Methodology
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In this decade environmental quality become a matter of great public concern, progress has been made, particularly in controlling the visible and obvious of forms of environmental pollution. Rivers and Lakes, being formed from water which has percolated through the surface soil contain dissolved salts, traces of organic matter, suspended matter, and such dissolved gases as oxygen, nitrogen, carbon-di-oxide. The activities of man, however, lead to quite marked alterations in the natural composition of river water.

The fundamental importance of water for life on the earth needs little justification. Although river pollution to be caused by physical and biological factors.

The accurate assessment of the concentration of pollutants present in water primarily depends upon the sample drawn for the analysis. The sample must be the representative in nature. The selection of sampling stations of Betwa River was carried out on the basis of reconnaissance survey and finally five stations were selected. The investigations were carried out lasted for the period of 2 years Jan. 2004 to Dec. 2005.

The sampling was carried out at monthly intervals in the second week of the month between 7 am. To 10 am.

Selection of study stations

The stretch of river Betwa under study is 22 Km. It was surveyed by boat and having in view the topography as well as village, city, Nala discharges cattle and human activities, washing ghat and confluence with Yamuna. The variations were considered in selection of five study stations which are :-
Sampling Station I Pothia Village

Sampling Station II Betwa Ghat at Hamirpur City
I - Pothia
II - Betwa Ghat
III - U.P. Stream
IV - down stream
V - Sangam (Near Meerapur-Bhilawan villages)

Water Sampling

During the present investigation water samples were collected in clean white plastic container from each of the stations, using standard methods of collection. Water samples were brought to the laboratory and kept in presser water at 4°C for farther analysis of various physico-chemical parameters ie. Turbidity, Carbon dioxide, Chloride, Total Alkalinity, Total Hardness, Calcium Hardness, Magnesium Hardness, Dissolved Oxygen (D.O.), Chemical Oxygen Demand (C.O.D.), Biological Oxygen Demand (B.O.D.), Ammonical Nitrogen (NH₄-N), Nitric Nitrogen (NO₂-N), Nitrate Nitrogen, (NO₃-N), Potassium (K). Whereas for the estimation of dissolved oxygen study the samples were collected in D.O. bottles of 300 ml capacity and it was fixed at the site as per APHA method.

Station I

This spot is located at pothia village, which is on Rath road and 15 Km away in the south west of Hamirpur district. The river bank is western side, It varies from 100 to 800 meters wide during different seasons. Flow rate is low in winter and summer. Here the river is less deep than other stations. Bed composed of mud, sand, slit and gravels.
Sampling Station III Upstream (Sewage Discharge)

Sampling Station IV (Down Stream) at Hamirpur
Station II

It is at Betwa Ghat, Which is located at Hamirpur and 14 Km. away from station I. and 1.5 Km. before Betwa road bridge which connects Mahoba to Hamirpur. Southern bank is sandy where digging of the sand is done, called Sankari Peepar khadan whereas Northern bank has inbackment.

Station III

It is on up stream, situated at the entrance of Hamirpur city near Betwa bridge about 1 Km away from Betwa Ghat. The river is deepest here and a continous discharge of city sewage on the north bank of the river.

Station IV

It is on down stream at Ramaini Pumpcanal situated near down stream. This spote is 1 Km on words from the Betwa Bridge. The river is 400-500 meter wide and flow rate is little fast than upstream. Southern side of the river bank many crops and vegetables are cultivated. Their leaching reaches in the river.

Station V

It is at the confluence point of Betwa and Yamuna which is known as Sangam near Meera pur and Bhilawan Villages. On the southern side of the river are riveines whereas northern side agriculture crops are grown by farmers. Water current is the fastest and width is in rainy season reaches 200-300 meters here.
Sampling Station V (Confluence with Yamuna)

Betwa River Confluence with Yamuna Near Railway Bridge
Water Analysis Methods

The water analysis of the collected samples of different spots were studied for various factors by the methods as per standard methods for the analysis of water and waste water *APHA* (1998). American Water Works Association (*AWWA*) and Water Pollution Control Federation (*WPCF*).

Physical Factors

Temperature

Temperature of water was measured on the spot using a Celsius degree centigrade therma meter. The temperature of the water was noted by dipping and keeping the thermometer in water for about a minute.

Turbidity

Turbidity was recorded by a systronics Nephloturbidity meter and expressed in NTU.

