The samples were collected along the entire stretch of 640 km from Amarkantak to Hoshangabad. For descriptive and analytical purpose, the river is divided into two zones. **ZONE A:** Stretch covering the hilly mountainous tract from Amarkantak to Jabalpur and **ZONE B:** Stretch after entrance of the river into plains, from Shahpura to Hoshangabad. The sampling periods were different in zone A and zone B. Surface water samples were collected at monthly intervals from April 1988 to June 1989 in zone A. Samples were not taken in August and September 1988 due to the flooded condition of the river. In zone B, samples were taken from November 1989 to June 1990. The difference of comparing two zones sampled in different years are negligible because, the hydrological and climatological parameters in their respective time and seasons were almost similar.

2.1 Sample Collection:

For physico-chemical analysis, water samples were collected in acid washed and thoroughly cleaned 2 liter polythene bottles. Following physico-chemical parameters were studied following the methods suggested by APHA (1980) and Golterman and Clymo (1969).

2.2 Physical Parameters:

(1) **Temperature:**

Temperature of both air and water were recorded in the field at the sampling station by using a centigrade thermomometer in °C (1/10 div.).
(2) **CONDUCTIVITY** :-

Specific conductivity was determined by a conductivity bridge (SYSTRONICS) and recorded in $\mu$mho cm$^{-1}$.

(3) **pH**

pH observed by a METREX made digital pH meter.

(4) **Turbidity** :

A 'SYSTRONICS' made Nephelo turbidity meter was used to record turbidity in NTU (Nephelo Turbidity Unit).

2.3 **Chemical Characteristics** :

(1) **Free CO$_2$** :

The analysis of free CO$_2$ was performed in the field immediately after sample collection.

In a 100 ml water sample 5 to 10 drops of phenolphthalein indicator was added. If the solution turned red, free CO$_2$ was absent. If the solution remained colourless, it was titrated against N/44 Sodium hydroxide solution until faint permanent pink colour appeared.

**Calculation** :

\[
\text{Free CO}_2 \text{ in ppm} = \frac{\text{ml of N/44 NaOH used in titration} \times 1000}{\text{ml of sample titrated}}
\]

(2) **Carbonate Alkalinity** (Phenolphthalein Alkalinity) :

To 100 ml fresh sample 5 to 10 drops of phenolphthalein indicator was added. If the solution remained colourless the carbonate alkalinity was absent. If the solution turned red titrated against N/50 H$_2$SO$_4$ until the pink colour just disappeared. The analysis was done at the spot immediately after sample collection.
Calculation:

\[
\text{Carbonate alkalinity} = \frac{\text{ml of titrant used \times 100}}{\text{ml of sample titrated}}
\]

(in ppm)

(3) Bicarbonate Alkalinity (Methyl Orange Alkalinity):

Two drops of methyl orange indicator was added to the solution in which the phenolphthalein alkalinity had been determined and titrated against N/50 H\(_2\)SO\(_4\) until the yellow colour changed to faint orange tint.

Calculation:

\[
\text{Bicarbonate Alkalinity} = \frac{\text{ml of titrant used \times 1000}}{\text{ml of sample titrated}}
\]

(4) Dissolved Oxygen:

For analysing dissolved oxygen Winkler (Azide Modification) method was used. The water samples were collected in 125 ml narrow mouthed glass stoppered bottles avoiding are bubbles. The samples was fixed at the spot immediately after collection. In 125 ml water sample one ml of MnSO\(_4\) solution was added just below the neck of the bottle and one ml of alkaline iodidazide solution at the surface. The content was thoroughly mixed by inverting and rotating the bottles several times for about 10 seconds and the precipitated samples were brought to the laboratory and dissolved by adding 2 ml of concentrated H\(_2\)SO\(_4\).

Fifty ml of this solution was then titrated with 0.025 N Sodium thiosulphate solution till it turns pale straw colour. Then one ml of freshly prepared starch solution was added and continued the titration until the blue colour disappeared.

