Chapter III

Methodology
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A- Collection of water samples:

The subsurface water (about 30 cm depth) from littoral region of selected ponds was collected monthly with the help of Ruttner water sampler bottle with the capacity of two litters during the period, from February, 2009 to January, 2010. Parameters like Air and Water temperature, Depth, Transparency, Conductivity, pH, D.O., free CO₂, Alkalinity were analysed between 8:00 AM and 9:00 AM at the site. For remaining physicochemical analysis, water samples were filled in cleaned plastic bottles and brought to the laboratory.

B- Collection of sediment samples:

Sediment samples were collected monthly between 9:00 AM and 10:30 AM with the help of Ekman-dredge of size 15 cm x 15 cm. After collection, sediment samples were kept in plastic bags, labeled and were brought to the laboratory. A part of the sediment sample was put in oven at 105 °C for drying and grinded in a Gate-Mortar for sediment analysis.

C- Separation of bottom fauna and Identification

For benthos analysis, samples were mixed and diluted with tap water to prepare slurry in a bucket and sticks, leaves, debris were removed. Then slurry was divided into ten subsamples. Each subsample was passed through sieves, B.S. No. 30 (mesh # 500 μm) and B.S. No. 72 (mesh # 200 μm) arranged former above the latter so that smaller organisms (meio) were retained on the smaller sieve. Sieving yielded residue including mixture of animals and sediment. The sample retained on first sieve was emptied in shallow dish and organisms were sorted by using brush, forceps and pipette against a white background. To facilitate the sorting few drops of 10% aqueous solution of Rose Bangal was added. Organisms retained on second sieve were washed into a tray and then samples were taken in vials and labeled. Organisms were preserved in 70 % ethyl alcohol solution for qualitative and quantitative analysis. For larger animals, insect larvae and oligochaetes about 2 ml of preserved sample was taken in a calibrated petri-dish and studied under dissecting microscope.
For smaller organisms, about 1 ml of preserved sample was taken on Sedgewick Rafter cell and studied under an inverted microscope (Metzer). Individuals were identified up to genus or species level as could be possible and number of each taxon was noted. Density was determined per meter square area and result was expressed as ind/m². Identification was done with the help of keys given in Edmondson (1959), Needham and Needham (1962), Pennak (1978) and Tonapi (1980).

**D- Water analysis**

Physico-chemical parameters were analyzed on monthly basis from February, 2009 to January 2010 in the three selected Ponds. Each chemical parameter was repeated at least three times and the average of the three readings was taken to minimize the error.

**Air and water temperatures** were recorded with the help of mercury thermometer graduated up to 100°C between 8.00-9:00 am.

**Conductivity** was recorded with the help of digital conductivity meter (Hanna instrument, No. S250178).

**The depth** at the sampling sites was obtained in cm by sounding the bottom with graduated nylon rope tied to a lead weight.

**Transparency**, the limit up to which light can penetrate in water body, was measured by using standard Secchi-disc having diameter of 20 cm.

**Dissolved oxygen (D.O.)** analysis was performed at the sites by Winkler’s modified technique (Trivedy and Goel, 1984).

**Free carbon dioxide (CO₂)** was determined by titrating 100 ml of water sample with N/44 NaOH using Phenolphthalein as an indicator (Theroux et al., 1943).

**pH** of the water was determined at the sites by using a portable electronic digital pH meter (Hanna instrument, No. S254992).
**Alkalinity** was estimated by titrating 100 ml water sample with 0.02 N Sulphuric acid using Phenolphthalein and Methyl orange as indicator (Theroux et al., 1943).

**Hardness** of water was estimated by titrating the water sample with 0.01 N EDTA solution using Murexide as an indicator (Trivedy and Goel, 1984).

**Calcium** and **Magnesium** present in water was estimated by titrimetric method (Trivedy and Goel, 1984).

**Chloride** was estimated by titrating the 50 ml sample with 0.025 N Silver nitrate using 5% Potassium chromate as an indicator (APHA, 1998).

