MATERIALS AND METHODS
A. Physico-chemical characterization of water

For physico-chemical analysis, samples were collected from the selected sites of the rivers on a monthly interval for two years from March, 1991 to February, 1993. Seven sampling sites were selected to cover the four rivers as shown in the figure (III-2). Composite surface water samples were collected using one litre polyethylene bottles and 250 ml glass bottles. All samplings were made before noon in the first week of every month. The samples were brought to the laboratory as early as possible and analyzed for its physico-chemical properties. The following parameters were analysed using the standard methods for examination of water and waste water (APHA, 1976) and as compiled by Trivedi and Goel (1984).
1. Temperature

Water temperature was recorded with centigrade thermometer in °C (1/10 divisions) at the site.

2. pH

The pH was measured by ELICO LI 10 T pH meter in laboratory by standardizing the pH meter with a buffer of known pH.

3. Total solids (T.S.)

In pre-weighed clean porcelain dish, 100 ml, unfiltered sample water was evaporated to dryness, on a boiling water bath. The amount of total solids was calculated from the difference of pre-weighed porcelain and the final weight of the porcelain, and is expressed as mg/1.

4. Dissolved Oxygen (D.O.)

The unfiltered samples were collected in 250 ml glass stoppered B.O.D. bottles. The sample was fixed at the site by adding 2 ml MnSO₄ (100 gm MnSO₄ in 200 ml d.w.) and 2 ml alkali iodide azide solution (100 gm KOH and 50 gm KI in 200 ml d.w.). The samples were then transported to the laboratory. The precipitate formed was dissolved by adding 2 ml Con. H₂SO₄. The
sample was titrated against 0.025 N sodium thiosulphate solution using starch (1gm in 100ml d.w.) as indicator. The end point was noted and calculated according to the following formula.

\[
\text{D.O. mg/l} = \frac{(\text{ml} \times \text{N}) \times \text{B} \times 1000}{\text{V} - \text{v}}
\]

\( V = \) volume of the sample bottle after placing the stopper

\( v = \) volume of MnSO₄ and KI added

5. Total Alkalinity (T.A.)

It was estimated by titrating the sample with 0.1 N HCl using methyl orange as indicator. At the end point the colour changes from yellow to pink. Total alkalinity was calculated using the following formula.

\[
\text{T.A. as CaCO₃ mg/l} = \frac{\text{(volume} \times \text{normality}) \times \text{HCl} \times 1000 \times 50}{\text{ml of the sample}}
\]

6. Hardness

To 50 ml of the filtered sample 1 ml buffer solution [(A) 16.9 gm ammonium chloride in 143 ml concentrated ammonium hydroxide, (B) 1.79 gm disodium EDTA and 0.78 gm MgSO₄.7H₂O in 50 ml d.w.; A & B were mixed and made upto 250 ml.] and 100-200 mg of Erichrome Black T indicator (0.40 gm of Erichrome Black T was grinded with 100 gm NaCl) were added and it was then titrated.
against 0.01 M E.D.T.A. solution. At the end point the colour changes from wine red to blue. Calculations were made using the following formula.

\[
\text{Hardness as mg/l CaCO}_3 = \frac{\text{ml EDTA used} \times 1000}{\text{ml sample}}
\]

7. Chloride

To 50 ml filtered sample, 2 ml of 5% \( K_2CrO_4 \) was added and titrated with 0.02 N AgNO\(_3\) until a persistent reddish brown tinge appeared. Calculations were made using the formula.

\[
\text{Chloride mg/l} = \frac{(\text{ml} \times N) \text{ of AgNO}_3 \times 1000 \times 35.5}{\text{ml sample}}
\]

8. Phosphate (Inorganic phosphorous)

To a known aliquot of filtered sample 2 ml of ammonium molybdate solution (\( a - 2.5 \text{ gm ammonium molybdate in } 17.5 \text{ ml d.w.}, \ b - 28.0 \text{ ml con. } H_2SO_4 \text{ to } 40.0 \text{ ml d.w.; } a \text{ and } b \text{ were mixed and made upto } 100 \text{ ml with d.w.} \) and 5 drops of stannous chloride reagent (2.5 gm stannous chloride in 100 ml glycerol) were added. The optical density of the blue colour developed was immediately recorded at 690 nm. A standard curve was prepared, using anhydrous potassium hydrogen phosphate (\( K_2HPO_4 \)). The values were calculated and expressed as mg/l.
9. Sulphate

To 50 ml of the sample 2 ml conditioning reagent (70 gm NaCl, 30 ml con. HCl, 100 ml 95% isopropyl alcohol, in 300 ml d.w. + 50 ml glycerol) and a pinch of Barium chloride crystals were added and stirred by using a magnetic stirrer. The reading was noted at 420 nm, exactly after 4 minutes. A standard curve was prepared using Na$_2$SO$_4$ as standard. The values were calculated and expressed as mg/1.