Colour

The colour of the water was observed by visual appearance only.

Water Current

Water current was measured by a mechanical current meter and expressed in current cm/Sec.
Chemical Factors

pH Value

The pH (Hydrogen ion concentration) of samples were measured on the spot by ‘Qualigens’ pH indicator papers and later it was confirmed by (systromic) digital pH meter MK VI in the Laboratory.

Total Dissolved Solids (T.D.S.)

Total dissolved solids are the portions of solids that pass through a filter of 2 µmhos under specific conditions. This was determined by evaporation of 100 ml of well-mixed sample in a weighted beaker and dried to constant weight at 180°C after filtration through a standard glass fiber filter (whatman filter paper no.42.) Increase in weight of beaker represents the total dissolved solids and it was calculated by this formula.

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

Where

- \( A \) = Weight of dried beaker + Residue in mg.
- \( B \) = Weight of beaker in mg.
- \( V \) = Sample volume in ml.

Total Alkalinity

Reagents :-

(a) **0.02 N (N/50 Sulphuric acid)** - 26.7 ml of sulphuric acid (Sp.gr. 1.84), was made on one litre with distilled water and standardized
against 1 N sodium carbonate solution. 0.02 N (N/50) sulphuric acid was prepared by making the calculated amount of standardised solution of sulphuric acid to one liter with distilled water.

(b) **Standard 0.02 N (N/50) Na₂CO₃**. To make 0.1 N Na₂CO₃ stock solution, 5.3 gm anhydrous, Na₂CO₃ was carefully dessicated and dissolved in one litre of distilled water. 0.02 N (N/50) Na₂CO₃ was prepared from the stock solution (0.1N, Na₂CO₃) diluting it to 250 ml.

(c) **Phenolphthalein indicator** - 50 ml of phenolphthalein indicator was dissolved in 100 ml of 50 % Ethyl alcohol.

**Methyl Orange** :- 50 mg of methyl orange indicator was dissolved in 100 ml of distilled water.

**Procedure :-**

(i) **Carbonate Alkalinity**- 4-6 drops of the Phenolphthalein indicator were added in an Erlenmeyer flask containing 100 ml. of sample water and placed on a white porcelain tile. If the sample remained colourless phenolphthalein alkalinity was zero. However, if the sample turned pink it was titrated with 0.02 N (N/50) sulphuric acid to a colourless a end point.

**Calculation :**

Phenolphthalein alkalinity mg/l = Number of ml of 0.02 NH₂SO₄ x 10

(II) **Bicarbonate alkalinity**- The bicarbonate MO (MO= Magnissium oxide) alkalinity was determined by the procedures (vide supra) but with methyl orange, as indicator. The colour of the sample was changed from yellow to faint orange, the end point was recorded.
Calculation:

\[ \text{MO alkalinity mg/l} = \text{No. of ml of 0.02 N H}_2\text{SO}_4 \text{ used} \times 10 \]

**Total Hardness:**

Total hardness was determined titrimetrically using \textit{EDTA} method (APHA 1998). To 50 ml of sample taken in a conical flak, one ml of ammonia buffer and pinch of “Eriochrome black T” indicator was added and titrated against \textit{EDTA} (Ethyl Diamine Tetra Aceticacid) till colour changed purple to blue.

\[ \text{M}^{++} + \text{EDTA} \rightarrow \text{(M.EDTA) complex.} \]

\[ \text{M}^{++} + \text{Eriochrome black T} \rightarrow \text{M.Eriochromeblack T} \]

(Wine red) complex.

Where \( \text{M}^{++} = \text{Ca}^{++} \) and other divalent metal ions causing hardness.

Calculation:

\[ \text{Total hardness (mg/1)} = \frac{A \times 1000}{\text{Vol. of sample (in ml)}} \]

Where, \( A = \text{ml of titrant used.} \)

**Calcium Hardness**

Calcium hardness was determined titrimetrically using EDTA method, one ml of 8 % Sodium hydroxideand 0.2 gram ammonium purpurated indicator
was added to 50 ml of sample and titrated against EDTA titrant till pink colour changed to purple in order to determine calcium as calcium carbonate.

