Chapter IV

MATERIALS & METHODS
Chapter-IV

MATERIALS AND METHODS

The present work was carried out for a period of two year’s from January, 2006 and December 2007. For the completion of exhaustive work compiled in this thesis the standard methods described for the purpose have been used. Certain permissible modifications according to the local condition have also been incorporated. A short description of materials and methods applied during the present investigation had been presented below-

IV.A: Sampling and Preservation:

Since the Chittaurgarh dam is a deep water body and with a slight inflow of water as runoff from the margin it normally overflows. If is also a fact that the geographical and topographical situation of this water body is quite different and therefore for the study of hydrobiological (physico-chemical and biological) condition of the water body and to assess the actual position of phytodiversity, the entire area of the dam has been taken in to account, For the purpose three sampling stations namely: littoral, pelagic and polluted were set, depending upon the degree of inflow and water turbidity. At the glance stations littoral and pelagic were marked non-polluted. The sampling station’s were marked by means of a weighted plastic float.

All samples for abiotic and biotic component (e.g., water sediment, planktons and macrophytes) of the dam were collected during the second
weak of each month between 8:00 to 11 A.M. They were taken from different sampling stations fixed up in littoral, pelagic and polluted regions and were transported to the Laboratory of Botany Department, M.L.K. (P.G.) College, Balrampur (U.P.) India, at the earliest for qualitative and quantitative estimations. Water samples were collected in three replicates from each of the site in clean plastic containers, using standard method of collection (APHA, 1998). The entry of scums and other surface materials in the samples were avoided. Prior to collection sample bottles were rinsed thoroughly with the sample water. Each bottle was clearly labelled and protected them from direct sunlight during transportation.

**Preservation:**

Prior to the use of preservatives or techniques, the samples so collected in the sampler were transferred to well rinsed, appropriately labelled suitable sample containers of borosilicate glass or polyethylene. Labels on different bottles clearly indicated the name and location of sampling station, date and time of sampling, station No. and depth.

These samples in well labelled and tightly capped containers were brought to the laboratory in an ice-box and kept in a freezer to check the biological activity and preserve them. The physico-chemical analyses of these samples were completed within a very short time to avoid chemical or biological deterioration of the sample (APHA-AWWA-WPCF-1992). Some techniques for the preservation of samples for chemical analyses have been given in Table IV.1.
Table IV.1: Preservation techniques of the water samples for chemical analysis.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sample Volume (ml)</th>
<th>Holding time</th>
<th>Preservation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidity</td>
<td>100</td>
<td>24 hr</td>
<td>Refrigerate 4°C</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>100</td>
<td>24 hr</td>
<td>Refrigerate 4°C</td>
</tr>
<tr>
<td>BOD</td>
<td>1000</td>
<td>6 hr</td>
<td>Refrigerate 4°C</td>
</tr>
<tr>
<td>COD</td>
<td>50</td>
<td>7 days</td>
<td>$\text{H}_2\text{SO}_4$ to pH less 2</td>
</tr>
<tr>
<td>Chloride</td>
<td>50</td>
<td>24 hr</td>
<td>Refrigerate 4°C</td>
</tr>
<tr>
<td>Colour</td>
<td>50</td>
<td>24 hr</td>
<td>Refrigerate 4°C</td>
</tr>
<tr>
<td>DO</td>
<td>300</td>
<td>-</td>
<td>Immediate</td>
</tr>
<tr>
<td>Hardness</td>
<td>100</td>
<td>7 days</td>
<td>Refrigerate 4°C</td>
</tr>
<tr>
<td>Metals</td>
<td>200</td>
<td></td>
<td>$\text{HNO}_3$ to pH less 2</td>
</tr>
<tr>
<td>Ammonia</td>
<td>500</td>
<td>24 hr</td>
<td>Refrigerate 4°C</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>500</td>
<td>7 days</td>
<td>$\text{H}_2\text{SO}_4$ to pH less 2</td>
</tr>
<tr>
<td>Nitrate</td>
<td>100</td>
<td>24 hr</td>
<td>Refrigerate 4°C</td>
</tr>
<tr>
<td>Nitrite</td>
<td>100</td>
<td></td>
<td>-do-</td>
</tr>
<tr>
<td>pH</td>
<td>100</td>
<td>-</td>
<td>Immediate</td>
</tr>
<tr>
<td>Phosphate</td>
<td>50</td>
<td>24 hr</td>
<td>Refrigerate 4°C</td>
</tr>
<tr>
<td>Conductance</td>
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<td>-do-</td>
</tr>
<tr>
<td>TDS</td>
<td>100</td>
<td>24 hr</td>
<td>-do-</td>
</tr>
<tr>
<td>Sulphate</td>
<td>50</td>
<td>24 hr</td>
<td>-do-</td>
</tr>
<tr>
<td>Sulphide</td>
<td>100</td>
<td>-</td>
<td>Immediate</td>
</tr>
<tr>
<td>Temperature</td>
<td>1000</td>
<td>-</td>
<td>Immediate</td>
</tr>
</tbody>
</table>