Calculation:

\[
\text{Dissolved Oxygen} = \frac{\text{ml of Na}_2\text{S}_2\text{O}_3 \text{ used \times N \times 8 \times 1000}}{\text{ml of sample titrated}}
\]
(5) **Total Hardness** :

To a 50 ml water sample one ml of buffer solution and 4-5 drops of indicator (0.5 g of Eriochrome black T and 4.5 g of hydroxylamine hydrochloride in 100 ml of alcohol) was added. The sample then titrated against standard EDTA (4.0 g of analytical grade disodium EDTA and 0.1 g of Magnesium Chloride-hydrate in 750 ml of distilled water) until the wine red colour changed to purple blue.

**Calculation** :

\[
\text{Total Hardness in ppm} = \frac{\text{ml of titrant used} \times 1000}{\text{ml of sample titrated}}
\]

(6) **Calcium Hardness** :

To a 50 ml water sample 2 ml of 1 N sodium hydroxide and 0.2 g of Murexide indicator was added. The solution was then titrated with EDTA (as mentioned in total hardness) until the pink colour changed to purple.

**Calculation** :

\[
\text{Calcium Hardness in ppm} = \frac{\text{ml of titrant used} \times 1000}{\text{ml of sample titrated}}
\]

(7) **Magnesium Hardness** :

Determined by subtracting calcium hardness from total hardness.

(8) **Chloride** :

To a 5 ml water sample 2 ml of Potassium chromate reagent was added and the solution was titrated against 0.02 N Silver Nitrate solution until reddish tinge appeared.

**Calculation** :

\[
\text{Chloride in ppm} = \frac{\text{ml of titrant used} \times N \times 35.5 \times 1000}{\text{ml of sample titrated}}
\]
(g) **Sulphate**:  
Sulphate was determined turbidimetrically using 'SYSTRONICS' turbidity meter.

To a 50 ml water sample 2.5 ml of conditioning reagent (Glycerine, NaCl, HCl and alcohol solution) was added and the reading was taken on turbidity meter. The sample was then stirred on a magnetic stirrer and during stirring 0.2 g of BaCl₂ were added to the solution. After one minute of stirring the reading was taken on turbidity meter. The difference between initial and final reading was calculated.

The concentration of sulphate in mg l⁻¹ was calculated using standard curve prepared by standared solution using the same procedure as for sample. The standared curve was prepared for 0-40 mg l⁻¹ sulphate (standard solution of Na₂SO₄) at an interval of 5 ml l⁻¹.

(10) **Sodium**:  
Analysis by 'SYSTRONICS' digital Flame photometer.

(11) **Potassium**:  
By the same method as sodium.

(12) **Phosphate**:  
To a 40 ml filtered water sample in a 50 ml volumetric flask 5 ml of Molybdate-Antimony solution and 2 ml of 0.1 M Ascorbic acid was added. The volume was made up to 50 ml. After 10 minutes the extinction was measured on a Spectrophotometer at a wave length of 720 μm.

The concentration of phosphate in mg l⁻¹ was then calculated using standard curve prepared by using different concentrations of standard KH₂PO₄ solution.
(13) **Ammonia**:  
Ammonia was determined by distillation method using Parnas-Wagner nitrogen distillation apparatus. 25 ml of water sample was taken in the distillation chamber and 1 ml of buffer solution was added. About 40 ml of distillate was collected in 50 ml volumetric flask having 2.5 ml of 0.04 NH$_2$SO$_4$. The distillate was diluted to 50 ml and one ml of Nessler's reagent was added in the distillate. The colour extinction was measured on a spectrophotometer at a wave length of 380 µm.

The concentration of ammonia in mg/l$^{-1}$ was then estimated by standard curve.

(14) **Nitrate & Nitrate**:  
The same sample in which ammonia has been distilled about 0.2 g of Devarda's alloy and 5 ml of 5 N NaOH solution were added. The distillate was collected in a 50 ml volumetric flask containing 2.5 ml of 0.04 N H$_2$SO$_4$ as in ammonia method. In this procedure the NO$_2$ and NO$_3$ are reduced to NH$_3$ which is distilled and the sample was then processed as in ammonia distillation method.