**Reaction:**

\[
\text{Metal indicator} \quad \rightarrow \quad \text{Metal indicator (Complex)}
\]

\[
\text{Metal indicator (Complex) + EDTA} \quad \rightarrow \quad \text{Metal-EDTA} \quad + \quad \text{Indicator (Complex)}
\]

**Calculation:**

\[
\text{Calcium Hardness (mg/l)} = \frac{A \times 1000}{\text{Vol. of sample (in ml)}}
\]

Where

\[
A = \text{ml of titrant}
\]

**Magnesium Hardness**

Magnesium hardness was calculated by deducting values of calcium hardness from total hardness.

**Magnesium Hardness (mg/l) = Total Hardness - Calcium Hardness**

**Chloride**

Chloride was determined by mohr's argentometry method (APHA, 1998) in such methods important reactions involved are:

\[
\text{NaCl} + \text{AgNO}_3 \quad \rightarrow \quad \text{AgCl} + \text{NaNO}_3 \text{ (till end point)}
\]

\[
2\text{AgNO}_3 + \text{K}_2\text{CrO}_4 \quad \rightarrow \quad \text{Ag}_2\text{CrO}_4 + 2 \text{KNO}_3 \quad \text{(Brick red ppt) (end point)}
\]
**Calculation:**

\[
\text{Chloride in mg/l} = \frac{T v \times N \times 35.45 \times 1000}{\text{Vol. of water sample (ml)}}
\]

Where,

\[ T v = \text{Vol. of titrant} \]

\[ N = \text{Normality of titrant.} \]

**Dissolved Oxygen (D.O.)**

The dissolved oxygen was estimated by winkler’s method using alsterberg azide modification.

The samples were carefully preserved at the sampling stations and the analysis was done with in 6 hour’s after collection to avoid any change.

**Reagents:**

(a) **Manganese sulfate solution:** Weight and dissolved 4800 gm of MnSO₄.4H₂O in distilled water, filtered transferred to a 1 litre volumetric flask, and diluted to the mark.

(b) **Alkali iodide azide:** Weight and dissolved 500 gm of NaOH and 135 g of NaI in distilled water, transferred to a 1 litre volumetric flask, and diluted to the mark.

(c) **Concentrated Sulphuric acid**

(d) **0.025 N (N/40) Sodium thiosulphate:** The standard solution (0.1/N) of sodium thiosulphate was prepared by dissolving 24.32 gm Na₂S₂O₃. 5H₂O in 700 ml of distilled water. Later 4 gm of borax (Na₂B₄O₇.10H₂O) was added as stabilizer. The solution was made to
one litre with distilled water. This solution was diluted four times to form 0.025 N Sodium thio Sulphate solution.

(e) **Starch Solution**: To a suspension of 2gm powdered starch in 350 ml of distilled water, 30 ml of 20 percent NaOH Solution was added. After stirring well, the alkali was neutralized with HCl using litmus as indicator. The starch solution was acidified with 1 ml of glacial acetic acid.

**Procedure**

Water sampling for the estimation of dissolved oxygen was done carefully, to avoid any mixing of free oxygen in the form of air bubbles. The water samples were collected from sub surface by the help of D.O. bottles of 300 ml capacity. The bottles were immersed in water and filled completely till the water had over flown twice of thrice the capacity of the bottles. The bottles were brought to the surface 2 ml each of manganous sulphate and alkaline iodide reagent were added by means of a pipette which was dipped to the bottom of the bottle and slowly taken out after adding the reagents and Stoppard. The bottle was inverted three or four times for homogenous mixing the reagents with water samples. The formation of a flocculent precipitate confirmed that the dissolved oxygen had been fixed.

The samples were analyzed in the laboratory for the quantitative estimation 2 ml cone sulphuric acid was added in each sample and the precipitate was dissolved by the gradual shaking of the bottle. The volume corresponding to 200 ml of original sample after correction had taken for titration.

The volume of treated sample to be taken was calculated by following formula:
Exact capacity of the bottle \times 200

\[ \text{Exact capacity} = 4 \text{ ml} \]

The calculated volume of the treated sample was titrated with 0.025 N sodium thio sulphate solution. Firstly few ml titrant was added till the colour of sample changed from brown to some what Pale, then 1 ml starch solution was added as indicator the colour become some what black, it was further titrated till the black colour changed from black to blue. The titration was completed when it turned colourless. The volume of sodium thiosulphate solution used up for the complete. Neutralization of released iodide was recorded.

