MATERIALS AND METHODS

The Angoori reservoir is an irregularly shaped manmade reservoir. Its construction started in the year 1992-93 on Angoori nalaa, tributary of Pahuj river. This reservoir has been constructed with the help of J.B.I.C. It was completed in the year 2004. This reservoir is situated in district Datia (M.P.) at longitude 78.28° and latitude 25.38° on the Datia (M.P.) Jhansi NH no.75. 9 km. from Datia head quarter. Delhi-Mumbai train way and NH 75 are on the North side, village Gandhari, Pisnari, Lamaych are on the South-West side of the reservoir and West side is covered with the rock. Derra and Chirulla villages are situated on the Eastern side of the reservoir.
**Morphometry:**

Catchment area of this reservoir is 162sq. km. It has capacity of 2.55 million cubic meters, while live capacity is 2.05 million cubic meters. Maximum water level is 235.60 sq. km, while river bed level is 230.5 million cubic meters. The reservoir has submerrged area of 197 hectares. The gross command area is 57683 hectares. The reservoir is an important source for irrigation in district Datia. About 246 villages are benefitted from this reservoir.

A systematic limnological study has been made to understand the physical and chemical characteristics of water and biotic components of the Angoori reservoir with special reference to fish diversity. Monthly observations were made for two years from January 2009 to December 2010. Four sampling stations were set up in varied ecological zones of Angoori reservoir. Station A was near of village Gandhari, Pisnari, Lamaycha on the South-West, Station B was on the West side of the reservoir covered with rock and unused land, Station C was on the North side of Delhi – Bombay train rout and NH 75 and the station D was on Eastern side of reservoir near Derra and Chirulla villages.
Setellite view of Angoori reservoir

Different sampling stations of Angoori reservoir
Sampling Station C

Sampling Station D
PHYSICOCHEMICAL METHODS

Surface water samples were collected from four sampling stations in morning hours between 8:00 to 9:30 am on every last Sunday of the month. They were collected in one litre polythene bottles and covered with tight cap. Some physico-chemical parameters were tested on the sampling stations such as pH, temperature, transparency, DO, free CO$_2$, and alkalinity. Samples were brought to laboratory immediately for testing of the chemical parameters like nitrate, sulphate, chloride, sodium, potassium, B.O.D., C.O.D., and T.D.S.

The analyses of samples was based on standard methodology recommended by APHA (1985), Trivedy and Goel (1986), and Adoni (1985). Biological study was made following Ward and Whipple (1959), and Palmer (1980). The detailed methodology is outlined below:

**Odour:**

The odour was recorded by simply smelling the water samples at sampling stations.

**Colour:**

The colour of water was observed by visual appearance only.

**Transparency:**

Transparency was measured with the help of “secchi disc”. Secchi (1865) an Italian scientist devised a method for studying the transparency of aquatic bodies. The secchi disc is a metallic plate of 20 c.m. diameter, with four quadrants on the upper surface and a hook in the centre to tie a nylon rope. The disc with centrally placed weight at the lower surface was lowered through a graduated cord at the sampling points. The depth at which it disappeared in the water, (D1)
and (D2) at which it reappeared after lifting were noted. The reading D1+D2 divided by two gave the transparency in cms. This was the measure of light penetration through surface water.

\[
\text{Transparency} = \frac{D1+D2}{2}
\]

**Temperature:-**

Surface water temperature was recorded in degree Centigrade by good grade mercury thermometer at each station by dipping the mercury bulb below the surface water and reading was recorded. The thermometer was checked periodically against other standard thermometers.

**Electrical Conductivity:**

Conductivity when measured between the electrodes having a surface area of 1 cm\(^2\) and placed at a distance of 1 cm. is called electrical conductivity. Conductivity is the numerical expression of water’s ability to conduct on electric current. It is measured in micro Siemens per cm and depends on the total concentration, mobility and temperature of the solution of ions. It is defined as the reciprocal of the resistance involved and expressed as \(\mu\text{mho}\) or Siemens (S).

