# Chapter – 2

# MATERIALS AND METHODS

## The Site

## Parametric Analysis

## Biodiversity Analysis

## Fisheries Data Analysis

# Materials and methods:

THE RESERVOIR ecosystem needs to be evaluated for its physico-chemical status as well as biological status to estimate potential for fisheries. Since ancient time civilization took place around rivers or other major water source and human developed skills for water storage and harnessing. Even though marine fisheries of India are well established, there is a necessity for better development of Inland fisheries.

## 2.1 The Site:

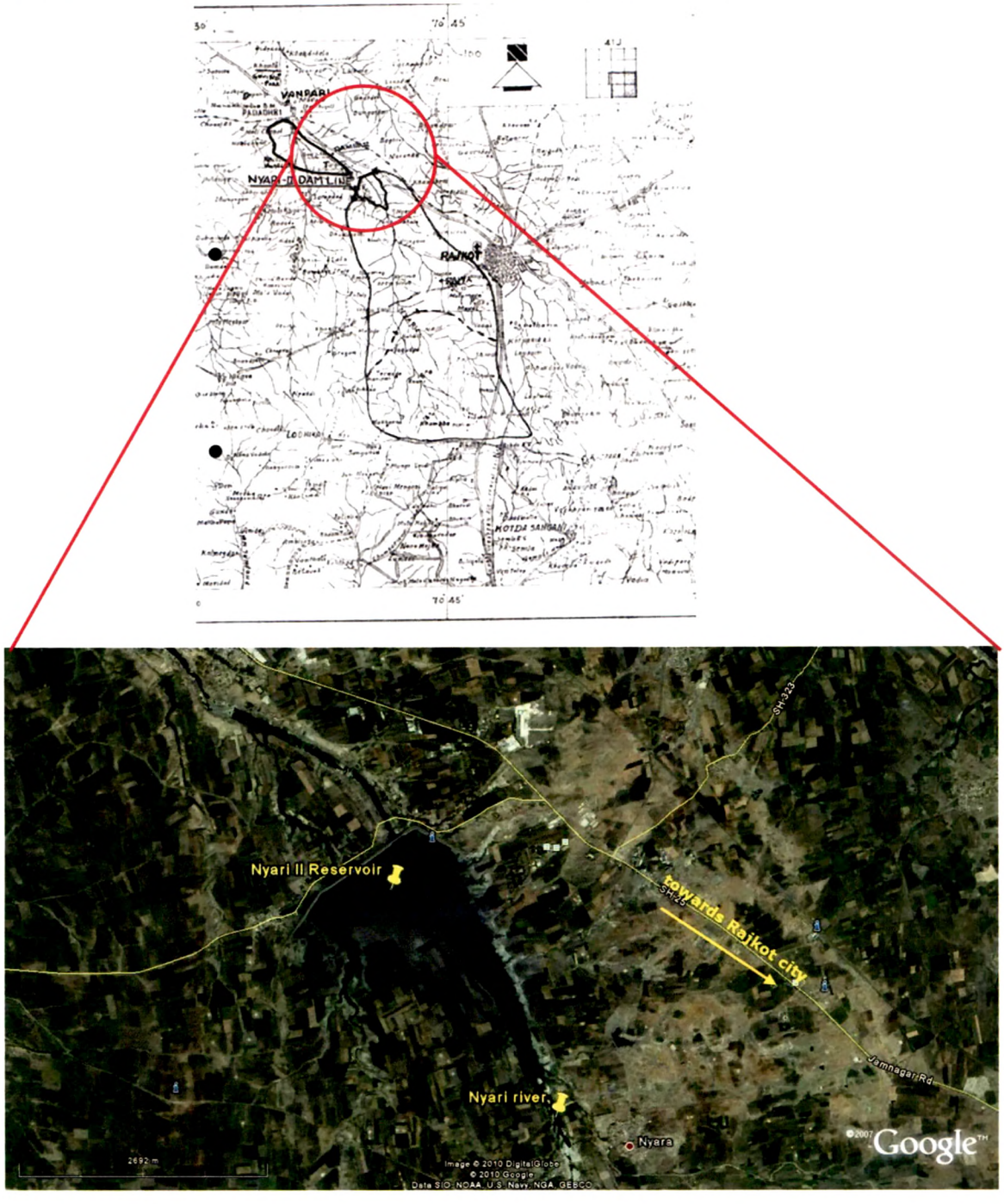
For aforementioned aim for estimation of fishery potential of a reservoir, suitable site was to be selected. The selection criteria was mainly the accessibility of the sampling site, utility of that water sheet, its fisheries status etc.

Inland reservoir, Nyari – II, is located in the Rajkot district of Gujarat state (Plate – 1). This perennial reservoir is rain fed as well as receives flood water through Nyari River and normally gets filled up in monsoon. Primarily the water resource is identified to utilize for irrigation and community water supply to RUDA (Rajkot Urban Development Area). This seasonal reservoir (Table – 2.1) has the storage capacity of 88.50 meters FRL (Full Reservoir Level) and the mean depth is ~ 45 meters, which is used for capture fisheries. Government of Gujarat Fisheries Department has under taken this reservoir for stocking of Carp seeds due to its good quality and quantity of water. As existing fish yield has been reported well from this reservoir therefore, it was planned to consider this site for detailed analysis to achieve the objectives of the present study.

**Table – 2.1: Data of Reservoir Nyari – II Dam.**

| Sr. No. | Detail | | Sr. No. | Detail | |
|---|---|---|---|---|---|
| 1 | Latitude | $22^0$-21'- 45" | 6 | Main Source | Nyari river |
| 2 | Longitude | $70^0$-40'- 15" | 7 | Length | 3855 Meters |
| 3 | Village | Rangpar | 8 | Width | 6 Meter |
| 4 | Teshil | Paddhari | 9 | Height | 14.35 Meter |
| 5 | District | Rajkot | 10 | Depth | 12.50 Meter |

## Plate – 1: Location of study site Nyari – II Reservoir



## 2.2 The parametric analysis:

To assess the ecological status of the reservoir, physico-chemical analysis of water and soil samples was carried out. The samples were collected monthly from the sampling site (Plate – 2). The biotic component was analyzed through estimation of productivity and biodiversity of aquatic resources of the reservoir.