Calculation:

\[ \text{D.O. mg/l} = \frac{\text{No. of ml of Na}_2\text{S}_2\text{O}_3 \text{ Used} \times 1000}{\text{Vol. of sample}} \]

**Biochemical Oxygen Demand (B.O.D)**

B.O.D. gives an idea about the extent of pollution for biochemical oxygen demand required by coliform and E.coli. Method from work book on limnology was adopted B.O.D was estimated by incubating the sample in B.O.D. Incubator for 5 days at 200C and after 5 days dissolved oxygen was fixed and estimated. Difference of initial \( D_0 \) & final \( D_5 \) gave the total biochemical oxygen demand.

Calculation

\[ \text{B.O. D in mg/l} = \frac{D_0 - D_5}{V} \]
Where,

\[ \text{Do} = \text{initial dissolved oxygen.} \]
\[ D_5 = \text{D.O. Calculated after 5 days} \]
\[ V = \text{Decimal volumetric fraction of sample used.} \]

**Chemical Oxygen Demand (C.O.D.)**

Chemical oxygen demand was determined by potassium dichromate Reflux method [NEERI, 1986]. 20 ml of sample water was taken in a 200 ml flask. The 10 ml of 0.25 N Potassium dichromate, 30 ml of cone. H\textsubscript{2}SO\textsubscript{4} a pinch of silver sulphate and mercuric sulfate were added and refluxed for two hours in a water bath. After two hours distilled water was added to make its volumes 140 ml. 2 to 3 drops of ferroin indicator was added to refluxed sample, mixed thoroughly and treated with 0.25 N ferrous ammonium sulphate till a brick red colour end point is obtained.

**Reaction:**

\[ 2\text{K}_2\text{Cr}_2\text{O}_7 + 8\text{H}_2\text{SO}_4 \rightarrow 2\text{K}_2(\text{SO}_4)_3 + 3\text{O}_2 + 2\text{Cr}_2(\text{SO}_4)_3 + 8\text{H}_2\text{O} \]
\[ \text{C}_6\text{H}_12\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O} \]
\[ \text{Cr}_2\text{O}_7^{2-} + 6\text{Cl}^- + 14\text{H}^+ \rightarrow 3\text{Cl}_2 + \text{Cr}^{3+} + 7\text{H}_2\text{O} \]
\[ \text{Hg}^{2+} + 2\text{Cl}^- \rightarrow \text{HgCl}_2 \]

**Calculation:**

\[ \text{C.O.D. in mg/l} = \frac{(A-B) \times N \times 8000}{\text{Vol. of sample (in ml)}} \]
Where,

\[ A = \text{ml of titrant used with sample.} \]
\[ B = \text{ml of titrant used with blank.} \]
\[ N = \text{Normality of FeSO}_4 (\text{NH}_4)_2 \text{SO}_4 \cdot 6\text{H}_2\text{O}. \]

**Carbon di-oxide (CO}_2\)**

As a free carbon di-oxide is liable to escape easily from the water, it is highly desirable that analysis be made soon after the collection of the sample (welch 1948). For this the analysis was done in the field according to the standard method of APHA.

**Reagents:**

(a) **N/44 Sodium hydroxide solution:** 0.1 N Sodium hydroxide solution was prepared by dissolving 4 gm. Sodium hydroxide (A.R.) in distilled water and than made to 1000 ml, which was standardised against 0.1 N sulphuric acid, Using phenolphthalein as indicator, 100 ml of this 0.1 N solution was dilute to 440 ml with distilled water, to form N/44 sodium hydroxide solution.

(b) **Phenolphthalein indicator:** 0.5 percent solution of phenolphthalein in indicator was prepared by dissolving 500 mg of phenolphthalein in 100 ml of 50 percent alcohol

**Procedure**

100 ml of sample was taken in a beaker and four drops of the phenolphthalein indicator were added to it. If the water turned pink it showed the absence of free CO\textsubscript{2}, on the other hand if remained colourless, the free
carbon di oxide was present. If the free carbon di oxide was present than the solution was titrated with N/44 sodium hydroxide solution till a distinct pink colour developed, which flashed for a few seconds through out the solution. The volume of the titrant used was noted and CO₂ estimated.

