CHAPTER IV

MATERIAL AND METHODS
I. Physico-Chemical Characters of the water:

The physical and chemical conditions such as depth, temperature, turbidity and light along with the chemical parameters, such as pH, oxity ($DO_2$), Free carbon dioxide ($FCO_2$), carbonate, bicarbonate, hardness, phosphate, silicate, nitrate and chloride are some of the most essential characteristics reflecting the water quality and its productivity. Water temperature depends upon sunlight, depth and climate and have much greater influence on the aquatic organisms. Other parameters also influence the biotic components of the water bodies individually and also collectively. The nutrient status influences the plankton and hence primary production. All these factors are interrelated and inter-dependent.

II. Chemical analysis of Soil:

Besides nutrient, one of the factors of importance in fish productivity is soil. A satisfactory pond bottom soil is that in which mineralisation of organic matter takes place rapidly, nutrients are absorbed, held and released slowly over a long period. Loose, friable and loamy soils falls under this category. According to Macan et al. (1942), mud bottoms are most-productive of all and inorganic bottoms of gravel, sand and
clay are very poor but can be improved by the application of stable manure of sewage sludge. In normal ponds, the physical and chemical properties of the pond water are more or less a reflection of the bottom soil. The soil samples were taken to study the seasonal variation of soil condition in both the ponds.

III. Plankton:

Plankton is free floating animal and plant organisms, whose locomotion remain almost at the mercy of currents and waves. Plankton plays a very important role as primary producers. The productivity of a water body is mainly influenced by the plankton density, some being indicators of pollution. In fish culture systems where fish are not provided supplemental feed, plankton forms the most-abundant base of the food web. Direct food web will produce a greater weight of fish per unit area. Plankton populations with time and space certainly influences the fish production of the ponds because there is a close relationship between plankton abundance and fish production.

IV. Stocking of Ponds and Fish Growth:

The standard technology of Composite fish culture as described by Sinha (1976) was followed. The physico-chemical conditions of the ponds water and soil were analysed employing standard methods (Piper, 1949 and A.P.H.A., 1955). The ponds were stocked with catla, rohu and mrigel at the rate of 6000-8000 fingerlings/ha.
1. Physical features of water:

The details of monthly observations on physico-chemical characteristics of the two ponds were conducted during April 1988 to September, 1989. For the estimation of various physico-chemical parameters, surface and subsurface water samples were collected generally between 08.00-10.00 Hrs. in the morning. Temperature, pH, DO₂ (Dissolved oxygen), FCO₂ (Free Carbon dioxide), Carbonate (CO₃⁻), bicarbonate (HCO₃⁻) alkalinity were estimated on the spot. Whereas the other physical and biological parameters were determined in the laboratory according to A.P.H.A. (1955).

Water samples from both the ponds were stored in clean glass bottles after preserving them by adding required Toluene were brought to the laboratory for analysis. Along with the regular collection of water samples from the surface, bottom samples of water were also studied for the first 12 weeks. As differences in the Physico-chemical factors from surface to bottom were not significant in respect to most of the factors, the practice of bottom sampling was discontinued.

Diurnal fluctuations of certain physico-chemical factors, such as temperature, pH, DO₂, FCO₂, CO₃⁻ and HCO₃⁻ of surface water of both the ponds was studied at intervals of 4 hours in 24 hours period. These diurnal studies were performed in the month of
May and August, 1988 and January, 1989 representing summer, monsoon and winter seasons respectively.

A. Physical Measurement

(i) Temperature: The water temperature readings were recorded using a mercury thermometer graduated $110^\circ C (1^\circ C$ divided into ten divisions at $0.1^\circ C$).

(ii) Turbidity: The transparency of water was measured with a 20 cm. Secchi disc provided with a graduated rope. The upper surface of which was divided into four equal quadrants, each of them being pointed black and white alternately while the lower side of plate painted black to eliminate reflection of light. The depth at which the disc disappeared was first noted and then by slowly raising the disc. The reading at which it reappeared was also recorded. The average of these two readings is given as the limit of viability and recorded as Secchi disc transparency (Welch, 1948).

B. Chemical Measurement

The analysis of water samples for various chemical characteristics was done as per methods laid by Mackereth (1963), Golterman and Clymo (1969) and Standard methods, A.P.H.A. (1955).

(i) Hydrogen-ion-concentration (pH):

A portable griph pH meter of 'Systronics Make' was used for determining pH.
(ii) **Oxygen** (DO$_2$):

Modified Winkler method was used for the estimation of dissolved oxygen. Samples were taken from just beneath the surface with least disturbance in the medium and were fixed in the field in 100 ml capacity glass bottles fitted with air tight stoppers by avoiding the trapping of any air bubble during sample collection. Fixatives used were 1 ml of manganous sulphate and 1 ml of alkaline iodite (KOH + KI). Then concentrated Sulphuric acid was added to the solution to dissolve the precipitate and then titration was done against standard N/40 Sodium thiosulphate using starch as the indicator. The volume of Sodium sulphate used in cc was equal to the amount of dissolved oxygen present expressed as parts per million (ppm).

