ipomea stems. 2 cm long stem pieces were cut at the same locality every time. The diameter of the stem was measured to calculate the surface area. The stems were heated in conc. \( \text{H}_2\text{SO}_4 \) and \( \text{KNO}_3 \) was added after boiling, to oxidize the organic matter. After removing the acid and making suitable dilution, the diatom cells were counted in the same way as the plankton.

CHEMICAL:

Pigment extraction:

A known amount of lake water (1000 ml) was filtered through a filter paper and the residue together with the paper was extracted with 80% aqueous acetone.
at room temperature. The extract was clarified by centrifugation (3000 r.p.m.) and the optical density was measured in an EEL absorption meter at 660 mp against a similarly treated blank.

The methods used for the chemical analysis of the water were largely based on those suggested by the American Public Health Association (1936), unless otherwise indicated.

1. H-ion Concentration:

The pH of the lake water was determined on the spot by the B.D.H. Universal indicator.

2. Solids

a) Suspended:

The suspended matter was estimated by filtering a known amount of water and drying the filter paper at 60°C to a constant weight.

b) Dissolved impurities:

100 ml of the filtrate from the above was evaporated to dryness in a weighed crucible. The increase in weight indicated the amount of dissolved matter.

3. Turbidity:

The turbidity was measured by a white marble slab tied to a measuring tape. The slab was not just visible. The length of the tape up to the surface of the water was taken as a measure of the turbidity (or visibility).
4. **Hardness:**

The total hardness in terms of calcium carbonate was determined by using a standard soap solution as suggested by the American Public Health Association (1936).

5. **Dissolved Oxygen:**

To 250 ml of sample were added 0.7 ml conc. H₂SO₄ and 1 ml of KMnO₄. After a thorough shaking, a further addition of 1 ml of KMnO₄ was made. After a subsequent addition of 1 ml of potassium oxalate, 1 ml of magnesium sulfate and 3 ml of alkaline potassium iodide were added and the precipitate was allowed to settle down. Then 1 ml of conc. H₂SO₄ was added and mixed thoroughly. Upto this stage, the procedure was followed at the spot and then the sample was titrated in the laboratory with 0.025N sodium thiosulphate, using starch solution as the indicator.

6. **Carbon dioxide:**

100 ml of the sample was titrated with N/50 H₂SO₄ using phenolphthalein as the indicator.

7. **Alkalinity and Acidity:**

These were determined according to the procedure suggested by the American Public Health Association (1936).

8. **Phosphate:**

Phosphorous was estimated by the method of Allen (1940), using 1-amino-2-naphthol 4-sulphonic acid,
instead of Amidol. The amount of phosphorous was read from a calibration curve obtained with standard solutions. The standard solution containing 1 mg/ml was prepared by dissolving 1.0967 g KH$_2$PO$_4$ (A.R.) (dried in air oven) in distilled water and diluting to 250 ml. Suitable standard solutions for the estimations were prepared by dilution of the stock solution.

9. Total iron:

Total iron was colorimetrically estimated by potassium thiocyanate method as recommended by the American Public Health Association (1936).

10. Nitrate:

Nitrate was determined by the phenoldisulphonic acid method. 100 ml of water was evaporated to dryness on a water bath. The residue was treated with 2 ml of phenoldisulphonic acid. After diluting a little with distilled water, the mixture was treated with a strong solution of potassium hydroxide, until a maximum yellow colour was obtained. The nitrate was colorimetrically read from a calibration curve obtained with the standard nitrate solutions.

11. Nitrite:

To 50 ml of the sample, 1 ml of sulphuric acid solution and 1 ml of α-naphthylamine acetate were added and thoroughly mixed. The developed colour was
colorimetrically compared with the similarly treated standards.

12. **Ammoniacal-nitrogen**

   The method employed was that suggested by American Public Health Association (1936).

13. **Silicon**

   When the concentration of silicon in the water was higher than 1 mg Si/l, the method of Wattenberg (1937) was followed. For silicon concentration below 1 mg Si/l, the method as described by Bunting (1944), using 1-amino-2-naphthol-4-sulfonic acid, as the reducing agent, was followed. The determinations were carried out photometrically with an EEL absorptionmeter. Calibrations were made with a standard silicon solution. The term 'silicon concentration' referred to in the present investigation refers to the quantity of silicon in the water which reacts with ammonium molybdate (see Jorgensen, 1957).

Silicate in water occurs in soluble and colloidal forms and it is suggested by many workers that only the soluble silicate reacts with ammonium molybdate. The colloidal silicate is, therefore, not considered in this investigation. According to Lewin (1955) colloidal silicate is not utilized by *Navicula pelliculosa*. 