For the estimation of conductivity, by digital conductivity meter (systronic make water quality analyser) with conductivity cell was used as per Adoni (1985) and Trivedy and Goel (1986). Adjusted the temperature compensation to the temperature of the sample. Kept the function switch to X 1000 position and calibrated to CAL mark. Dipped the conductivity cell in the sample and connected the terminals to the sockets. Whenever the deflection was negligible, disconnected the cell.
and calibrated to CAL for X 100 position. Reconnected the cell and the reading was recorded. In case the deflection was still found negligible, moved the switch to 10, calibrated again and then the reading was recorded. Turned the switch to “off” position, after disconnecting, it washed the electrode with distilled water. Multiplied the dial reading by the value of the selector switch to get the result in μmhos/cm

**Total Dissolved solids:-**

Total dissolved solids are determined as the residue left after evaporation of the filtered sample.

**Requirement:-**

Evaporation dish (100 ml. beaker), Oven, Chemical balance, Whatman filter paper No.4,

**Method:**

Weight of an evaporating dish of 100 ml. was noted. Filtered sample was evaporated on water bath in the evaporating dish. Weight of dish was noted after cooling. This was repeated till the constant weight was achieved. Calculated the total dissolved solids and expressed in mg/l.

**Calculation:**

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

Where,

- \(A\) = final weight of dish (g)
- \(B\) = initial weight of dish (g)
- \(V\) = volume of sample taken (ml.)
**Hydrogen ion concentration (pH):**

pH of water samples was observed with the help of digital tripper meter model 181 Electronic India. Before using, the pH meter was calibrated with the buffer solutions. After setting the pH, electrode was removed from buffer solution and washed with distilled water and dried and placed in to the sample to be tested. pH of the sample was read on the pH meter directly expressed in mols / litre.

**Dissolved Oxygen:**

Dissolved oxygen is a very important parameter for water analysis as it serves as an indicator of the physical, chemical and biological activities of water body. Winkler idometric method was used to measure the dissolved oxygen in water sample. DO of water sample was measured by precipitating as manganese basic oxide which was dissolved by concentrated sulphuric acid forming magnus sulphate. It immediately reacted with potassium iodide already present, liberating iodine which was determined by titrating with sodium thiosulphate (0.025N) using starch as an indicator.

**Reagents:**

**Sodium thiosulphate (0.025N):**

24.82 g of sodiumthiosulphate was dissolved in boiled distilled water and made up the volume to 1 litre. 0.4 g pallet of NaHO was added and stabilized. This is 0.1 N stock solution. It was diluted to 4 times with boiled distilled water to prepare 0.025 N sodium thiosulphate solution and kept in a brown glass stoppard bottle.
Alkaline potassium iodide solution:-
100g of KOH and 50g of KI was dissolved in 200 ml. of boiled distilled water.

Manganous sulphate solution:-
100g of manganous sulphate was dissolved in 200 ml. of boiled distilled water and filtered.

Starch solution:-
1g of starch powder was dissolved in 100 ml. of warm (80°c -90°c) distilled water and few drops of formaldehyde solution were added

Sulphuric acid:-
H₂SO₄ concentrate (A.R. Grade).

Procedure:-
300 ml. of sample was taken in a glass stoppered bottle (BOD bottle) carefully avoiding trapping of any air bubbles. 1 ml. of MnSO₄ and alkaline KI solutions was added with the help of pipette, stoppered and shook well and kept the bottle for some time to settle down the precipitate. After that 1 ml. of concentrated H₂SO₄ was added and shaken well to dissolve the precipitate. 50 ml. of digested sample was taken in a 100ml. conical flask and titrated with 0.025N sodium thiosulphate until the yellow colour appeared. After this 4-5 drops of starch indicator were mixed, the yellow color changed to wine-red color. Again titrated with 0.025 N sodium thiosulphate until the wine-red color disappeared. Reading was noted and calculated with the formula:

\[ \text{DO mg/l} = \frac{\text{ml of titrant} \times N \times 1000 \times 8}{\text{ml. of sample}} \]
Biological oxygen demand:-

Biological oxygen demand is the measure of the degradable organic material present in a water sample, and can be defined as the amount of oxygen required by the microorganisms in stabilizing the biologically degradable organic matter under aerobic conditions, (Trivedi and Goel 1984).

Requirements:-

A. BOD bottles having 300 ml. capacity

B. BOD incubator set at 20°C

C. Phosphate buffer: - 8.5gm KH₂PO₄, 21.75gm K₂HPO₄, 33.4gm Na₂HPO₄.7H₂O and 1.7 gm NH₄Cl was dissolved in distilled water to prepare 1 litre of solution, pH was adjusted to 7.2.