**Calculation :** Free CO₂ mg/l = No. of ml of N/44 NaOH used x 10.0

**Ammonical Nitrogen (NH₄-N)**

Direct nesslerization method was adopted for this parameter. To 50 ml of sample, 5 drops of Rochelle salt solution and two ml of Nessler’s reagent was added. After ten minutes intensity of colour was measured on spectrophotometer at 420 nm wave length. The value of NH₄-N was obtained from standard curve.

**Nitrite Nitrogen (NO₂-N)**

1 ml of each sulfanilic acid Naphthylamine Hydrochloride & sodium acetate solutions in sequence were added in 50 ml of colourless filtered sample. A wine red colour of Nitrite appeared and determined at 520 nm wave length & the value of NO₂-N was calculated in mg/l directly from the standard curve.

**Nitrate Nitrogen (NO₃-N)**

Phenol di sulphonic Acid method was chosen for this parameter 50 ml of water sample was evaporated in water bath. Residue was dissolved in 1,2,4 phenol di sulphonic acid on addition of ammonia solution yellow colour alkaline salt was formed and determined at 510 nm wave length. Value of NO₃-N in mg/l was found out by standard curve.
Phosphate

Phosphate was determined on Spectrometer by stannous chloride method. To 50 ml of sample, 20 ml of Ammonium Molybdate solution and 6 drops of stannous chloride was added and colour was allowed to develop for five minutes. Absorbance was noted on spectrophotometer at a wave length of 690 nm and concentration of phosphate in mg/l was calculated from standard graph.

Sulphate

40 ml water sample was taken into 100 ml cylinder, followed by 10 ml. Barium chlorid solution. The sample was shaken and kept standing for 15 min. The reading of developed turbidity was measured on UV-VIS spectrometer at 420 nm. The concentration of sulphate was calculated by standard curve. Results were expressed in mg/Litre.

Sodium

Sodium determination was carried out by Using systronics make flame photometer. The instrument was calibrated with standard sodium chloride solution. Then the sample was sprayed in ten gas flame and the excitation was measured at 589 nm.

Potassium

Potassium determination was carried out by using flame photometer. The instrument was calibrated with standard solution of potassium chloride. The potassium determination was carried out at 768 nm.


Fluoride

Reagents:

(a) Standard fluoride solution: Diluted 100 ml stock fluoride solution to 100 ml with distilled water. 1.00 ml = 10.0 µg $F^-$. 

(b) SPADNS Solution: Dissolved 958 mg SPADNA, Sodium 2- (Parasulfo phenylazo) 1,8-dihydroxy - 3, 6 - naphthalene disulfonate, also called 4 - 5 dihydroxy - 3 - (Para sulfophenylazo) - 2, 7 - naphthalene disulfonic acid trisodium salt, in distilled water and diluted to 500 ml. 

(C) Zirconyl acid reagent: Dissolved 133 mg zirconyl chloride octahydrate, ZrOC$_2$.8H$_2$O in about 25 ml distilled water. Added 350 ml cone HCl & diluted to 500 ml with distilled water. 

(d) Acid zirconyl-SPADNS reagents: Mixed equal volumes of SPADNS solution and Zirconyl acid reagent. 

(e)Reference Solution: Added 10 ml SPADNS solution to 100 ml distilled water. Diluted 7 ml conc. HCl to 10 ml and add to the diluted SPADNS solution. 

(f) Sodium arsenite solution: Dissolved 5.0g NaAsO$_2$ And diluted to 1 liter with distilled water. 

Procedure

(a) Preparation of standard curve: Prepared fluoride standard in the range of 0 to 1.40 mg F/L diluting appropriate quantities of standard Fluoride solution to 50 ml with distilled water. Pipette 5.00 ml each of SPADNS solution and Zirconyl acid reagent or 10.00 ml mixed acid Zirconyl SPADNS
reagent to each standard and mixed well. Avoided contamination; Set spectra photometer to Zero absorbance with the reference solution and obtained absorbance readings of standards. Plotted a curve of the milligrams fluoride absorbance relationship. Prepared a new standard curve whenever a fresh reagent is made or a different standard temperature is desired. As an alternative to using a reference, set spectrophotometer at some convenient point (0.300 or 0.500 absorbance) with the prepared 0 mg. F⁻/l standard.

(b) Sample pretreatment: If the sample contained (Sodium arse) residual chlorine, removed it by adding 1 drop (0.05 ml) NaAsO₂ solution/0.1 mg residual chlorine & mixed.