### Water quality analysis:

The water samples were collected from the reservoirs for each month in the morning hours. Three samples were collected randomly from various zones of the reservoir and were pulled together and final sample was drawn for the analysis. These samples were processed, preserved etc. for further detailed quality estimations in the laboratory. All the quality parameters were analyzed as per methods (APHA, 1998), details of the same presented in table 2.2.

### Physical parameters:

### Temperature:

For determining water temperature of surface water samples, a centigrade thermometer graduated at 1.0°C interval was used. The thermometer was dipped directly in to the water at surface, kept for about 1 minute and temperature reading was noted.

### Electrical Conductivity (EC):

The reading of electrical conductivity was measured directly through conductivity meter in the laboratory. The electrode of the instrument was immersed in the water sample and the reading was recorded.

### Turbidity:

The nephlometric method was used for turbidity measurement. This method is based on a comparison of the intensity of light scattered by a sample and a standard reference under same conditions. Higher the intensity of scattered light higher the turbidity.

**Plate – 2: Water and Soil samplings for physico-chemical analysis**

**Table – 2.2: Methods for various water quality parameters**

| Sr. No. | Parameter | Method | Instrument |
|---|---|---|---|
| **Physical parameters** | | | |
| 1 | Temperature | Laboratory or Field method | Mercury Thermometer |
| 2 | Electrical Conductivity | Instrumental method | Conductivity meter |
| 3 | Turbidity | Nephlometric | Nephlometer, Turbidity meter |
| 4 | TDS, TSS, SS | Filtration method | Oven, Beaker |
| **Chemical parameters** | | | |
| 6 | pH | Electrometric method | pH meter |
| 7 | DO | Winkler's method - Azide modification method | BOD bottle, Titration assembly |
| 8 | BOD | Winkler's method - Azide modification method | BOD bottle, Titration assembly |
| 9 | COD | Titrimetric Open Reflux Method | COD digestion apparatus |
| 10 | Alkalinity | Titrimetric | Titration assembly |
| 11 | Chloride | Argentometric Method (Titrimetric method) | Titration assembly |
| 13 | Nitrogen | Colorimetric method (PDA) | Spectrophotometer |
| 14 | Phosphate | Stannous Chloride Method | Spectrophotometer |
| 15 | Sulphate | Turbidometric Method | Spectrophotometer |
| 16 | Hardness | Titrimetric Method | Titration assembly |
| 17 | Free $CO_2$ | Titrimetric Method | Titration assembly |
| **Biological parameters** | | | |
| 18 | Chlorophyll - a, b, c. | Spectrophotometric and Trichromatic (Golterman et.al.,1978) | Spectrophotometer |
| 19 | Primary Productivity N.P.P. & G.P.P. | Titrimetric Method, DO – Difference | BOD bottle, Light & Dark bottle |

**Procedure:** Switch on the instrument and select calibration curve for desired range. Place the filter frame in position. Fill the sample in clean and dry turbidity meter tube up to the mark and then lower the plunger into the sample tube carefully, Place it in the circular groove of the mirror tube. Close the door of the apparatus. Switch on the instrument. Immediately balance the light intensity of the central spot with the surrounding field with the dial knob and read the scale on the dial. Determine the turbidity directly from the selected graph and if dilution is made multiply by dilution factor.

## Total Dissolved Solids (TDS):

Filtration method was employed for measurement of Total Dissolved Solids (TDS). Filter measured volume of well mixed 50 ml sample through glass fiber filter, wash with three successive 10 ml volumes of distilled water, allowing complete drainage between washings and continue suction for about 3 minutes. After filtration is complete transfer filtrates to a weighed evaporating dish or a beaker and evaporate to dryness on a steam bath. If filtrate volume exceeds dish capacity add successive portions to the same dish after evaporation. Dry for at least 24 hour in an oven at 103° C ± 2° C, cool in a desiccators to balance temperature and weight. Repeat cycle of drying, cooling, desiccating and weighing until a constant weight is obtained or until weight lost is less than 4% of previous weight or 0.5 mg, whichever is less.

Calculation: TDS mg / l $= \frac{(A-B) \times 1000}{\text{Sample taken (ml)}}$

Where, A = Weight of dried residue + beaker/evaporating dish (crucible),

B = Weight of empty beaker or evaporating dish (crucible)

## Total Suspended Solids (TSS):

A filtration method was employed for the estimation of Total Suspended Solids (TSS). Assemble filtering apparatus, begin suction wet filter with a small volume of distilled water to seal it. Filter a measured volume well mixed sample through the glass fiber filter, wash with three successive 10 ml volume of distilled water, allowing complete drainage between washings and continue suction for about 3 min. after filtration is complete. Carefully remove filter from filtration apparatus and transfer to petridish. Dry for at least 1 hour at 103° C to 105°C, in an oven, cool in a desiccators to balance temperature and weight.

Calculation: TSS mg/l = $\frac{(B-A)\times 10^6}{\text{Sample taken (ml)}}$

Where, A = Weight of empty filter, B = Weight of filter + dried residue

## Chemical parameters:

## pH:

The pH is determined by measurement of the electromotive force of a cell comprising an indicator electrode, an electrode responsive to Hydrogen ions (glass electrode) immersed in the test solution of a reference electrode. Contact between the test solutions of the reference solution is achieved by means of a liquid junction; in the reference electrode. The electromotive force is measured with a pH to read directly as pH value.

## Dissolved Oxygen (DO):

To determine dissolved oxygen in the water sample titrimetric method of Winkler's Method (Azide modification) was used in the laboratory. The water samples were collected in 300 ml BOD bottle by avoiding contact with air and agitation. The dissolved oxygen was tapped by:

- Add 2 ml MnSO4 (Manganese Sulphate) solution.
- Add 2 ml Alkali iodide azide solution.
- Shake well and allow settling (brown ppt).