Always used P.G. type of container.
IV.B. Water Sample Analysis:

The procedures described by Michael (1984), Manivaskam (1987) and Trivedy et. al. (1987) have been adopted in the analytical techniques. A brief description of the methods employed are given here:

(a) Physical Parameters:

(1) Depth: Depth was measured by a weighed handline marked in meters. Handline was lowered until the weight touches the bottom of the water level. Such observations were made always at the same place and were recorded in meters.

(2) Temperature (°C): For determination of temperature, soon after the collection of sample in the polyethylene bottle, a mercury thermometer of 0.1-0.2°C accuracy was inserted in the bottle up to mercury level and was recorded separately.

(3) Colour: The colour was determined by platinum-cobalt method. Collected samples were centrifuged to remove suspended matters and turbidity free samples were obtained as (A). Stock solution of platinum-cobalt colour standard was prepared by dissolving 1.246 gram of potassium chloro platinate (K₂PtCl₆) and 1 gram crystalline cobaltous chloride (COCl₂. 6H₂O) in distilled water having 100 ml of concentrated HCl and it was made to 1 litres. This solution has 500 mg/l of platinum and about 250 mg/l of metallic cobalt, equivalent to 500 colour units. (B). Colour Units of 5, 10, 20, 25, 30, 35, 40, 45, 50, 60 and 70 were prepared by diluting the stock colour standard (B) and made 50ml each with distilled water in Nessler’s tubes as (C). For colour determination, 50ml turbidity
free sample (A) was taken in Nessler’s tube compared with colour units (C) prepared from stock colour standard (B) by looking vertically through the tubes above white surface. Matching colour was recorded.

(4) Transparency: Water transparency was measured by a Secchi distance of 20 cm diameter, having alternate quadrants of white and black colours, attached to a graduated rope. The disc was lowered in the water till it disappeared visually and then it was slowly lifted till it become visible again. The depths of disappearance of Secchi disc were noted and the average of the two was taken as Secchi disc transparency.

\[ \text{S.D. Transparency (c.m.)} = \frac{A + B}{2} \]

Where: \[ A = \text{depth at which Secchi disc disappears (cm)} \]
\[ B = \text{depth at which Secchi disc reappears (cm)} \]

(b) Chemical parameters:

1. Hydrogen ion concentration (pH): The pH was measured by Electrometric method using Century CP-901 digital pH meter. The instrument was adjusted according to the instruction manual issued by the manufacturer with the help of buffer of pH 4.0 to 9.20.50 ml of sample was taken in a 100 ml clean beaker and the pH electrode was inserted into it. The reading of the meter was noted as the pH of the sample.

(2) Free carbon dioxide (Free CO₂): 50 ml of sample was taken in a conical flask and few drops of phenolphthalein indicator (1 g phenolphthalein + 100 ml alcohol + 100 ml distilled water) was added to it. Sample remained colourless. It was titrated against 0.05 N NaOH(40g NaOH dissolved in CO₂ free distilled water and diluted to 1 liter. 50ml of
this solution was again diluted to 1 litres and it was standardised with HCl. At the last, a pink colour appeared.

\[
\text{Free } \text{CO}_2 \text{(ml)} = \frac{V_1}{V_2} \times 1000 \times 44
\]

Where, \( V_1 = \text{N of NaOH} \) \( V_2 = \text{Volume of sample} \)

3. **Dissolved Oxygen (DO):** The dissolved oxygen was measured by Winkler’s Iodometric method. A glass stoppered BOD bottle of known volume (300ml) was taken and filled with sample avoiding any bubbling. 2 ml each of manganese sulphate solution (100g MnSO\(_4\) \( \cdot 4H_2O \) dissolved in 200 ml boiled water and filtered) and alkaline potassium iodide solution (100g KOH and 50g K dissolved in 200ml distilled water) using separate pipette well below the surface from the walls.