(iii) **Free Carbon-dioxide** (FCO$_2$):

It was determined by titrating the 100 ml water sample against N/44 Sodium hydroxide (NaOH) using phenolphthalein as indicator. The amount of the alkali used for 100 ml was multiplied by 10 to obtain the value for a litre of the sample which was expressed in ppm.

(iv) **Alkalinity** (Carbonate and Bicarbonate):

The amount of total alkalinity (Carbonate and Bicarbonate) was determined by titrating 100 ml water sample against N/50 Sulphuric acid solution using phenolphthalein and methyl orange
as indicator respectively. The results expressed as Carbonate
(CO$_3$) and bicarbonate (HCO$_3$), taking into consideration the
amount of acid used and multiplying by a factor 10, the result
being expressed as ppm.

The samples for the estimation of total hardness, calcium
hardness, chloride, silicate, phosphate, nitrate, nitrogen,
sodium, potassium, calcium and magnesium were fixed in
chloroform and sealed on the spot and were carried to the
laboratory for analysis.

(v) **Total hardness:**

It was determined by titrating 50 ml of the well mixed
sample with standard EDTA (0.01 N) solution using 1-2 ml ammonia
buffer solution and total hardness tablets as indicator.

(vi) **Calcium Hardness :**

Calcium hardness was determined by adding about 1 ml of
NaOH solution to 50 ml of the sample and then titrating it with
standard EDTA solution using calcium hardness tablets as indicator.

(vii) **Chloride (Cl$^-$):**

Chloride was estimated by titrating 50 ml of sample with
standard (0.05 N) silver nitrate solution using potassium
chromate as indicator.

(viii) **Silicate (SiO$_3^{2-}$):**

Silicate was estimated by colorimetric method using picric
acid solution as standard (equivalent to 0.001 mg silica/ml) 
(Jhingran, Natarajan, Banerjee and David, 1969).

(ix) **Phosphate** (PO$_4^{3-}$):

Phosphate was determined with the help of a photoelectric 
colorimeter (Systronics Model Type No. 101-T Sr. No. 805) with 
660 mm, wave length filter. The sample was treated with ammonium 
molybdate sulphuric acid solution and stannous chloride as 
indicator. The colorimeter reading was compared with the standard 
curve and using a distilled water blank (Wilde, Voigt and Iyer, 
1972).

(x) **Nitrate nitrogen** (NO$_3^-$ - N):

Nitrate nitrogen was estimated by colorimetric method 
using phenol disulphonic acid solution, and 12N sodium hydroxide 
solution. Potassium nitrate was used as a standard solution 
(equivalent to 0.001 mg N/ml) (Jhingran, Natarajan, Banerjee and 

(xi) **Sodium** (Na$^+$):

Sodium was determined with the help of a flame photometer 
(Systronics Type 125 MK-I/MK-II) using stock sodium solution 
(equivalent to 1 ml = 1 mg sodium) and deionised distilled water.

(xii) **Potassium** (K$^+$):

Potassium was determined with the flame photometer using 
stock potassium solution and deionised distilled water.
(xiii) Calcium (Ca⁺⁺):

Calcium was obtained by calculation (APHA, 1955) -

\[ \text{Calcium mg/l} = \frac{A \times B \times 400.8}{\text{ml sample used}} \]

Where,

\[ A = \text{ml titration for sample and} \]
\[ B = \text{mg CaCO}_3 \text{ equivalent to 1.0 ml. EDTA titrant at} \]
\[ \text{the calcium indicator and point.} \]

(xiv) Magnesium (Mg⁺⁺):

For the estimation of magnesium concentration in water, calculations were made from the EDTA total hardness and calcium hardness titration.

\[ \text{Magnesium mg/l} = (\text{total hardness as mg/l CaCO}_3) - \]
\[ \text{Calcium hardness as mg/l CaCO}_3) \times 0.244. \]

The coefficient correlations (r) value of various physico-chemical parameters were worked out and given (Table XIX).

(xv) Diurnal variations:

Diurnal variations were recorded at 4 hours intervals from 08.00 hours of one particular day to 04.00 hours next day in both the ponds. Experiments in both the ponds were conducted on 28-29 May, 1988 representing summer season, 20-21 August, 1988 representing rainy season and 14-15 January, 1989 representing winter season. Like monthly sampling, in diurnal
variation studies too, the representative water samples for
certain physico-chemical analysis of each pond were collected
and composite sample for each pond were obtained and on the
spot analysis was conducted. Results of water temperature,
transparency, oixy and Free Carbon dioxide analysis are
presented as average value.