Procedure: Add 2 ml Conc. $H_2SO_4$ in the preserved sample and mix it thoroughly to dissolve brown precipitate and take 100 ml out of 300 ml in 250 ml flask and titrate with 0.0125 N Std. $Na_2S_2O_3$. When pale straw color persists, add 1-2 ml of fresh starch solution, blue colour will appear, and continue the titration to the first disappearance of the blue color and read the final burette reading.

Calculation: 1 ml 0.0125 N Std. $Na_2S_2O_3$ = 0.1 mg DO

$$DO = \frac{0.1 \times \text{ml reading} \times 1000}{100 \text{ ml taken sample}}$$ DO mg /l = ml Reading

## Biochemical Oxygen Demand (BOD):

BOD is defined as the Oxygen required by the living organisms in the utilization and stabilization of Oxygen matter present in the water or wastewater.

Calculation: BOD mg/l = $\frac{\text{Actual Difference in DO} \times 100}{\text{Sample taken (ml)}}$

Actual Difference = (0 Day DO – 5 Day DO) – Blank difference.

## Chemical Oxygen Demand (COD):

Open Reflux Method was used to estimate Chemical Oxygen Demand (COD) for the collected water samples. COD test determines the Oxygen required for chemical oxidation of Organic matter with help of strong chemical oxidant.

**Procedure:** Take 10 ml Potassium Dichromate in COD tube add 30 ml Conc. $H_2SO_4$ containing $Ag_2SO_4$ , add 1 gm Mercuric sulphate and appropriate ml of sample (maximum 20 ml sample). Shake well the mixture and reflux for about 2 hours at 150 °C. Titrate against 0.1 N Ferrous Ammonium sulphate using Ferroin Indicator. Note down the burette reading. Also run the reagent blank.

Colour change: Green – Bottle green – Red.

Calculation: Normality of FAS = $\frac{\text{N of Potassium Dichromate} \times \text{Vol. of } K_2Cr_2O_7}{\text{ml of FAS used}}$

Factor = N of FAS X 8000 COD mg/l = $\frac{\text{A-B} \times \text{Factor}}{\text{Sample taken (ml)}}$

## Alkalinity:

Alkalinity of the water samples was determined by simple titration.

### Phenolphthalein Alkalinity:

Add 3-4 drops of Phenolphthalein Indicator to 100ml sample and titrate against 0.02 $NH_2SO_4$ to the coloration corresponding to the proper equivalence point of pH – 8.3. If there is no colour change the Phenolphthalein Alkalinity is zero. Colour change is from pink to colourless and note down the burette reading as (A).

**Total Alkalinity:**

Add few drops of Methyl Orange Indicator to the sample in which the Phenolphthalein Alkalinity has been determined or a sample of suitable size 50ml or 100ml. Titrate against 0.02 $NH_2SO_4$ to the proper equivalence point. The indicator changes to orange at pH 4.6 and pink at pH 4.0. Colour change from pale yellow to orange and note down the burette reading as (B).

Calculation:

Phenolphthalein alkalinity as $CaCO_3$ mg/l $= \frac{A \times N \times 50,000}{\text{Sample taken (ml)}}$

Total Alkalinity as $CaCO_3$ mg/l $= \frac{B \times N \times 50,000}{\text{Sample taken (ml)}}$

Where, A = ml titration for sample to reach the phenolphthalein end point

B = total ml titration for sample to reach the second end point

N = Normality of acid

## Chloride ($Cl^-$):

The chloride in water sample was determined by Argentometric Method (Titrimetric method).

**Procedure:**

- Take pretreated sample (Adjust the neutral pH) & add 1 ml Potassium chromate indicator & titrate against Silver nitrate (0.0282 N).
- Also run the charcoal / reagent blank
- Colour change : Yellow to Reddish orange

Calculation: Chloride mg/l = $\frac{A \times \text{Normality of Silver nitrate}}{\text{Taken sample (ml)}}$ X Dilution

## Nitrate ($NO_3^{-2}$):

Colorimetric Method (Phenol Di – Sulphonic Acid Method) was used for estimation of Nitrate.

**Procedure:** Take decolourized 50 ml of sample in 100 ml beaker and dry at $105^0$ C in oven for 12 hrs. After cooling add 2 ml PDA solution and rub the residue thoroughly,

dilute with 20 ml distilled water. Transfer in 100 ml in Nesseler tube then add 10 ml Liquor Ammonia solution and make up final volume 100 ml with distilled water. Measure O.D. at 410 nm. It is necessary to make a standard curve before the analysis of sample. Also run the reagent blank.

Calculation: $NO_3^-$ N mg/l = $\frac{O.D. \times Factor}{Taken\ sample\ (ml)}$

## Phosphate ($PO_4^{-3}$):

Phosphate in sample water was determined by spectrometric method using Stannous Chloride reagent.

Reagents:

- Conc. Sulfuric acid
- Ammonium – Molybdate reagent
- Stannous Chloride reagent
- Glycerol

**Procedure:** Take 100 ml of sample in conical flask. Add 4 ml strong acid and digest it on hot plate up to the volume of 15 to 20 ml and cool it. Then make volume to 100 ml in Nesseler tube with distilled water and add 4 ml Ammonium Molybdate and add 10 drops $SnCl_2$. Now put it for 10 minutes for colour development. Blue colour will appear. Measure O.D. at 690 nm. It is necessary to make a std. curve before the analysis of sample. Also run the reagent blank.

Calculation: $PO_4$ mg/l = $\frac{O.D. \times Factor}{Taken\ sample\ (ml)}$

## Sulphate ($SO_4^{-2}$):

Sulphate was estimated by turbidometric method, wherein turbidity of precipitates of sulphate salts has been created through chemical reaction and this turbidity was measured by spectrophotometer.

Reagents:

- Conditioning reagents (30 ml conc. HCl add 300 ml DW and 100 ml 95% Ethanol add 75 gm $NaCl_2$ and 50 ml Glycerol)
- $BaCl_2$ powder or saturated solution of $BaCl_2$.

- Standard Sulphate solution.