D. Magnesium sulphate: - 82.5gm MgSO₄.7H₂O was dissolved in distilled water to make 1 litre of solution.

E. Calcium chloride: - 27.5gm of anhydrous CaCl₂ was dissolved in little distilled water and made up to one litre.

F. Ferric chloride: - 0.25g FeCl₂.6H₂O was dissolved in distilled water to prepare 1 litre of solution.

G. Sodium sulphate dolution (0.025):- 1.575gm Sodium sulphate was dissolved in distilled water. Volume was made up to 1 litre.

Procedure:-

Prepared dilution water was aerated in a glass container by bubbling compressed air in distilled water for about 30 minutes. 1ml each of phosphate buffer, magnesium sulphate, calcium
chloride, and ferric chloride solution were added for each litre of dilution water and mixed thoroughly. The sample was neutralized to pH around 7.0 by using 1 N NaOH or H₂SO₄. Since the DO in the sample is likely to be exhausted, it is usually necessary to prepare a suitable dilution of the sample according to the expected BOD range. Dilutions were prepared in a large glass trough and the contents were mixed thoroughly. 2 sets of the BOD bottles were filled. One set of the bottles was kept in BOD incubator at 20°C for 5 days, and the DO was determined in another set immediately. DO in the other sample was determined after the completion of 5 days incubation. Similarly for blank, 2 BOD bottles were taken for dilution water. DO content of one bottle was determined immediately and the other was incubated with the sample to determine DO after 5 days.

Calculation:

\[
\text{BOD (mg/l)} = (D_0 - D_5) \times \text{dilution factor}
\]

Where’

\[
D_0 = \text{Initial DO in the sample}
\]

\[
D_5 = \text{DO after 5 days incubator}
\]

**Chemical oxygen demand:**

Chemical oxygen demand is the important parameter for estimation of the carbonaceous fraction of the organic matter much closer to the actual amount. The amount of organic matter in water is estimated by their oxidability by chemical oxidants, such as potassium permanganate or potassium dichromate. In the permanganate method the organic matter is first oxidized with
a known amount of KMnO₄ and then the excess of oxygen is allowed to react with potassium iodide to liberate iodine in amounts equal to the excess oxygen, which is estimated titrimetrically with sodium thiosulphate solution with starch as indicator.

Reagents:

A. Potassium permanganate solution (0.1N):-

3.16 g KMnO₄ and 16 g NaOH was dissolved in distilled water and made up to one litre.

B. Sodium thiosulphate solution (0.1M):-

15.81 g of Na₂S₃O₃ was dissolved in little CO₂ free distilled water and made up to 1 litre. 1 g of Na₂CO₃ was added as preservative.

C. Sulphuric acid (2M):-

900 ml distilled water was added cautiously to 100 ml of AR grade sulphuric acid.

D. Potassium iodide solution (10%):-

10 g of KI was dissolved in 90 ml distilled water.

E. Starch solution (1%):-

1 g starch was mixed in 100 ml distilled water and heated until dissolved.
Procedure:-

25ml sample was taken in a 50ml flask and simultaneously took distilled water in another flask to run as control. 2.5ml of KMnO₄ solution was added and placed the flasks in water bath for one hour at boiling temperature. Samples were cooled for 10 minutes at room temperature. 2.5ml of KI solution and 5ml of H₂SO₄ were added in flasks and titrated with 0.1M sodium thiosulphate solution until pale yellow colour appeared. After that 1ml of starch solution was mixed. The solution turned blue. Titration was continued until the blue colour disappeared completely and noted the reading.

Calculation:-

\[
\text{COD of sample mg/l=} \frac{(A-B) \times N \text{ ferrous ammonium sulphate} \times 1000 \times 8}{\text{ml of sample}}
\]

Where=

A= Volume of titrant used for blank in ml.
B= Volume of titrant used for sample in ml.

Free carbon dioxide:-

Samples were titrated with 0.05 N sodium hydroxide by using phenolphthalein as an indicator.