A precipitate was appear. The stopper was put tightly and the bottle was shaken vigorously. 2ml conc. \( H_2SO_4 \) was added to it and the ppt. dissolved. The whole content was then transferred to a conical flask and a few drops of starch indicator (1g starch indicator + 100 ml warm distilled water at 80-90 °C + few drops of formaldehyde solution) was added to it.

This solution was titrated against 0.025 N sodium thiosulphate solution (6.205 g sodium thiosulphate dissolved in boiled distilled water and made up the volume to 1 litres. 0.4 g Borax was added as stabiliser. This was 0.1 N solution. This solution was diluted 4 times and 0.025 N solution was obtained and was kept in brown glass Stoppered bottle).
$$D.O.(mg/l) = \frac{V_1 \times N \times 8 \times 1000}{V_2 \times V_3}$$

Where

$$V_1 = \text{Volume of titrant (ml)}$$

$$V_2 = \text{volume of sample bottle after placing the stopper (ml)}$$

$$N = \text{normality of titrant (0.025)}$$

$$V_3 = \text{volume of manganous sulphate + Potassium iodide solution added (ml)}$$

4. **Total Alkalinity:** Immediate analysis of the sample after collection be made. 50 ml of sample was taken in a flask and 2-3 drops of phenolphthalein indicator were added. Slight pink colour indicated phenolphthalein alkalinity while its absence marked the presence of free CO$_2$. The solution was titrated against 0.02 H$_2$SO$_4$ (2.8 ml conc. Sulphuric acid was diluted marked the presence of free CO$_2$. The solution was titrated against 0.02 H$_2$SO$_4$ (2.8ml conc. Sulphuric acid was diluted to 1litres with distilled water. 200ml of this stock solution was again diluted to water and 0.02 N solution was prepared. This was standardised until it become colourless. The reading noted. 2-3 drops of methyl orange indicator (0.1g methyle orange + 200 ml distilled water) in he same flask and titrated again with sulphuric acid until it turned orange. This was also noted. The volume of titrant used for both the titrations were recorded.

$$\text{Total alkalinity (as CaCO}_3\text{ mg/l)} = \frac{T \times 1000}{S}$$

Where,

$$S = \text{volume of sample}$$

$$T = \text{total volume of titrant used for the two titrations}$$
Carbonate alkalinity = 2 \times \text{ Phenolphthalein alkalinity}

Bicarbonate alkalinity = \text{Total alkalinity} - \text{Carbonate alkalinity.}

5. **Chloride**: 10 ml of sample was kept in a flask and 5-6 drops of potassium chromate indicator (10 g potassium chromate + 20 ml distilled water) was added to it. The colour of the sample became yellow. This was titrated against 0.025 N silver nitrate solution (3.397 g AgNO₃ dissolved in distilled water and diluted to 1 litres) unit a persistent brick red colour appeared. The end point reading was noted.

\[
\text{Chloride (mg/l)} = \frac{V \times N \times 35.457 \times 1000}{S}
\]

Where,

- V = volume of titrant (ml)
- N = normality of titrant
- S = volume of sample (ml)

6. **Nitrate (NO₃⁻)**: It was estimated by Brucine method. 10 ml of sample was taken in a Erlenmeyer flask and 2 ml of sodium chloride solution (300g NaCl + distilled water diluted to 1 litres) was added to it. The flask with contents was shaken and kept on a cool water bath. 10ml of H₂CO₂ (500ml conc. H₂SO₄ in 125ml distilled water) was added slowly. 0.5ml brucine sulphameric acid solution (1g brucine sulphate + 0.1g sulphameric acid in 50 ml of warm distilled water + 3 ml conc.HCl and diluted to 100 ml) was also added. This was shaken and the flask was kept on a hot water bath with boiling point for 20 minutes. The contents were cooled and the absorbance (s) was recorded on spectrophotometer at 410 nm. The distilled water was used as blank.
The standard nitrate solution (0.722g anhydrous KNO$_3$ + distilled water made 1 litre, equivalent to 100 mg NO$_3$ N/l or 443 NO$_3$ ions/l) was processed in similar manner and the absorbance (s) of the standard nitrate solutions of different strengths (0.0 to 1.0mg NO$_3$ N/l) at the intervals of 0.1. A standard curve between absorbance (S) and concentration of standard solution was prepared and the value of NO$_3$-N in samples (S) with the standard curve was deducted and the resulting expressed in my NO$_3$N/l.