(c) Colour development: Used a 50.00 ml sample or a portion diluted to 50 ml with distilled water. Adjusted sample temperature to that used for the standard curve. Add 5.00 ml each of SPADNS solution and Zirconyl acid reagent, or 10 ml acid zirconyl. SPANDS solutions and zirconyl acid reagent, or 10 ml acid zirconyl-SPADNS reagent mixed well and read absorbance, First setting the reference point of the photometer as above. If the absorbance falls beyond the range of the standard curve, repeat using a diluted sample.

Calculation:

\[ \text{mg F}^-/\text{L} = \frac{A}{\text{ml sample}} \times \frac{B}{C} \]

Where,

- \( A \) = \( \mu g \) F⁻ determined from plotted curve,
- \( B \) = final Volume of diluted sample, ml and
- \( C \) = Volume of diluted sample used for colour development, ml.
When the prepared 0 mg F⁻/L standard is used to set the photometer, alternatively calculate fluoride concentration as follows:

\[ \text{Mg F}^{-}/\text{L} = \frac{A_0 - A_2}{A_0 - A_1} \]

Where,

- \( A_0 \) = absorbance of the prepared 0 mg F⁻/L standard.
- \( A_1 \) = absorbance of the prepared 1.0 mg F⁻/L standard.
- \( A_2 \) = absorbance of the prepared sample

**Biological Analysis**

**Bacteriological Examination**

**Sample for Bacteriological Examination**

Extreme precaution is needed for collection of samples for bacteriological analysis. Usually the samples are collected from the marginal waters, which was badly contaminated and disturbed due to various human activities.

I collected samples from marginal water and about 100 meters away from the margin between Jan 2004 to Dec 2005.

**Estimation of total coliform:** To detect the bacteria in water. The chief objectives were to identify that water contains faecal pollution and was unsafe for consumption. Coliforms were gram negative bacteria, which were rod
shaped and usually inhabited the gastrointestinal tract. The coliforms which were present in the faecal waste, were called the faecal coliforms. Faecal and non faecal coliforms are together called total coliforms. The most important and convenient is the Most Potable Number (M.P.N.) technique which was followed for the present research.

M.P.N. Procedure

In this procedure water samples in to a no of tubes of medium (McConkey’s Broth). 10 ml, 1 ml and 0.1 ml amount was inoculated in to sterilized lactose broth tubes. The ignition tube was placed in each test tube. The tubes were incubated at 35 °C to 37 °C. Caliform organisms were identified after 24 hours by their production of gas from the lactose. By refereeing to a M.P.N. table, a statistical range of the number of coliforms were determined by observing how many broth tubes showed gas formation. The tubes showing the positive tests were subjected to a confirmatory test.

The presence of coliforms were confirmed by streaking samples form the positive lactose broth cultures on to Eosin Methylene Blue agar (E.M.B.). Faecal coliforms showed a characteristics greenish metallic seen, the non faecal coliform exhibited dark reddish colonies whereas the non coliform showed colourless colonies.

Calculation:

\[
\text{MPN/100 ml} = \sqrt[ \text{Total sample (ml) in negative tube} \times \text{Total sample in test} ]{ \text{No. of positivetube} \times 100 }
\]
Phyto and zooplankton

As regards the phyto-Zooplankton and fishes, their identification was done, with the help of taxonomy books standard methods (APHA 1995).

The plankton density usually diminishes from the phyto-zooplankton margin to mid stream and also in down wards surface.

Maximum population was recorded in the marginal and surface water but this may not give the over all picture as some of the plankton prefer surface region. Moreover vertical migration of plankton keeps on fluctuating with the light intensity temperature and co-contents during the diel cycle. There was also some variation is plankton density on the two opposite banks of the river. It was generally higher on that side of the river which has greater human settlement correct conclusions about plankton density and diversity can be down only when the sample were collected from the cross section of the river. Therefore the samples were collected from the cross section of the river.

