

METHODS

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SAMPLING OF WATER

The water was sampled by gently wading as far as possible into the water at certain sites where no other approach is possible.

The phytoplankton samples were also collected in the similar way. The sediment samples were collected by using an Eckman dredge. The sampling was carried out at monthly interval.

PROCESSING OF THE SAMPLES

pH, Water temperature and dissolved oxygen were measured at the field, for remaining parameters the water samples were immediately preserved. The suitable container, time and chemicals of preservation were selected as per APHA (1981). For phytoplankton 500 ml sample was taken and immediately preserved in Lugol's solution (Vollenweider 1969). The samples were later concentrated in the laboratory at 1500 r.p.m. in a clinical centrifuge and preserved in a mixture of formalin and Lugol's solution.

For soil, samples were brought to the laboratory in polythene containers, air dried (Allen et al. 1974) and stored after labelling for further analysis.

PHYSICO-CHEMICAL ANALYSIS OF WATER

WATER TEMPERATURE : It was recorded using a centigrade thermometer with a precision of 0.1°C

TRANSPARENCY : It was measured by the secchi disc made up of a 20 cm diameter circular metal disc painted black and white alternatively in radial sections. It was lowered in^{to} the water with the help of a string and the transparency was recorded as the mean of the depths at which the disc disappears and reappears after raising.

pH : The pH was recorded on a digital pH meter (Elico make).

CONDUCTIVITY : It was recorded on a conductivity bridge fitted in a water quality analyzer of Elico Pvt.Ltd.

TOTAL SUSPENDED SOLIDS : They were determined as the difference between the total solids and total dissolved solids.

DISSOLVED OXYGEN : It was determined at the field by using modified Winkler's method. The samples were taken into the ground glass stoppered 300 mL corning make BOD bottles preventing any bubbles. 2 mL of saturated solution of manganous sulphate and potassium iodide + potassium hydroxide solution were added after the settlement of the precipitate. It was dissolved in sulphuric acid and titrated against 0.025 N sodium thiosulphate solution.

COD : It was determined by refluxing the sample or an aliquot of that with potassium dichromate and sulphuric acid with ammonium ferro sulphate using ferrous phenanthroline as an indicator.

$\text{NH}_3\text{-N}$: It was estimated by microkjeldahl distillation method. The distillate was collected in a 4% boric acid solution mixed with methyl-red and bromocresol green and was then titrated against 0.01 N HCl.

NITRITE-N : It was determined by colorimetric method. The wine red colour was developed by addition of solution of EDTA, sulphanilic acid, alpha naphthylamine hydrochloride and sodium acetate to colourless filtered sample. The transmission was obtained at 520 nm wave length.

TOTAL KJELDAHL NITROGEN : It was estimated by microkjeldahl distillation apparatus after digesting the sample with sulphuric acid, potassium sulphate, copper sulphate and sodium chloride. The value of dissolved organic nitrogen was then obtained by subtracting $\text{NH}_3\text{-N}$ from obtained nitrogen value.

DISSOLVED INORGANIC PHOSPHORUS : It was estimated by colorimetric method using ammonium molybdate and stannous chloride for development of a blue colour. Transmission was observed at 690 nm.

ORGANIC DISSOLVED PHOSPHORUS : It was estimated by molybdate method after digestion of the filtered sample as was done for organic nitrogen ion and deducting the organic phosphorus from the obtained values.

PARTICULATE-P : It was determined as the difference between the total phosphorus in unfiltered and filtered samples.

CHLORIDES : They were determined by titrating the sample against silver nitrate using potassium chromate as an indicator.

CALCIUM : It was determined titrimetrically by EDTA method using murexide as an indicator.

MAGNESIUM : It was also determined by EDTA method using Eriochrome black-T as an indicator.

HARDNESS : It was determined by EDTA method.

SODIUM & POTASSIUM : Determined flamephotometrically.