7. Calcium: It was estimated by Titimetric method of Jackson (1967). A 50 ml sample was taken in an Erlenmeyer flask. 1 ml of 8% NaOH solution (8g NaOH + distilled water made 100 ml) and a pinch of muroxide indicator (0.2g ammonium purpurate + 100g NaCl, grinded thoroughly) were added. This was titrated with EDTA solution and the reading, when pink colour changed purple was, recorded.

\[
Ca(\text{mg/l}) = \frac{T \times 400.5 \times 1.05}{V}
\]

Where,

\(T = \text{total hardness}\)

\(V = \text{volume of sample}\)

8. Phosphate: The water samples were analysed in the laboratory for the phosphates by stannous chloride method of APHA (1998). First of all, 2 ml ammonium molybdate reagent was added into 100 ml of water sample. Then 0.5 ml of freshly prepared stannous chloride solution was added which caused the appearance of blue colour. Density was read in a spectrophotometer against a distilled water blank. Prepared a calibration
curve and find out the number of micrograms of phosphate equivalent to the observed optical density of the sample. Expressed the result as mg phosphate of sample.

9. **Total Nitrogen:** 50 mg of sample was taken in an evaporating dish and evaporated it to dryness. 4 ml of digestion mixture (K₂SO₄, mercuric oxide, sulphuric acid in distilled water) was added to the residue and dissolved it in about 20 ml of distilled water. Heated the solution to fuming for over 15 minutes and cooled it. Then transferred the digest to microkjeldahl distillation assembly and added about 3.5 ml of hypo solution. 5 ml of boric acid solution was taken, containing 2-3 drops of mixed indictor (methyl red, 0.5% bromocresol green solution) in a conical flask placed below the condenser of assembly, so that the tip of outlet of condenser is dipped in contents of conical flask. Heated the boiling flask of assembly so that the steam passed into sample through chamber of assembly and distillation was continued for about 10 minutes. The conical flask with distillate was removed titrated that distillate against HCl. Turning of blue colour to pink indicated the end point. A distilled water blank was run in similar way

\[
\text{Total nitrogen (mg/l)} = \frac{(A - B) \times N \times 1000 \times 14}{V}
\]

Where,

A = volume of titrant used against sample (ml)

B = volume of titrant used against blank (ml)

N = normally of titrant (0.04)

V = volume of sample (ml)
10. **Total organic matter:** It was analysed according to standard method of American Public Health Association (1992).

11. **Total Solids (T.S.):** The unfiltered known volume of sample was kept in an evaporating silica dish (V) and weighed. The weight of the dish noted as (A). The dish was kept on a water both for evaporation and the final weight of the dish was recorded as (B)

   \[ TS = \frac{A-B}{V} \times 1000 \text{ mg/l} \]

12. **Total Dissolved Solids (TDS):** An evaporating silica dish was taken and weighed. The initial weight was recorded as (A). Filtered sample of known volume (V) kept in the dish and evaporated on a water both and weighed finally after evaporation as (B)

   \[ TDS = \frac{B-A}{V} \times 1000 \text{ mg/l} \]

13. **Total Suspended Solids:**

   It is the difference of TS and TDS.

   \[ \text{T.S.S.} = \text{T.S.} - \text{T.D.S. (mg/l)}. \]

**IV.C. Sediment Analysis:**

The samples were taken with the help of Ekman dredge and kept in 0.5 litre wide-mouth bottles. A known amount of mud sample was kept for drying to constant weights in an oven at 105-110°C. The dried samples were lightly grind in mortar passed through 2 mm sieve and stored in polythene bags for physico-chemical analysis. Samples have been taken monthwise during the tenure of research work. Soil suspension was prepared in 1:5 ratio of soil and water. The estimation of following parameters was done by different methods as under:
1. **Temperature** was recorded on the spot by the help of soil thermometer.

2. **PH** value was recorded electrometrically with the help of pH meter (Elico make, model L-12).

3. **Conductivity** was recorded (in mmho) with the help of conductivity probe of water analyser kit.

4. **Total alkalinity** was analysed titrametrically by following standard method of Piper (1968).

5. **Organic matter** was determined by the standard method of Piper (1968).

6. **Total nitrogen** was analysed by Micro-Kjeldahl method (Misra, 1968) by digesting 10gm powdered and sieved soil sample in 20ml concentrated H₂SO₄ using catalyst mixture.

7. To determine **available phosphorus**, 5 gm sediment was weighed and analysed by chlorostannous-reduced molybdophosphoric blue colour method (Jackson, 1967).

8. The content of **calcium** of the filtrate was determined with Systronic Flame Photometer-127.

9. **Nitrate-nitrogen** was estimated by treatment of soil extract with nitrophenol disulphuric acid and measuring colour intensity at 420 nm in flame photometer.