Reagents:-

A. 0.05 N NaOH:-

1.0 N NaOH was prepared by dissolving 40gm. of NaOH in CO₂ free boiled distilled water to make 1 litre of solution. 50
ml. of 1.0 N NaOH was diluted with 1 litre distilled water. It was standardized with H$_2$SO$_4$

Phenolphthalein indicator:-
100 mg. phenolphthalein was dissolved in 100 ml. of 95% ethyl alcohol.

Procedure:-

25 ml. of sample was taken in a conical flask and few drops of phenolphthalein indicator were added and titrated with 0.05N NaOH till pink color appeared. Noted the reading and calculated with the help of the following formula.

\[
\text{Free CO}_2 \text{mg/l} = \frac{\text{ml. of titrat} \times \text{N of NaOH} \times 1000 \times 44}{\text{ml. of sample}}
\]

Total alkalinity:-

Reagents,

A. Phenolphthalein indicator:-

1.25 gm phenolphthalein was diluted with 125 ml. ethyl alcohol. 125 ml. of distilled water was added. After stirring some drops of 0.02 N sulphuric acids were added.

B. 0.02N H$_2$SO$_4$ titrant:-

200 ml. of stock 0.1 N sulphuric acid was diluted with one litre of distilled water (0.1 N stock solution was prepared by diluting 2.8 ml. of concentrated sulphuric acid to one litre with distilled water).
C. Methyl orange indicator:-

0.1 gm methyl orange was dissolved in 200 ml. of distilled water.

**Calcium hardness:-**

Calcium hardness was measured by EDTA titrimetric method and same reagents were used in the calcium hardness testing.

A. 1 N NaOH:-

40 gm NaOH was dissolved in one litre of distilled water.

B. 0.01M EDTA solution:-

3.723 gm of EDTA disodium salt was dissolved in one litre of distilled water and stored in polyethylene bottle.

Procedure:-

25 ml. samples was taken in a 100 ml. conical flask and 2 ml. of 1 N NaOH and a pinch of muroxide indicator were added, and it was titrated with 0.01 M of disodium salt solution titrant until the pink colour changed to dark purple colour. (Trivedi and Goel 1986) Calcium hardness was calculated by the formula.

\[
\text{Calcium hardness mg/l} = \frac{\text{ml. of titrant} \times 1000}{\text{ml. of sample}}
\]

**Magnesium hardness:-**

Magnesium was determined by calculation method using following relation. (Adoni 1985)

Magnesium mg/l = total hardness – calcium hardness × 0.243
**Total hardness:-**

The total hardness is defined as the sum of calcium and magnesium concentrations. It was calculated by the method of ammonia buffer and EDTA solution.

Reagents:-

A. Ammonia buffer solution:-

   16.9 gm. of ammonium chloride was dissolved in 143 ml. of concentrated ammonium hydroxide and made up to 250 ml. by distilled water and added 1.179 gm. disodium salt.

B. Eriochrome Black “T” indicator:-

   0.5 gm eriochrome Black T was mixed with 100 ml. of 80% ethyl alcohol

Procedure:-

25 ml. of well mixed sample was taken in a flask and 0.5 ml. of ammonia buffer solution was mixed and 2 to 3 drops of eriochrome Black T indicator were added and the solution was titrated with 0.01m disodium salt solution until the wine-read color changed to clear blue. The total hardness was calculated with the following formula:

\[
\text{Total Hardness mg/l} = \frac{\text{ml of titrant} \times 1000}{\text{ml of sample}}
\]

**Chlorides:-**

Argentometric method was used for chloride estimation. Sample was titrated with silver nitrate and potassium chromate was used as indicator.
Reagents:-

A. Standard silver nitrate titrant:-
   2.395 gm. of silver nitrate was dissolved in distilled water and made up to one litre and stored in dark bottle.

B. Potassium chromate indicator:-
   10 gm. potassium chromate was dissolved in a little distilled water and silver nitrate solution was added to produce red precipitate. It was filtered after 24 hours and the filtrate was made up to 200 ml. with distilled water.

Procedure:-

25 ml. of sample was taken in a 100 ml. conical flask and 1 ml. of potassium chromate indicator was added. The sample was titrated with 0.0141 N standard AgNO$_3$ titrant until a brick red colour appeared and noted the reading. Chloride content was calculated in mg/l by the formula given below:

$$\text{Chloride mg/l} = \frac{\text{ml. of titrant} \times \text{Normality of titrant} \times 1000 \times 35.5}{\text{ml. of sample}}$$

Sulphates:-

Turbidimetric method was used for sulphate detection.