(15) **Bio Chemical Oxygen Demand**:  
Water with an appropriate dilutions were incubated for 5 days in dark at 20°C in BOD incubator. Each dilution of sample were initially fixed. Titration analysis was made after 5 days following Winkler method. The reduction in DO concentration during the incubation period yield a measure of BOD.

(16) **Chemical Oxygen Demand**:  
After placing 4 gm of Mercuric sulphate (HgSO$_4$) in a
reflux flask, 20 ml of water sample added followed by 10 ml Potassium dichromate solution and 30 ml con. \( \text{H}_2\text{SO}_4 \) to this. It was mixed thoroughly and refluxed for 2 hours using Silver sulphate as a catalyst. The excess dichromate was titrated with standard Ferrous ammonium sulphate using Ferroin as indicator.

**BIOLOGICAL PARAMETERS :-**

(1) **Zooplankton :-**

The monthly quantitative zooplankton samples were collected by filtering the 100 liters of water through a plankton net made up of bolting silk having 100 meshes cm\(^{-1}\) and collected in polythene bottle of 100 ml. The samples were immediately fixed in 4 % formalin. The qualitative samples were collected by maintaining the plankton net in flowing water for 10 min or holding net in running boat across the transect of the river. The 50 % of the samples were fixed while 50 % brought live for examination of different taxonomic characteristics of the species. The qualitative samples gives an idea about the general composition and association of various planktonic organisms.

The counting of the zooplankters were done as per the method suggested by Welch (1952). As the random distribution exist within the shaken sample hence, for counting purpose 10 ml subsample were taken from thoroughly mixed sample. The subsample was concentrated with the help of centrifugation and 1 ml concentrated sample was counted in a Sidgewick-Rafter cell under binocular research microscope. The individuals were counted and multiplied by the concentration factor and result express per 100 liters. As per the need the sample were either diluted or
concentrated at different stations but result always expressed per 100 lit. The qualitative sample were examined three to four time so as to know the general composition of the zooplankton.


The identification of rotifera and cladocera were conformed by preparing the temporary mount in Polyvinyl Lactophenol tinted with lignin pink. Identification was done under higher magnification and diagnostic features were drawn with the camera lucida and measurements were taken using a calibrated ocular micrometer.

(2) Macrobenthos :-

The sampling of the zoobenthos were only possible during the summer particularly in April and May when water level reduces. However at pool zone N₅ Ekman grab sampler used but the zoobenthos were not recorded and quantification was not possible. The qualitative benthic samples were collected by disturbing the bottom where ever possible.

2.5 BIOTIC INDICES :

Some biological indices were calculated in order to know the changes in the community structure of the zooplankton assemblages at different stations of zone A. The biotic indices at different stations were calculated as below.
(1) Sorensen (1948) Similarity Index:

\[ S = \frac{2c}{a + b} \times 100 \]

s = similarity index

a = Number of species present at one station
b = Number of species present at another station
c = Number of species common to both station

(2) Shannon and Weaver (1963) Species Diversity Index:

\[ \bar{H} = \sum i \left( \frac{n_i}{N} \right) \log_2 \left( \frac{n_i}{N} \right) \]

OR

\[ \bar{H} = \sum p_i \log p_i \]

ni = Importance value for each species
N = Total number of individual
pi = Importance probability for each species.

The \( \log pi \) values were calculated on the basis of table for the calculation of the information theory of diversity index.

(3) Pielou (1966) Equitability Index:

\[ e = \frac{\bar{H}}{\log S} \]

e = Equitability index

(\( \bar{H} \)) = Species diversity index
S = Total number of individual

2.6 STATISTICAL ANALYSIS:

The statistical analysis were carried out for establishing the relationship between different hydrobiological characteristics and computed as per the method suggested by Sokal and Rohlf (1981).