**Time of sampling:** Planktons are very sensitive to water temperature, solar radiations, D.O. and nutrient levels of the water. I have observed a marked variation in the vertical movement of plankton during the diel cycle which is obviously on account of variations in some of the important physico-chemical aspects of water practically all the groups of phyto-plankton are sensitive to light, therefore by and large their activity was brisk during the morning hours. The time consider with the feeding time of the zooplankton in early hours. It may be pointed out that the plankton population during the noon diminishes due to down word migration of zooplankton. Moreover due to changes in the osmoregulatory process and buoyancy of the phyto-plankton round the clock has shown that maximum plankton density was between 8.00 am to 9.30 am while stock. Therefore the sample the study of planktons were collected by
boat from such places related to the different stations between 8.00 am to 9.30 am which gave a general feature of the stretch of the river under study.

Collection of Phyto & Zooplankton

Plankton samples were collected from mid stream sub surface water by means of planktonnet, having in view the above mentioned conditions. The most standard convenion and widely used planktonet is made of bolting silk no 25 (65 μm) and comprises a metal ring of 15 c.m diameter attached to a metal handle of one metre length, Bolting silks cloth is tightly stitched around the metal ring in a manner to shape it like a truncated cone. At the tapering end of the cone a specimen tube (10ml) with a rim at the top is strongly tied. At the time of collection the net is dipped in the water, so that the ring is completely immersed below the water surface.

This dipping was done from the sides of the boat subsequently the dipped net moved against the water current about a meter and it was quickly lifted in this manner two to six successive hauling was made. Haulings were both the sides of the boat.

The contents of specimen tube are subsequently transferred to other specimen tube having 4% formalin one fraction of the sample is life for the microscopic examination and identification of plankton density usually the plankton density is determined by micro transect method Lackey (1938). The details of the method are as follow:

The qualification and quantitative studies were made and for their identification the literature was consulted by Goyal and Trivedi (1986).

The concentrate of Zooplankton was thoroughly mixed, then one drop of it was put on the clean side by a pipette (one drop = 0.1 ml) and covered with 22 x 22 mm glass cover slip. Their counting of organisms were made in five
strips along the length or breath of the cover slip under the microscope five such drops were examined. The width of microscopic field was measured with the help of stage micrometer.

Each transect was represent a definite fraction of area under cover slip, hence a definite volume of sample. The no. of plankton per drop was calculated as follows:

\[ \text{Total no per drop} = \frac{\text{Area of cover slip} \times \text{Average no. of plankton per transect}}{\text{Area of transect}} \]

The No of zooplankton per litre was calculated by the following formula.

\[ \text{No. of zooplankton per litre} = \frac{q \times V^l}{V^{ll}} \]

Where,

\[ q = \text{number of plankton per ml.} \]
\[ V^l = \text{Volume of concentrate} \]
\[ V^{ll} = \text{Volume of water filtered through the net.} \]

The volume of water filtered through the net was calculated by using the following formula.

\[ V^{ll} = rl \]

Where,

\[ V^{ll} = \text{Volume of water filtered} \]
\[ r = \text{Radium of planktonnet ring} \]
\[ I = \text{Column of water filtered.} \]
Fishes

They were collected during night and also in early morning by drag net and also with vertical nets (100’ X 5’’) with a mesh (3’ X 5’’) in diameter and they were identified with the help of Francis DayFauna and also with Gopal Ji Srivastava for identification of fishes.

Aquatic Weeds

The sample of aquatic weeds collected and their abundance was assessed by visual observations and identified up to Subramanyan (1962).

Meteorological data are recorded for the period of two years i.e. (2004 – 2005) from Collectrate office at Hamirpur and their means values were calculated.

Mathodology for determining the correlation among various parameters:

Correlation analysis is a discussion of the degree of closeness of the relationship between two variables. According to prof. Bodding “wherever some define connection exists between two or more groups, classes or series of data, there is said to be a correlation.” In the present study the method for determining the correlation between two parameters is the Karl Pearson’s method. It is the most widely used method and also known as Peronian coefficient correlation. It can be calculated by following formula.

\[
\text{Correlation coefficient} = \frac{\sum (dx \cdot dy)}{\sqrt{\sum (dx^2 \cdot dy^2)}}
\]
Where,

- $x$ and $y$ are two series of variables and
- $dx = \text{deviation from } x^1 \ (X - x^1)$
- $dy = \text{deviation from } y^1 \ (Y - y^1)$
- $x^1 = \text{mean of series } X$
- $y^1 = \text{mean of series } Y$

The value of the coefficient always lies between +1 and -1. When it is +1, then there is a perfect positive relation and if it is -1, then there is perfect negative relationship. If it is 0 then there is no relationship between two variables.