PHYTOPLANKTON :

Since direct count was not possible with most samples, they were centrifuged at 1500 r.p.m., a suitable volume was made-up, the quantity of the preservatives adjusted and kept for further work. The phytoplankton were identified at genus and species level to a maximum possible extent using the keys and flora of Philipose (1959), Kamat (1963, 1968, 1974), , Palmer (1968), Kamat (1985), Nygaard (1949) & Weber (1971).

The microscope was calibrated for counting and an ocular micrometer was placed in the eye piece of the microscope permanently to measure the dimensions of the phytoplankton simultaneously. The counting was done in a haemocytometer. The care was taken that at least 100 individuals of important species are counted.

The phytoplankton data was subjected to the analysis of various indices, the calculation procedures for them are mentioned.

PHYTOPLANKTON BIOMASS : The phytoplankton biomass was estimated according to Lohmann (1980). The linear dimensions of the phytoplankton units were measured by whipple micrometer disc (Ocular disc). The average cell volume of each unit was computed after giving it a proper geometric shape (Vollenweider 1969, Dowing & Rigler 1984). The biomass of phytoplankton was calculated taking the density as 1.01 - .03 (Hutchinson 1967). The final results of the biomass were expressed as ug/L (Spondnniowska 1974 and Jumpp anen 1976).

PHYTOPHANKTON INDICES :

DIVERSITY INDICES : Two types of diversity indices have been calculated.

Shannon Weiner's diversity index : It is calculated by using following formula.

$$D = -\sum p_i \log_2 p_i$$

Where D = Diversity Index , $p_i = n_i/N$

(n_i = number of individuals in species i

N - total number of Individuals in the sample)

ODUM'S INDEX :

$$\text{Odum's Index} = \frac{\text{Total No. of species}}{\text{Total Density}} \times 1000$$

PLAMER'S ALGAL GENUS INDEX : Palmer index (Palmer 1969) is based on the assumption that certain species can flourish well in organically rich waterbody and can be used as indicators of organic pollution. A value of 20 or more for a sample is indication of organic pollution. Score of 15 to 19 is considered as evidence of high organic pollution. Lower values of this index indicate that organic pollution is not high or the sampling has not been representative.

NYGARD'S TROPHIC STATE INDICES : Nygard (1949) propose five indices to evaluate the organic pollution of a water body on the basis of algal groups (Myxophycean index, chlorophycean index, diatom index, Euglenophycean index and compound index). These indices have been developed on the basis of the fact that various algal species have different tolerance to organic pollution and nutrient enrichment. As a general rule, the cyanophyta, Euglenophyta, the centric diatoms and most of the chroococcales are

commonly found in eutrophic waters, while the pennate diatoms and desmids are commonly found in oligotrophic waters. For the calculation of the indices, the number of species of various algal groups is taken into consideration. Following table provides details of calculation of Nygaard's indices and classification of water on that basis.

However, those indices could not be calculated in several months due to the absence of particular group (desmids).

Nygaard's trophic state indices.

Index	Calculation	Oligo-trophic	Eutrophic
Myxophycean	<u>Myxophyceae</u> * (<u>Cyano phyceae</u>) Desmideae	0.0-0.4	0.1-3.0
Chlorophycean	<u>Chlorococcales</u> Pennate diatoms	0.0-0.7	0.2-9.0
Diatom	<u>Centric diatoms</u> Pennate diatoms	0.0-0.3	0.0-1.75
Euglenophycean	<u>Euglenophyta</u> Myxophycea + Chlorococcales	0.0-0.2	0.0-1.0
Compound	Centric diatoms + <u>Euglenopyta</u> Desmideae	0.01-1.0	1.2-2.5

* or Cyanophyta

SEDIMENT SAMPLING & ANALYSIS

The analysis of soils and sediments were made following the methods given in Piper (1950), Jackson

(1958) Allen et al. (1974) and Trivedy & Goel (1984). Following is a summary of methods used.

pH of the soil was determined with the help of a pH meter in 1:5 soil solution.