**Procedure:** Take 50 ml distilled water in the flask and add 5 ml Conditioning reagent and one spatula $BaCl_2$ powder (or 2 ml of $BaCl_2$ solution). Put the prepared flask on magnetic stirrer and add the sample until turbidity appears. Then add distilled water (Total volume of sample and distilled water must be equal to 50 ml) and measure turbidity at 420 nm. It is necessary to make a std. curve before the analysis of sample. Also run the reagent blank.

Calculation: $SO_4$ mg/l = $\frac{O.D. \times Factor \times 100}{Taken\ sample\ (ml)}$ X Dilution

## Hardness:

EDTA Titrimetric Method was followed to estimate hardness in the water sample.

**For Total Hardness:** If necessary, remove the colour of the sample by charcoal and take appropriate sample add ammonium buffer solution. ( up to pH 10.5) add 0.2 – 0.4 gm Eriochrome Black T indicator and titrate against 0.02 N EDTA immediately until the last reddish tinge change to blue colour is observed and note down the burette reading (A). Also run charcoal / reagent blank.

**For Calcium:** If necessary, remove the colour of the sample by charcoal and take appropriate sample add 3 ml 0.1 N NaOH (up to pH 12) add 0.2 – 0.4 gm Murexide indicator and titrate against 0.02 N EDTA until the pinkish tinge colour disappears and violet (purple) colour is observed and note down the burette reading (B). Also run charcoal / reagent blank.

Calculation:

Total Hardness as $CaCO_3$ mg/l = $\frac{Burette\ reading\ (A) \times 1000}{Taken\ sample\ (ml)}$ X dilution

Calcium Hardness as $CaCO_3$ mg/l = $\frac{Burette\ reading\ (B) \times 1000}{Taken\ sample\ (ml)}$ X dilution

Magnesium Hardness as $CaCO_3$ mg/l = $\frac{Burette\ reading\ (A-B) \times 400.8}{Taken\ sample\ (ml)}$ X dilution

$Ca^{++}$ mg/l = $\frac{Burette\ reading\ (B) \times 400.8}{Taken\ sample\ (ml)}$ X dilution,

$Mg^{++}$ mg/l = $\frac{(A-B) \times 243.2}{Taken\ sample\ (ml)}$ X dilution

## Free $CO_2$:

Waters having pH more than 8.3 do not contain appreciable amount of free $CO_2$. Therefore, Phenolphthalein indicator, which has end point at this pH value, is used for estimating the amount of $CO_2$ in a water sample for titration with a standard alkali.

$$2\ NaOH + CO_2 = Na_2CO_3 + H_2O$$

Procedure: Take 100 ml of the freshly collected water sample in a white porcelain basin. Add 3 drops of Phenolphthalein indicator into the water. If the sample turns pink, the pH of water is above 8.3 and free $CO_2$ is not present. If the solution remains colourless after addition of indicator, titrate with N/44 NaOH solution with gentle stirring with a glass rod till the colour turns pink.

Calculation: Concentration of free $CO_2$ (ppm) = ml of N/44 NaOH required for titration X 10.

## Biological parameter:

## Chlorophyll Estimation:

Procedure: Concentrate the sample by centrifuging. Often 0.5 liter water is enough for chlorophyll estimation. Use only 100 ml in highly green, algal rich waters. Transfer the pellet to a pestle – mortar; add few ml of 90 % acetone. Grind the contents until thoroughly macerated. Pour ground sample to a graduated centrifuge tube. Rinse mortar and pestle with sufficient acetone and 0.2 ml $MgCO_3$ suspension and bring the volume to 10 ml. Keep the sample at 4°C for 4 – 6 hrs for pigments to elute. Keep this time constant for all samples from one area. Centrifuge at 2500 – 3000 rpm for 20 minutes. Decant the extract into a 15 ml centrifuge tube and measure the volume or make up the final volume of extract to 10 ml. Take absorbance at 663, 645 and 630 nm. Calculation: Chlorophyll a, b and c can be calculated by using following equations.

$$\text{mg pigment/m}^2 = \frac{\text{Ca} \cdot \text{Ve}}{\text{Vs} \cdot \text{L}}$$

Where,

Ve = Volume of acetone extract, ml and

L = Light path of cell, cm (in Spectrophotometer)

Vs = Volume of water centrifuged,

C = Ca, Cb or Cc obtained respectively from

Ca = (for chlorophyll a) = 11.64 E 663 – 2.16 D 645 + 0.10 D 630

Cb = (for chlorophyll b) = 20.97 E 645 – 3.94 E 663 + 3.66 D 630

Cc = (for chlorophyll a) = 54.22 E 630 – 4.81 E 645 - 5.53 E 663

E 663, E 645 and E 630 are optical density values of the extract at these respective wave lengths.

## Primary Productivity:

Primary productivity of a water body is usually determined from the differences in DO values of water samples incubated in bottles under light and dark conditions thus allowing photosynthetic activity to take place in one bottle and the same to be restricted under the other. The decrease in DO content in the dark bottle, as compared to initial value represents the amount of DO consumed by respiration by all the biomass in the bottle. The increase in DO content in the light bottle indicates that amount of DO in water which exceeded oxygen consumption by respiration. Both GPP and NPP can be calculated from the differences in these DO values.

**Procedure:** Take in water sample cautiously as well as uniformly in three bottles (initial, light and dark) from the desired depth of water. Add 1 ml of manganese sulfate and alkaline iodide each in the initial bottle. Invert the stopper bottle a few times. Take the other two bottles and dip in the water under the depth from which water samples were collected. Incubate the water samples under water for some period which should not normally be less than three hrs. After the time period is over, take out both of the bottles and fix oxygen as before. Determine DO values of water in all the bottles.

Calculation:

$$GPP = \frac{LB - DB}{T} \times \frac{0.375}{1.2} \times 1000 \text{ mg cm}^{-3} \text{ h}^{-1}$$

$$NPP = \frac{LB - IB}{T} \times \frac{0.375}{1.2} \times 1000 \text{ mg cm}^{-3} \text{ h}^{-1}$$

Where,

LB = DO (ppm) in light bottle, DB = DO (ppm) in Dark bottle

IB = DO (ppm) in initial bottle, T = Time (h) of incubation

0.375 = ratio of the weight of C and O, 1.2 = photosynthetic co – efficient.