Requirements:-

A. Spectrophotometer.

B. Conditioning reagent: - 75gm NaCl, 30ml conc. HCl and 100ml of 95% ethyl alcohol were mixed in 300ml of distilled water.
water. 50ml of glycerol was added to this solution and mixed thoroughly.

C. Barium chloride crystals (BaCl$_2$):- in dried form.

D. Standard sulphate solution: - 0.1479g of anhydrous Na$_2$SO$_4$ was dissolved in distilled water to make 1 litre of solution. This solution contained 100 mg/l of sulphate. Standard solution can also be prepared by diluting 10.41ml of 0.02N H$_2$SO$_4$ to 100 ml with distilled water. It also contains 100mg/l of sulphate.

Procedure:-

100 ml sample was taken in a conical flask; 5ml of conditioning reagent was added and then shaken vigorously. Sulphates were determined by turbid-metric method. The contents were mixed in magnetic stirrer and retreated by one spoonful of barium chloride crystals and stirred for one minute. Solution was taken in cuvettes and read at 420 nm. at intervals of 30 seconds, upto 4 minutes, the maximum reading was noted. Concentration of sulphate was obtained from the standard curve. Final value was calculated in mg/l by the relation:

\[
\text{mgSO}_4/\text{l} = \frac{\text{MgSO}_4 \text{ from curve}}{100} \times 1000
\]

**Sodium and Potassium:-**

The quantity of sodium and potassium was recorded by digital flame photometer calibrated with the help of standard sodium
chloride and potassium chloride solutions and washed with distilled water.

Reagents:

Standard Solution:

25 ml. of the sample was filtered through filter paper.

First the flamephotometer was started and the compressor maintained at air pressure at 10 pounds and gas flame set at blue conical flame. Now the blank was fed to set zero. After that flamephotometer was standardized with stock sodium and potassium chloride solutions (40%, 60%, and 100%). After standardization the numlaras tube was washed with distilled water and set again at zero and the sample was fed and readings of Na, and K samples were read and expressed in mg/l. with the help of standard curve.

Nitrates:

Nitrates were estimated using brucine sulfanilic acid method.

Requirement:

A. Spectrophotometer.

B. NaCl Solution: - 300gm NaCl was dissolved in 1 litre distilled water.

C. Sulphuric acid solution: - 500 ml conc.H₂SO₄ was diluted in 125 ml of distilled water.

D. Brucine sulfanilic acid solution: - 0.1gm sulfanilic acid was dissolved in 70 ml of hot distilled water. 3ml conc. HCl was
added and the solution was made up to 100ml by distilled water.

Procedure:-

10ml sample was taken in Nessler’s tube and 2 ml of NaCl solution was added. The tube rack was placed in cool water bath. Contents were mixed and 10ml of sulphuric acid solution was added and contents were cooled in water bath. 0.5ml of brucine sulfanilic acid solution was added and mixed thoroughly. The tubes rack was placed in boiling water bath at 95°C. After 20 minutes the rack was cooled to room temperature and reading was recorded at 410 nm. The value of NO₃⁻N was obtained directly from the standard curve:

\[
\text{Mg/l Nitrate} = \frac{N \text{ mg NO}_3^- \text{ N}}{10}
\]

**Biological parameters:-**

Biological monitoring of water is essential for human use to have a check on pathogenic organisms. All natural waters have a variety of organisms, both plants and animals as the natural flora and fauna. The qualitative and quantitative evaluation of plankton was done to assess the structure and variation of plankton community of Angoori reservoir and to understand their relationship with the abiotic components.

**Phytoplankton:-**

Samples for the phytoplankton estimation were collected every last Sunday of the month from the sampling stations. One litre of water sample was taken in polyethylene bottles and fixed for 24 hours with
Lugol’s iodine solution. Supernatant was siphoned off carefully and 10 ml of concentrate was preserved for identification.