10. **Chloride** was analysed by titration with standard silver nitrate solution using Potassium chromate as indicator (Trivedy *et. al.*; 1987).
IVD. Biological Analysis:

IV.D.1. Macrophytes:

(a) Collection:

The macrophytes of the chittaurgarh dam in different month of investigation were collected from prepared floating and sinking type of quadrates (1x1 meter size) for their systematic study and identification. The free floating smaller plants were collected by long handled net (r = 15 cm.) and submerged plants by Ekman dredge of 25 ×25 cm² size. The macrophytes thus collected were washed thoroughly to remove animals, and other debris and kept in polythene bags. They were brought to laboratory for further analysis.

(b) Systematic study:

In the laboratory, the collected macrophytes were segregated species wise in water to avoid dehydration and recorded their nature *viz-* marshy, emergent, submerged, attached floating and free floating. The identification was made with the help of pertinent literature (Agharkar, 1923: Hogweg *et. al.*, 1969).

(c) Phytosociological Studies:

Studies were made according to the method described by Puri *et. al.* (1968) by recording presence (+) or absence (-) of different species occurring in and around the chittaurgarh dam during 2006-2007. Frequency of distribution and degree of heterogeneity was worked out according to Misra (1968).
IV.D.2. Plankton Analysis:

Collection of sample for biological studies of phytoplankton (especially algae) and zooplankton was done by using a plankton net (64 nm) of ‘Bolting silk’ 5 liter of water were seaved from each sampling sites on each sampling time. The plankton concentrates thus obtained we divided into two sub samples in ratio 3:1 volumetrically. The larger sub samples were used for chemical estimation and smaller for direct counting. Samples were studied under inverted microscope in a Sedgiwick-Rafter cell. Plankton counts were made by plankton counting cell.

Identification was done by using standard methods by Palmer (1969) and for zooplankton analysis the standard monographs of Pennak (1978) and Tonapi (1981). The colonial alga like *Volvox* and *Anabaena* etc. were counted as individual units which were numerically compared in the data to single celled organisms such as *Chlamydomonas* and *Euglena* etc. (Sinha, 1968). The counts were converted to number of units per litre or per C.C. the following groups were enumerated.

Myxophyceae (Cyanophyceae), Diatomaceae and Chlorophyceae amongst phytoplanktons and Rotifera, Cladocera and Copepoda amongst zooplanktons.

IVD.3 Nutrient dynamics analysis:

For the analysis of dynamics experimental pot approximate 5 gms. of fresh macro and microphytes were introduce separetely. The selected eutrophic plants were put in respective sets and allow to grow for only 10 days. At 11\textsuperscript{th} day after termination of the experiment the plants were
harvested and chlorophyll, dry weight, protein and NPK contents were recorded. The Nutrients occur in plant samples were carried out following the method of Trivedy et al. (1987). The total chlorophyll content was estimated following the method of Arnon (1951). The total protein was estimated in accordance with Lowry (1958). The nitrogen and phosphorus contents were estimated Subba Row (1997). Potassium was estimated by flame photometer (AIMIL). The data obtained were statistically analysed through graphic presentation following Dospekhov.

IVD.4. Eutrophic gradient analysis:

Naygaard (1949) proposed five trophic states to evaluate eutrophic gradient and level of organic pollution. In this regard he considered cyanophycean index, Bacillariophycean index, Euglenophycean index and compound index.

Naygaard's trophic state indices:

Cyanophycean index = \( \frac{\text{Cyanophyceae}}{\text{Desmideae}} \)

Chlorophycean index = \( \frac{\text{Chlorococcales}}{\text{Desmideae}} \)

Diatom index = \( \frac{\text{Centric diatoms}}{\text{Pennate diatoms}} \)

Euglenophycean index = \( \frac{\text{Euglenophyta}}{\text{Cyanophyceae+Chlorococcales}} \)

Compound index = \( \frac{\text{Centric diatoms} + \text{Euglenophyta}}{\text{Desmideae}} \)
IV.G. Conservation perspectives:

Regular visits were made in all the months and seasons of the study years. The surveys were conducted and broad map of the dam was prepared. The complete panoramic view, photograph of the dam under investigation was prepared. The villagers and visitors were surveyed regarding their wish for the dam development. They were interviewed and proposed tourism, notional park, bird sanctuary facility oriented map was prepared depicting the different sites like botanical gardens, resorts, restaurants, parking communication and transportation need for ecotourists and pilgrims of the dam. We have also conducted a number of seminars on the conservation perspectives of the dam.