### Soil quality analysis:

The soil samples were collected from the immersed part of the periphery of the reservoir in triplicate and carried in polythene bags to the laboratory for further analysis. Prior to analysis all the soil samples were dehydrated, grinded and sieved to prepare the proper sample. The quality parameters were analyzed as per standard methods reported in table 2.3.

### Physical parameters:

### Soil Particle Density and Porosity:

The Particle density of a soil is referred to the mass of a unit volume of soil particles (Soil Solids). It is determined by measuring the mass and volume of soil solids.

**Procedure:** Weigh empty dry Pycnometer. Fill the dry Pycnometer with water completely. Wipe outer side of the Pycnometer with a piece of filter paper and weigh it. Pour out water and dry it from outside with a piece of filter paper. Put 10 g of oven – dry soil in to the Pycnometer. Fill the Pycnometer to about half with water using the pipette and wash with a jet of water to remove any particles sticking to the inner side of the neck. Expel the entrapped air by gently boiling the contents. Allow the contents to cool to room temperature and fill the Pycnometer to the brim/ mark with boiled and cooled water. Fix the stopper well. Clean the outer side of the Pycnometer with a filter paper and weigh it. Relationship between porosity and density of soil:

$$\% \text{ Solid space} = \frac{\text{Bulk density}}{\text{Particle density}} \times 100$$

Since, % pore space + solid space = 100

OR % pore space = 100 – % solid space

OR % Pore space = $100 - \frac{\text{Bulk density}}{\text{Particle density}} \times 100$

% Pore space = $100 \left[1 - \frac{\text{Bulk density}}{\text{Particle density}}\right]$

**Table 2.3: Methods for analysis of physical parameters of soil.**

| Parameters | Method / Reference | Instruments |
|---|---|---|
| Particle density and Porosity | Richard (1954) | Pycnometer |
| Bulk density | Page et al. (1986) | Large weighing bottle |
| Maximum water holding capacity | Piper (1950) | Brass cup |
| Particle size distribution (Mechanical analysis) | International pipette Method Piper (1950) | Filtration apparatus, Sedimentation cylinder |

## Bulk density:

The mass of the oven dry soil which fills the container of a known volume is determined by weighing. The volume of the packed soil will equal to the capacity of the container. Bulk density is then calculated as the ratio of mass of soil to its volume.

**Method:** Weigh an empty large weighing bottle of about 50 ml capacity or specific gravity bottle without the stopper. Fill the bottle with dry soil up to the brim and tap it 15 – 20 times by letting it fall gently on the table from a height of approximately 2.5 cm each time. (This tapping is assumed). Empty the bottle and find its volume by filling it completely with water from a burette. Measurement of porosity of soil: Porosity of a soil sample is the volume which is occupied by air and water or porosity of a soil is the fraction of soil volume not occupied by soil particles.

- Determine the soil bulk density (Db) and particle density (Dp)
- Calculate the total porosity.

## Maximum water holding capacity:

Maximum water Holding capacity is the amount of moisture absorbed (i.e. macro and micro pore space is completely filled with water) per unit weight of dry soil, when placed in water saturated condition. It is expressed in terms of percentage. It is also

called as maximum water retention capacity of soil. The moisture retained is governed by inherent characteristics of soil. The Maximum water holding capacity is of great value in practical agriculture, since it provides a simple means of determining soil moisture content required for good plant growth. When a thin layer of soil is allowed to absorb water from a free water surface, total pore space gets completely filled with water gradually. The amount of water thus retained in the soil helps in calculating maximum water holding capacity of the soil.

**Procedure:** Weigh an empty brass cup and filter paper. Fill up the dry soil in brass cup with gentle taping. Tap the brass cup and level the soil with a help of spatula. Remove the outer soil with the help of brush. Weigh the brass cup with soil. Place the brass cup in a petridish containing water. (See the brass cup should be submerged in water up to $1/4^{th}$ depth). Allow the brass cup with soil in water for one and half hour or till the soil becomes completely saturated with water i.e. soil surface becomes shining. After complete saturation, take up the brass cup, wipe off the outer surface of cup with blotting paper and weigh it. Subtract the moisture absorbed by the filter paper to correct the results and calculate the maximum water holding capacity of the soil.

Weight of oven dry soil = $W2 - W_1$ = ----- g

Weight of actual water absorbed by soil = $(W3 - W_2) - W_4$ = ----- g

Maximum water Holding capacity of soil % = $\frac{\text{Moisture held by Soil}}{\text{Weight of dry soil}}$ X 100,

$= \frac{(W3 - W2 - W4}{W2 - W1}$ X 100 = ----%

## Mechanical analysis:

The international pipette method is a standard method for particle size analysis of soils because of its accuracy. The following soil fractions can be determined by this method.

**For Coarse Sand:**

1 Weight of dish = $W_1$ = ----------g.

2 Weight of dish + dry coarse sand = $W_2$ = ----------g.

3 Weight of coarse sand = $W_2 - W_1$= ----------g.

Percentage of coarse sand on air dry basis = $\frac{W2 - W1}{30}$ X 100

**For Silt + Clay:**

1 Weight of dish = $W_1$ = ----------g.

2 Weight of dish + Silt + Clay (in 25 ml suspension) = $W_2$ = ----------g.

3 Weight of Silt+Clay (in 25 ml suspension) = ($W_2$–$W_1$) = ----------g.

Percentage of Silt + Clay in the soil = $\frac{W_2 - W_1 \times 1000 \times 100}{25 \times 20}$

(25 is the suspension taken and 20 is the weight of soil sample taken)

**For Clay:**

1 Weight of dish = $W_1$ = ----------g.

2 Weight of dish + Clay (in 25 ml suspension) = $W_2$ = -------g.

3 Weight of Clay = ($W_2 - W_1$) = ----------g.

Percentage of Clay in the soil = $\frac{W_2 - W_1 \times 1000 \times 100}{25 \times 20}$

**For Fine sand:**

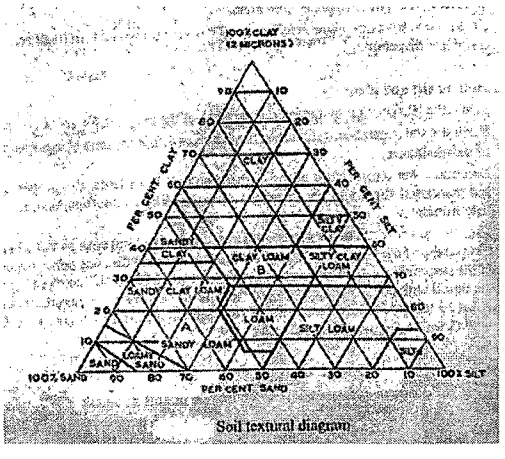
1 Weight of dish = $W_1$ = ----------g.