2. Sampling and Chemical analysis of Soil:

Soil samples of both the ponds $T_1$ and $T_2$ were collected
once in a month during the period of study (April, 1986 to
September, 1989). Samples collected from 2 spots of each pond,
were mixed together on a plastic surface and composite samples
were obtained. Samples were air dried in laboratory, powdered
and seived in 100 mesh sieve. About one Kg of the fine powder
was packed in plastic bags and leveled for detailed analysis.
Chemical analysis of soil samples, covering the aspects of soil
texture, pH, CaCO$_3$, organic matter, available phosphorus,
nitrogen and Potash was done as per methods recommended by
International Bureau of Soil Science, with slight modifications
as necessary (Piper, 1949). Soil analysis was done with the
courtesy of Indian Agriculture Research Institute, Pusa,
New Delhi.

3. PLANKTON:

Plankton samples were collected regularly once in a month
from both the ponds $T_1$ and $T_2$ for a period of study from April 1988 to September, 1989 on the sampling day between 7 A.M. to 8 A.M. during summer and rainy season and 9 A.M. to 10 A.M. during winter season. The samples were collected by hauling 60 litres of water through a Plankton net made of standard bolting silk cloth having mesh size 0.03-0.04 mm. The plankton collected were preserved in 4% formalin for subsequent quantitative and qualitative analysis in the laboratory.

The quantitative and qualitative analysis of phyto-plankton and Zooplankton were made with the help of binocular compound microscope following Lackey drop microtransect counting method (1938) as modified by Edmondson (1971).

One drop from the thoroughly shaken plankton sample was kept on a clean microslide with the help of a vertically held dropper and was covered by coverslip (22 mm size) in such a way that it covers the drop completely and no sample runs out. After enumeration of the larger forms in the low power of the compound microscope, the smaller forms were counted in about 20 Visual 'fields' (focus) under the high power. The process was repeated at least five times and number of individual species per litre were calculated with the help of the following formula:
Number of individuals per drop = \( \frac{\text{Area of cover glass}}{\text{Area of focus}} \times \text{Average number of individuals per focus.} \)

Individuals per litre = \( A \times \frac{1}{L} \times \frac{VV}{V} \)

Where,

\( A = \text{Number of individuals/drop.} \)
\( V = \text{Volume of one drop (ml).} \)
\( VV = \text{Total volume of concentrated sample (ml)} \)
\( L = \text{Volume of the original sample (l)} \)

The identification was mainly based on Carter (1926), Needham et al. (1930), Venkataraman (1939), Smith (1950), Ward and Whipple (1959), Desikachary (1959), Fritsch (1959), Philipose (1967), Victor and Fernando (1979) and Tonapi (1980). The coefficient correlation \( r \) value of certain physico-chemical parameters and plankton were worked out and given (Table XX).

4. **Stocking of Ponds and Fish Growth:**

Both the ponds selected for study were at the Shahdara Fish Farm, under the administrative control of Fisheries Department, Delhi Administration. The size of ponds is 30 m x 15 m x 2.5 m each and are completely drainable by operating the outlet sluice gates of each pond. The loss of water through evaporation and slight seepage is made up letting of water as and when desired. There is assured water supply for the twelve months of the year. The minimum water level of about 1.60 m was
maintained in both the ponds during summer months.

Both the ponds were prepared by dewatering and refilling. Liming (@ 1000 kg/ha), Cowdung (@ 3000 kg/ha), Urea (@ 200 kg/ha) and Single Superphosphate (@ 250 kg/ha) were applied before stocking the ponds. Thereafter both organic and inorganic fertilisers, alternating with each other, were applied in Pond (T1) during the course of study @ 1000 kg/ha/month and 35 kg/ha/month respectively (Sinha and Saha 1980). A judicious mix of three major carps fingerlings *Catla catla* (108 nos), *Labeo rohita* (81 nos) and *Cirrhina mrigala* (81 nos) i.e. total of 270 nos. each in the size range of 95 mm-160 mm were stocked in both the ponds under study during March, 1988. Thus a stocking rate of 6000 per hectare was followed.

The weight of the stocked fish fingerlings (Catla/Rohu/Mrigala) at the time of stocking in both the ponds (T1) and (T2) varied from 35-60 gms each fish. After stocking of the ponds in March, 1988, required supplementary feed in the form of powdered and served mixture of Mustard Oil Cake (MOC) and Rice Bran (RB) in the ratio of 1:1 was broadcast regularly (Once in a week) in one of the ponds (T1) under study at the rate of 0.6-3.6% of the body weight of fishes according to consumption. The feeding rate in pond (T1) was uniform and given either in one or two equal instalments throughout the period of study. Besides, other meticulous aquaculture operations were also used in this pond (T1) to achieve the highest production possible per unit
water area. While the other pond (T2) remained undisturbed from any deliberate management operations.

Fishes were sampled every month from both the ponds by drag netting for measuring their length range, average length, weight range and average growth. All these data as recorded in each sampling are given in Tables XVII and XVIII and Figures 25 to 28. Fishes sampled every month were measured on the usual fish-measuring board, divided into millimeters. The fishes were weighed on a platform balance to the nearest 1/10th of a gram. The conditions of fish were checked periodically by sampling few samples of fishes. Measures against parasitic infections, unsatisfactory growth rate etc. were taken (Das et al., 1982).