For **Total Solids (TS)** analysis, 200 ml water sample was taken. The residue left after the evaporation of 200 ml sample of unfiltered water was taken as the amount of total solids (TS) present. The residue left after the evaporation of 200 ml sample of filtered water was taken as the amount of the **total dissolved solids (TDS)** present. The difference between TS and TDS were taken as the amount of **total suspended solids (TS-TDS=TSS)** present. The results were expressed in mg/L.

**Nitrate-nitrogen (NO₃-N)** was determined following the Phenol-disulphonic acid method (Triedy and Goel, 1984).

**Phosphate- phosphorus (PO₄-P)** was estimated by Ammonium-molybdate-blue method using stannous chloride (SnCl₂) as an indicator (Barnes, 1959).

**E- Sediment analysis**

For sediment analysis, 1:5 ratios of sediment and water were made. Twenty gm of air-dried sediment was taken in a dried beaker and 100 ml of distilled water was added to it without stirring till a glistening layer appeared on the surface of soil. Now the sample was stirred well with the help of glass rod to form a uniform paste the pH, alkalinity and water temperature of sediment were analysed as mentioned for water analysis.
**Composition of Sand, Silt and Clay**

The sediment samples were also analyzed according to Michael (1984) for soil texture viz. sand, silt and clay and the type of sediment texture was determined according to soil texture.

**Organic matter and organic carbon**

Organic matter and Organic carbon was determined by Walkley and Black methods as described by Trivedy and Goel (1984).

Five gm of over-dried sediment sample, passed through 0.5 mm nonferrous sieve, was taken in 500 ml conical flask. To this, 10.0 ml of 1N K$_2$Cr$_2$O$_7$ solution and 10 ml of conc. H$_2$SO$_4$ were added and mixed thoroughly by gentle swirling. The whole content was kept for 30 minutes to complete the reaction. After the reaction was over, 200 ml of distilled water and 10.0 ml of phosphoric acid was added followed by 1.0 ml Diphenylamine as an indicator. The whole content then was titrated with 0.4N Ferrous ammonium sulphate. A blank was also used with the same quantities of chemicals but without sediment. Calculation was made using following formulae:

\[ \% \text{ C} = 3.951/g \times (1 - T/S) \]

\[ \% \text{ organic matter} = \% \text{ C} \times 1.72 \]

Where \( g \) = weight of sample in gm,

\( S \) = ml of ferrous ammonium sulphate solution used in blank titration,

\( T \) = ml ferrous ammonium sulphate solution used in sample titration.

(The factor 1.724 is based on the assumption that carbon is only 58 % of the organic matter).
Diversity

For Benthic species diversity following measures of diversity were analysed:

**Shannon-Wiener’s Index** (Ludwig and Reynolds, 1988)

\[ H = - \left( \sum pi \ln pi \right); \text{ where} \]

\[ Pi = \frac{n}{N} \]

\[ n= \text{ total number of individual of a taxon} \]

\[ N= \text{ total number of individuals of all taxa} \]

**Percentage similarities** Sorenson’s index (Sorenson, 1948) formula

\[ Cs= \frac{2C \times 100}{a+b} \]

\[ C= \text{ species common in two samples} \]

\[ a= \text{ number of species of one collection} \]

\[ b= \text{ number of species of next collection} \]

**Menhinick’s Index** (Menhinick, 1964)

\[ Dmn = \frac{S}{\sqrt{N}}; \]

Where; \[ S= \text{ Total number of species} \]

\[ N= \text{ total density of all the species} \]

**Evenness**

\[ E_1 = \frac{H^1}{\ln S} \] (Pielou, 1969);
Where $H^1= \text{species diversity}$

$S= \text{species richness}$

**Statistical analyses**: diversity indices, Cluster analysis, MDS and Similarity were calculated and plotted with the help of Primer v5 (version 5.2.4). Correlation analysis, Regression lines were calculated with the help of SPSS (v20). Pi figures, Histograms were plotted with the help of Microsoft excel (2007).