2 Weight of dish + fine sand = $W_2$ = ----------g.

3 Weight of fine sand = $W_2 - W_1$= ----------g.

Percentage of coarse sand on air dry basis = $\frac{W_2 - W_1}{20}$ X 100

Textural triangle and its usage: A procedure for using the textural triangle to determine the textural class of a soil which contains 55 percent clay, 32 percent silt and 13 percent sand is as follows:



Soil textural diagram

**(Source: Boyd et al., 2002)**

1 Take the percent clay (55) on the left side or clay side and draw a line parallel to the bottom or sand side of the triangle.

2 Take the percent silt (32) on the right side or silt side and draw a line parallel to the left side or clay side of the triangle.

3 The area in which intersection of two lines occurs gives the textural class or texture of the soil in this case, it is clay.

4 As a check, the percent sand (13) and draw a line parallel to the right side of the triangle.

5 If all the three lines intersect at the same point the class name has been determined correctly.

## Chemical parameters:

The chemical parameters of soil analysis comprise of pH, Electrical conductivity, available Potassium, available Phosphorus, soil organic carbon, micronutrients etc. The methods followed were documented in the table – 2.4.

**Table 2.4: Methods for analysis of chemical parameters of soil.**

| Sr. No. | Parameters | Method | Instruments |
|---|---|---|---|
| 1 | pH | Richard (1954) | pH meter |
| 2 | Electrical conductivity | Richard (1954) | Conductivity meter |
| 3 | Available Potassium | 1N $NH_4OAC$ Method Jackson (1973) | Flame photometer |
| 4 | Available Phosphorus | Olsen's Method (1954) | Kleft summerson colorimeter |
| 5 | Organic Carbon | Chromic acid Method Walkley and Black (1935) | Titration assembly |
| 6 | Available Nitrogen | Alkaline Permanganate Method Subbaih and Asija (1956) | Glass beads & Distillation apparatus |
| 7 | Available micronutrients. (Zn, Fe, Mn, Cu) | DTPA extractable Lindsay and Norvell (1978) | Atomic absorption spectrophotometer |

## Determination of Soil pH:

Soil pH was determined by using analytical pH meter. This instrument is composed of two electrodes.

- Glass electrode or indicator electrode.
- Calomel electrode or reference electrode.

When the both electrodes are dipped in aqueous solution under test, the potential difference between both the glass electrode and the calomel electrode is measured.

**Procedure:** Take 10g soils in 50 ml beakers add 20 ml of DW and stir intermittently with glass rod for 30 minutes. Determine pH of the soil suspension with the pH meter as per directions with the instrument. Stir the suspension in each beaker with a glass rod again just before taking the pH reading wash the electrodes with DW after each determination. Express pH to the nearest tenth of pH unit.

## Determination of Electrical Conductivity of Soil:

The solution offers some resistance to the passage of electrical current through them depending upon the concentration and the type of ions present. Higher the salt content, lesser the resistance to the flow of current.

The R is defined by ohm's low as the ratio of electrical potential in volt (E) and strength of current in ampere (I).

$$R = E / I = \frac{\text{Volts}}{\text{Current}} = R \text{ in Ohm.}$$

Electrical Conductivity or conductance is the reverse of resistance.

$C = 1/2 = 1 / R = 1 / \text{Ohm} = \text{Ohm}^{-1} = \text{mho}$ (reverse of ohm).

**Procedure:** Calibrate instrument at 0 and 100 or as per directions supplied with the instrument. Check the instrument with the saturated solution of calcium sulphate (EC–2.2 mmhos/cm – $dsm^{-1}$ 25°C) OR 0.01 N solution (EC–1.41 mmhos/cm – $dsm^{-1}$ 25°C) before proceeding with the samples.

The same soil suspension prepared for the determination of pH is also used for salinity determination. After recording the soil pH, allow the soil suspension in the beaker to setter (or take to gram soil in 50 ml beakers and add 20 ml of distilled water and stir with glass rod intermediately for 30 minutes). Pour the supernatant liquid in

to the conductivity tube after ringing it with the same supernatant solution. Dip the conductivity cell and read on direct conductivity meter. Express conductivity in $dsm^{-1}$ (millimhos / cm) at $25^0$ C to the nearest first decimal.

Conclusion: mmhos / cm = 1 $dsm^{-1}$ = 100 ms/m at $25^0$ C

- µmhos / cm = 0.001 $dsm^{-1}$ = 0.1 ms/m.
- $dsm^{-1}$ X 640 = ppm

## Determination of Available Potassium in soil:

A soil is taken with a neutral normal $NH_4OAC$ solution, the ammonium ion of acetate exchange with the exchangeable potassium ions of the soil. Then the potassium content of the extract is estimated with a flame photometer.

Being the almost similar ionic radia, $K^+$ is more effectively replaced by $NH_4^+$

**Procedure:** Weight 5g of soil in 150ml conical flask. Add to it 25 ml of nN $NH_4OAC$ solution. Shake it on an electrical shaker for 5 minutes and filter the contents through a filter paper No. 2. Add 2 drops of alcohol to each filtrate. Feed the filtrates into the flame photometer and note the reading. Place 5 g soil (2mm sieved) in a dry 100 ml conical flask, and add 50 ml of 1 n $HNO_3$. The contents are heated on a hot plate (low heat) under reflux (fitted with cork & long glass tube) for 10 minutes after boiling starts. On cooling, the suspension is filtered through dry Whatman filter paper No.1 and potassium as estimated flame photometrical after appropriate dilution. The non exchangeable K is obtained by subtracting the ammonium acetate K from this value.