(1) Analysis of Variance:

Single classification ANOVA with unequal sample size.
The use of ANOVA is to testify whether two or more sample means have obtained from observation with the same parameteric mean. Following formula was used for calculation:

Eq 1 - Grant total = (εX)
Eq 2 - Sum of the Square total = (εX)^2
Eq 3 - Sum of the square group each divided by sample size = (εX)^2/n_i
Eq 4 - Grant total square and divided by total sample size = (εX)^2/Σn_i
Eq 5 - S.S. group = Eq2 - Eq4
Eq 6 - S.S. group = Eq3 - Eq4
Eq 7 - S.S. group = Eq5 - Eq6

ANOVA Table

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Sources of variation</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Among stations</td>
<td>a - 1</td>
<td>Eq.6</td>
<td>6/a-1</td>
<td>M.S.gr M.S.Wt</td>
</tr>
<tr>
<td>2</td>
<td>Within months</td>
<td>n - a</td>
<td>Eq.7</td>
<td>7/ n-a</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Total</td>
<td>n- a</td>
<td>Eq.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a = Number of stations. n = Total number of observations.
d.f.= Degree of freedom.

The F.S. probability test applied as per following table.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Observations</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p &gt;</td>
<td>0.05 *</td>
</tr>
<tr>
<td>1</td>
<td>F (5:65)</td>
<td>2.4</td>
</tr>
<tr>
<td>2</td>
<td>F (8:67)</td>
<td>2.3</td>
</tr>
<tr>
<td>3</td>
<td>F (9:105)</td>
<td>1.8</td>
</tr>
</tbody>
</table>
(2) **Product-moment Correlation Coefficient** (p):

The degree of relationship between two variables is established by calculating a coefficient known as correlation coefficient (r). The correlation coefficient gives a quantitative measure of the degree of closeness of the linear relationship. It was calculated as follows.

\[
(p) = \frac{\Xi(y - \Xi x)\cdot(y - \Xi y)}{\sqrt{(\Xi x^2 - \Xi x\cdot\Xi x)(\Xi y^2 - \Xi y\cdot\Xi y)}}
\]

x and y are variables. The student t test was calculated directly from the (p) values suggested by Mishra and Mishra (1983) as below:

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Station</th>
<th>n</th>
<th>d.f.</th>
<th>0.05</th>
<th>0.01</th>
<th>0.001***</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,2,11,14,15,19</td>
<td>7</td>
<td>6</td>
<td>0.707</td>
<td>0.834</td>
<td>0.925</td>
</tr>
<tr>
<td>2</td>
<td>12,13,16,17,18</td>
<td>8</td>
<td>7</td>
<td>0.666</td>
<td>0.798</td>
<td>0.898</td>
</tr>
<tr>
<td></td>
<td>7,8,9,10.</td>
<td>11</td>
<td>10</td>
<td>0.576</td>
<td>0.708</td>
<td>0.823</td>
</tr>
<tr>
<td>4</td>
<td>3,4,5,6.</td>
<td>12</td>
<td>11</td>
<td>0.553</td>
<td>0.684</td>
<td>0.801</td>
</tr>
</tbody>
</table>

(3) **Linear Regression**:

The study of simultaneous variation of two variables or the dependance of one variable on the other involves the study of regression analysis. The regression analysis was carried out between zooplankton (y) and abiotic characteristics (x).
Regression coefficient \( b = \frac{\text{(\( \leq \) \( xy \) - \( x \leq y \))}}{\text{(\( \leq y^2 \) - \( y \leq y \))}} \)

Regression equation \( Y = a + b \times x \)

\( a = y \) intercept \( x \) and \( y \) are variables.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>n</th>
<th>df</th>
<th>0.05 *</th>
<th>0.01 **</th>
<th>0.001 ***</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>9</td>
<td>1.83</td>
<td>2.80</td>
<td>4.50</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>10</td>
<td>2.20</td>
<td>2.50</td>
<td>3.80</td>
</tr>
</tbody>
</table>