The counting of phytoplankton was done by Sedgwick rafter cell. Cover slip was put diagonally on the rafter cell cavity, shook the sample gently and transferred quickly 1 ml of it in to the cavity with the help of a graduated pipette. The Cover slip was properly adjusted to cover the cavity without air bubbles. Focussed on one edge of the cavity under the microscope and moved the slide horizontally, simultaneously counting the plankton till the other edge, and examined this way 2 to 4 such transects. The identification of planktons was made with the help of pertinent literature (Mervin and Palmer 1980), Ward and Whipple (1959), Garnett (1965) and Smith (1950). The number of planktons was calculated with the help of the following formula:

\[
\text{Organisms/l (N)} = \frac{C \times 1000 \text{mm}^3}{L \times D \times W \times S}
\]

Where;

- \(C\) = Number of organisms counted in all transacts.
- \(L, D, W\) = Length, Depth and Width of a transact.
- \(S\) = Number of transacts counted
- \(l\) = Volume of the original water expressed in litre.

**Zooplankton:-**

25 litre of water sample was filtered for zooplanktonic study through plankton net made up of bolting silk cloth and preserved with 5% formalin solution. After this 10 drops of glycerin were also added to it.
Counting of zooplankton was made by Sedgwick rafter cell and identification of zooplankton was done as per Sehgal (1983), Ward and Whipple (1959), and Garnett (1965). The quantitative estimation was done with the help of the following formula:

\[ N = \frac{a \times c}{l} \]

Where:

- \( N \) = number of plankton per litre of the sample.
- \( a \) = Average number of plankton in all counts in counting unit of 1 mm\(^3\) capacity
- \( c \) = Volume of original concentrate in mm\(^3\)
- \( l \) = Volume of the original water expressed in litre.

**Primary Production:**

Primary production is the most important biological phenomenon in nature on which the entire diverse array of life depends, either directly or indirectly. Phytoplanktonic productivity is the rate of primary production by phytoplanktonic populations and can be measured by “Light and Dark Bottle Technique”. This method was first employed by Gaarder and Gran in 1927, wherein photosynthesis and respiration are measured in terms of oxygen production and oxygen consumption respectively, by incubation of the sub-samples of phytoplanktonic populations in light and darkened bottles in natural conditions. Measurement of dissolved oxygen was done using either Winkler’s method.
Requirement:

300 ml BOD bottles (Eight light and Four blackened with tape or black painted), nylon rope, necessary reagents and glassware.

Method:

All the bottles (avoiding bubbling) were filled with water sample collected from known depth and stoppard tightly. Determined the dissolved oxygen concentration in four of the light bottles immediately by Winkler’s method and noted the initial oxygen content in initial bottles (IB), hanged the four dark bottles (DB) and remaining four light bottles (LB) dipped in the surface water. Took them out after exactly 4 hours and estimated the D.O. content with the help of winkler’s method:

Calculation:

Gross primary productivity = LB- DB x 0.375/h x 1000 (in mgc/m³/h⁻¹)
Net primary productivity = LB- IB x 0.375 /h x 1000 (in mgc/m³/h⁻¹)
Community respiration rate = IB-DB x 0.375 /h x 1000 (in mgc/m³/h⁻¹)

Fish fauna:

The composite fish culture is practiced at Angoori reservoir by the local fishermen. Monthly fish collection at fishing sites and fish marketing surveys were made with the help of local fishermen and M. P. fisheries department of Datia district. For the study of fish diversity and taxonomic study of fishes, adult male and female fishes available on the fishing sites were collected. The fish collection was also noted on monthly basis during the whole study period i.e. from January 2009 to December 2010. Available fish specimens were properly preserved in
10% formalin solution for further study. This solution was prepared by diluting one part of commercial formalin with nine part of distilled water. Fishes to be preserved were dropped in this solution after giving an inch cut on the mid ventral line of the body, so that preservative may enter the body. For proper and better preservation fish were kept in this preservative for at least 4 to 5 hours. Fishes were removed from this solution and kept in freshly prepared another formalin solution. The following tools were used for collection and preservation of fish specimens.

- Hand net, trap net, fasla and kudar nets, drug net and cast net with the help of local fishermen.
- Enamel trays.
- Measuring tape and marking material.
- Polythene bags.
- Note book.
- Camera for photography.
- A boat with fishermen.

Fishes collected were identified and classified under different species, genera, families and orders etc. following the methodology suggested by Day (1958), Jhingran (1985), Jayaram (1999) and Shrivastava (1980). Data collected were subjected to statistical analysis.