Observation & Calculations:

1 Weight of the sample : 5 g
2 Volume of nN $NH_4OAC$ added : 25 ml
3 Reading of the flame photometer for the test solution : X
4 Ppm, as read from the std. curve (for X) : Y
5 Ppm of available K in soil = Y x dilution (25/5) Z = Y x 5
6 kg/ha of available K = Z x 2.24
7 $K_2O$ – kg /ha = K kg/ ha x 1.22

## Determination of Available Phosphorus in soil:

The 0.5 M $NaHCO_3$, with pH 8.5, is designed to control and decrease the ionic activity of Ca, through the solubility products of $CaCO_3$ during the extraction of calcareous soils. This may be resulted because of raising the carbonate activity in soil. And hence, some phosphate from the surface of calcium phosphate is brought in to solution. As $Ca^{++}$ activity decreases, phosphate activity increases. Sodium bicarbonate solution also extracts some phosphates from the surface of Al and Fe phosphate by repressing the activities of Al & Fe (The Al by aluminates complex formation and Fe by precipitation as the oxide) (due to high P). The low activity of Ca presents the re precipitation of the liberated phosphate as calcium phosphate.

The heteropoly complex are thought to be formed by coordinated of Molybdate ions, with phosphorus as the central coordinated atom, the oxygen of the Molybdate radicals being substituted for that of $PO_4$.

- $\underline{H_3P\,(MO_3O_{10})_4 + 12\,H_2O}$
- Phosphomolybdate

This heteropoly complex is faint yellow due to their water solution, which on reduction with stannous chloride give a blue colour. The intensity of colour, which is proportionate to the concentration of phosphate, is read from a colorimeter at a wavelength of 660 μm or using a red filter.

- Phosphomolybdate $\rightarrow$ Reduced Phosphomolybdate
  Light yellow Blue colour

**Procedure:** Weight 5 g of soil (2mm sieved) in to a 250 ml. conical flask or plastic bottle. Add 1 tea spoon of activated charcoal and 10 ml of 0.5 m $NaHCO_3$ solution (Soil: Solution ratio 1:20). Mix well and shake on an electrical shaker for 30 minutes. Filter the suspension through Whatman No.42 filter paper.

Observation and Conclusion:

1 Weight of the sample: 5.00 g.
2 Volume of 0.5 m $NaHCO_3$: 100 ml solution added.
3 Volume of aliquot taken: 5 ml.
4 The final volume after the colour development: 25 ml.
5 Transmittance or Absorbance of test solution ; Reading : A

6 Blank Reading (if taken) : y

7 Actual Reading : X = A – Y

ppm of available P = G.F. x X x 25/5 x 100/5

P kg/ha = ppm x 2.24

$P_2O_5$ kg/ha = P kg/ha x 2.29

## Determination of soil organic carbon (Chromic acid Method):

Principle: A known weight of the soil is treated with an excess volume of standard $K_2Cr_2O_7$ solution in the presence of conc. $H_2SO_4$. The soil is slowly digested at the low temperature by the heat of dilution of $H_2SO_4$ and organic carbon in soil, and thus, oxidized to carbon dioxide. The excess of $K_2Cr_2O_7$ unused in oxidation is titrated back against a std. solution of ferrous ammonium sulphate in presence of NaF or phosphoric acid and diphenyl amine solution as indicator.

NaF and $H_2PO_4$ make the distinct change in colour because of their flocculating effect. At the end point, the colour of the suspension changes from violet, through blue, to bright green.

**Procedure:** Take an accurately 0.5 g of soil (less than 1% organic matter) which has passed through a 0.2 mm sieve (80 meshes per inch), in a conical (500ml) flask. Add 10 ml of 1 N $K_2Cr_2O_7$ solution by pipette and shake to mix. Then add 20 ml of conc.$H_2SO_4$ swirling the flask during addition and mixed by gentle rotation for 1 minute, to insure complete contact of the reagent with the soil, with care to avoid throwing soil up on to the sides of the flask out of contact with the reagent. When $H_2SO_4$ is added a lot of heat is produced, because the dilution of $H_2SO_4$ is an exothermic reaction. Cool the contents of the flask and allow for 30 minutes completing the reaction on the asbestos sheet.

Back titration:

1. Add 200 ml of DW, 10 ml of $H_3PO_4$ 85% of 0.5 g of sodium fluoride and shake vigorously to mix.
2. Now, add 10 drops of the indicator diphenyl amine (it will give a violet colour to the suspension).
3. Titrate the contents of the flask against 0.5 N Ferrous ammonium sulphates (FAS) solution. Till the colour changes from violet to bright green.

4. Note the volume of the ferrous ammonium sulphate (FAS) solution used.
5. Carry out a black titration in a similar manner. (If more than 1 ml of the dichromate solution is consumed, repeat with smaller quantity of soil 0.25 g).

Calculation:

1. Weight of the sample taken = 0.5g
2. Volume of 0.5 N FAS solution used for the blank titration = X ml
3. Volume of 0.5 N FAS solution used for titrating the excess sample = Y ml
4. Volume of 1 N $K_2Cr_2O_7$ solution used for the oxidation of carbon=(X–Y )=Z
   1 ml of 1 N $K_2Cr_2O_7$ solution = 0.003 g of OC

% of O.C. in soil (A) = Z X 0.003 X 100/S, % of organic matter in soil = A x 1.724

**Estimation of available Nitrogen of soil by alkaline permanganate Method:**

The alkaline $KMnO_4$ is a mild oxidizing agent and can extract the relatively easily oxidizable fractions of organic nitrogen. A known weight of the soil is treated with an excess of alkaline $KMnO_4$ & distilled. The ammonia gas evolved is absorbed in boric acid and titrated with standard sulphuric acid using mixed indicator.