ELECTRICAL CONDUCTIVITY : It was also determined in 1:5 soil solution with the help of a conductivity meter fitted in a water quality analyser of ELICO (model PE-132).

CHLORIDES : Chloride was determined titrimetrically by titrating the 1:5 soil solution with standard silver nitrate using potassium chromate as an indicator.

CALCIUM & MANGESIUM : Calcium and magnesium were determined after leaching of soil with ammonium acetate solution. The leachate was titrated with EDTA solution with murexide and Erichrome Black T indicator separately to find out calcium and magnesium.

SODIUM & POTASSIUM : Sodium and potassium were obtained by flamephotometric method in the ammonium acetate leachate of the soil.

TOTAL NITROGEN : It was determined by Kjeldahl distillation method. The soil sample was digested with concentrated sulphuric acid, selenium powder and potassium sulphate. The digest was distilled with 40% sodium hydroxide and the distillate was collected in 4% boric acid with mixed indicator. The contents were then titrated with 0.01N hydrochloric acid.

ORGANIC MATTER : It was estimated by Walkley and Black's rapid titration method. The organic matter was oxidised by potassium dichromate and sulphuric acid mixture and the residual potassium dichromate was titrated with ferrous ammonium sulphate using diphenylamine as an indicator.

AVAILABLE PHOSPHORUS : It was determined by ammonium molybdate colorimetric method after its extraction from the soil by 0.002 N sulphuric acid.

BACTERIOLOGICAL EXAMINATION OF WATER

The bacteriological examination of water was limited to the enumeration of Most Probable Number of Coliforms (MPN of coliforms). It was estimated by Multiple tube method provided in APHA (1981). The sampling was done by using standard methods. The BOD bottles were sterilized. The samples were collected after opening the bottle below water and closing the bottle, there itself. The samples were brought to the laboratory within 8 hours of collection and were processed immediately.

The test is carried out inoculating water sample into test tube containing a suitable liquid medium (Brilliant Green Lactose Bile Broth, BGLB, in this case).

The composition of media at :

Peptone	10 _g
Lactose	10.0
Oxgall	20.0
Brilliant green	0.0133 _g
Distilled Water	1 lit.

In all nine test tubes were selected for 1 sample out of which 3 tubes contained double strength media while 6 tubes contained single strength, each 10 mL quantity. Each tube contained an inverted Durham vial. The tubes were plugged with cotton and sterilized for 15 minutes. The sample is added in 1 mL and 0.1 mL amount in 6 tubes of single strength and 10 mL added in the tubes with double strength. The number of tubes producing gas were noted. The MPN table given in APHA (1981) was consulted and MPN of coliforms was calculated per 100 mL.

HEAVY METAL ANALYSES

Well mixed samples of each site were taken and acidified by Conc HNO₃. Estimation of heavy metals was done on Atomic Absorption spectrophotometer.

PRIMARY PRODUCTION

The phytoplanktonic primary production was estimated by the light and dark bottle method of Gardner and Gran (1917). Light and dark, bottles were suspended at surface and at a depth of about 50 cm (exact depth being recorded) for an hour interval. Gross Primary Production, Net Primary Production and Respiration of the community were computed from oxygen values in light and dark bottles before and after incubation. Oxygen values were converted in to carbon values by the following formula.

$$\text{g C/M}^3/\text{Hr} = \text{O}_2 \text{ mg/L/hr} \times 0.375$$

The values were recorded in morning, afternoon and evening.

PERIPHYTON :

The algae on all submerged substrates in water, e.g. mud, rocks, sand, macrophytes, sticks, etc. are commonly called as periphytic algae. In shallow waterbodies and running waters they are important contributors to biota and primary productivity, and are also known to respond to pollution quickly and distinctively. In past few decades several advancements have been made in periphytic algal studies. In present study the samples were collected by conventional method of scrubbing from various substrata. The samples were collected in 100 mL glass bottle. The samples were washed and freed of debris and mineral matter. They were preserved in the similar way as that of phytoplankton. The samples were further studied for the species composition and relative abundance only.