**Procedure:** Weigh out 20g of the sample of the soil and put it in to the distillation flask. Moist the sample with distilled water (about 20 ml) and fix the distillation assembly. Add 100 ml of 0.32 % $KMnO_4$ for solution and 100 ml of 2.5 % NaOH solution prevention of the frothing and bumping during boiling add liquid paraffin (1 ml) and few glass beads, respectively. The contents are distilled in a distillation assembly at a steady rate and the librated ammonia is collected in a 250 ml conical flask or beaker containing 20 ml of boric acid solution with mixed indicator (4 drops) – Dip the end of the delivery tube into solution with the absorption of ammonia the pinkish colour turns to bluish green. Nearly 100 – 150 ml of distillate is to be collected in about 30 minutes and then tube is disconnected. The boric acid is back titrated with a std. sulphuric acid. At the end point, the blue colour just disappears one drop in excess and turns solution pink. Blank correction should be run as per above procedure without soil.

Calculation:

1. Weight of the soil sample = W (20g)
2. 0.02 N $H_2SO_4$ used. During the back titration = (Z) ml
   a. Blank reading = X ml
   b. Reading for test solution = Y ml
3. 1 liter 1 N $H_2SO_4$ = 14g N
   1 ml 1 N $H_2SO_4$ = 0.014g N
   1 ml 0.1 N $H_2SO_4$ = 0.0014g N
   1 ml 0.02 N $H_2SO_4$ = 0.00028g N

Available N % = $\frac{(X-Y) \times 0.00028 \times 100}{W\ (20g)}$

Available N in ppm = % X 10,000

Available N in kg/ha $^{-1}$ = % X 22,400, ppm X 2.24

## Determination of available micronutrients by Atomic Absorption Spectrophotometer:

This method consists of shaking a few grams of soil with a buffered solution containing DTPA (Diethylene triamine penta acidic acid). This chemical acts as a mild chelating agent which extracts the easily soluble Zn, Fe, Mn and Cu. These dissolved elements are then measured by the atomic absorption spectrophotometer.

The extraction solution is buffered at pH 7.3 by triethanolamine (TEA) and in addition includes calcium chloride to prevent the dissolution of calcium carbonate. These conditions permit the right amount of Zinc, Iron, Copper and Manganese to be dissolved to indicate their availability in the soil. The function of TEA and $CaCl_2$ is to stabilize the pH of the extractant.

**Procedure:** Take 10g of soil in 100 ml conical flask, in it 20 ml DTPA reagent is added and shaken for two hours. The extract is filtered Whatman No. 40/42. Take 1 ml of aliquot and make 10 ml of volume, micronutrients are estimated with the help of atomic absorption spectrophotometer

Standard curve:

Zinc (Zn): 1 g of pure zinc metal is dissolved completely in minimum amount of dilute HCl and made up to 1 liter with double distilled water in an already used (not new) volumetric flask (corning/pyrex) and then transferred to a plastic bottle. This stock solution contains 1000 µg zn/ml.

In six 100 ml volumetric flasks, required aliquots are taken with standards of 0, 0.05, 1.0, 1.5, 2.0 & 2.5 ppm zinc solution are made and the standard curve prepared against the reading of the AAS after necessary setting and calibration of instruments.

Copper (Cu): A stock solution of 1000 ppm Cu is obtained by dissolving exactly 1 g of the pure metal (wire or turning) in 50 ml of dilute (1:1) $HNO_3$ and finally diluting to 100 ml with double distilled water (0.25, 0.5, 2.0, 2.5 & 3.0 ppm) and are prepared in 100 ml volumetric flasks.

Iron (Fe): To prepare a stock solution of 1000 ppm Fe, exactly 1 g of AR grade Fe metal is dissolved in 50 ml of dilute $HNO_3$ and made to 1 liter with double distilled water from this working solution (0, 1, 2, 4, 6, 8 & 10 ppm) are made in 100 ml volumetric flasks by appropriate dilution.

Manganese (Mn): The 1.583 g of $MnO_2$ (AR) pr 1.0g of pure metal distilled in 50 ml of dilute $HNO_3$ and made up to 1 liter which gives a stock solution of 1000 ppm, Mn in 100 ml volumetric flasks, requisite quantity of a stock solution are dilute to obtaining working concentration of 0, 1, 2, 4, 6, 8 & 10 ppm Mn.

Observation and Calculation:

1. Weight of the taken soil sample = W (20g)
2. Volume of DTPA added = x = 10ml
3. Dilution: Aliquot taken = 1 ml, Volume made = 10 ml
4. Reading on AAS:
5. Reading for test solution = X, Blank = Y
6. Actual Reading = Z = X – Y
7. ppm in soil = Z x GF x 10/1 x 20/10

## Biodiversity analysis:

The reservoir provides healthy environment to various floral and faunal forms through ecologically balanced condition. The flora of the reservoir play significant role in its productivity is consumed by inhabiting fauna. The algae and macrophytes were collected manually from the reservoir and were dried for preparation of herbarium. Then all these samples were identified in the laboratory through appropriate literature. The planktonic forms were collected from the surface of the reservoir water with plankton net of 20μ mesh size nylon cloth. The plankton samples were preserved for laboratory analysis. The phytoplankton were preserved in Lugol's iodine solution while zooplankton samples were preserved in 5.0% formalin. The quantitative analysis of phytoplankton was done by Lacky's drop count method (Lacky, 1938). The concentrated samples were preserved by adding 4% Formalin and 1 ml. of Lugol's iodine. The results were expressed as no/l. Phytoplankton were identified following Fritch (1944) Adoni et al. (1985) and Cox (1996). The molluscan fauna (shells) were collected from the periphery of the reservoir. The variety of fishes was collected from the fish catch hauled by fishermen. All the samples of mollusca and fishes were preserved in formalin for further laboratory analysis. The identification of these samples was done through various identification keys (Day, 1980 and Rao, 1989).

## Fisheries data Analysis:

This reservoir has been utilized for capture fisheries as well as for stocking of fish seeds of Indian Major Carps. The data for fish catch and other fisheries activity were collected from local fishermen and from the local fisheries office of the Department of Fisheries, Government of Gujarat. The fishery potential was estimated for this reservoir as Morpho-edaphic Index (MEI) as per Ryder (1965).