10. Nitrate (Nitrate Nitrogen)

To an erlenmeyer flask 50 ml of the filtered sample was taken. A known amount of silver sulphate solution (0.44 gm AgNO$_3$ in 100 ml d.w.) equivalent to chloride was added; (1mg/l Cl = 1ml AgSO$_4$) and the precipitated silver chloride was removed by filtration. The filtrate was then evaporated to dryness in the porcelain dish, on a waterbath. The residue was dissolved in 2ml phenol disulphonic acid (25 gm phenol dissolved in 150 ml concentrated sulphuric acid and 75 ml fuming sulphuric acid was added) and the volume made up to 50 ml with d.w. About 6 ml of liquid ammonia solution was added to develop yellow colour. The readings were taken at 410 nm. A standard curve was prepared using KNO$_3$ as standard. The values were calculated and expressed in mg/1.
11. Reactive silica

To 50ml of the filtered sample, 1 ml of HCl (1+1 with d.w.) 1.5 ml of oxalic acid (10 gm oxalic acid in 100 ml d.w.) and 2 ml ammonium molybdate (10 gm Ammonium molybdate in 100 ml d.w.) solutions were added. The optical density was noted at 410 nm. The values were calculated from the standard curve which was prepared using Na₃SiF₆ as standard and expressed as mg/l.

12. Calcium

To filtered 50 ml sample 100 mg meruxide indicator (0.2 gm ammonium purpurate grinded with 100 gm NaCl) was added to develop a pink colour. It was then titrated against 0.01 M EDTA solution. At the end point, the pink colour, changed to dark purple. The readings were noted and calculated as follows.

$$\text{Calcium mg/l} = \frac{\text{ml of EDTA used} \times 400.8}{\text{ml of sample}}$$

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

Since the D.O. of the sample is likely to be exhausted, suitable dilutions of the sample was made, as per the table given below, with well aerated distilled water.
Table for sample dilution, to bring it to the appropriate B.O.D. range.

<table>
<thead>
<tr>
<th>Expected range of BOD, mg/l O₂</th>
<th>Dilution</th>
<th>Sample volume in 1 litre of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 6</td>
<td>No dilution</td>
<td>1000</td>
</tr>
<tr>
<td>4 - 12</td>
<td>50</td>
<td>500</td>
</tr>
<tr>
<td>10 - 30</td>
<td>20</td>
<td>200</td>
</tr>
<tr>
<td>20 - 60</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>40 - 120</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>100 - 300</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>200 - 600</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

The samples were diluted accordingly to appropriate BOD range. 1000 ml of the diluted sample was taken in a large glass trough and 1 ml each of phosphate buffer of pH 7.2 (0.05 gm KH₂PO₄, 2.17 gm K₂HPO₄, 334 gm Na₂HPO₄.7H₂O and 0.17 gm NH₄Cl in 100 ml d.w. and the pH was adjusted to 7.2) magnesium sulphate (8.25 gm MgSO₄.7H₂O in 100 ml d.w.), Calcium chloride (2.75 gm CaCl₂ in 100 ml d.w.) and ferric chloride (0.025 gm FeCl₃.6H₂O in 100 ml d.w.) were added, and mix thoroughly. The pH was then neutralized to 7.0 using 1N NaOH/H₂SO₄. The sample was then filled in 2 sets of BOD bottles. One set was kept in BOD incubator at 20±2°C for 5 days and in the second set D.O. was determined.
immediately. The first set which was kept in the incubator was taken out soon after the completion of 5 days and the D.O. was measured. The BOD was calculated as follows.

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

where \( D_0 = \) Initial D.O. of the sample

\( D_5 = \) D.O. after 5 days

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

20 ml sample was taken in 250ml C.O.D. flask and 10ml 0.25 N potassium dichromate reagent was added. To it a pinch of \( \text{Ag}_2\text{SO}_4 \) and \( \text{HgSO}_4 \) were added. Thereafter 30ml sulphuric acid was added and the mixture was refluxed for 2 hours on a heating mantle with heibg reflux condensor. The flasks were then cooled and the volume was made to 140 ml with distilled water. It was then titrated against 0.1 N ferrous ammonium sulphate using feroin indicator (1.48 gm 1,10 phenonthroline and 0.675 gm ferrous sulphate in 100 ml d.w.). A blank was prepared using distilled water instead of sample using the same amount of chemicals. The COD was calculated as follows.

\[ \text{COD mg/l} = \frac{(b-a) \times N \times K_2\text{Cr}_2\text{O}_7 \times 1000 \times B}{mL \text{ sample}} \]

where \( a = \) ml of the titrant with sample

\( b = \) ml of the titrant with blank.
B. Phycological characterization

For phycological evaluation of various habitats algal samples along with water samples were collected every month, from and around the sampling sites. The algal samples were also collected from nearby soils enriched by river water. Algal samples from both the soil and water were collected carefully. For better representation, the sampling was done from different places of the site. The samples were collected in plastic bottles with proper labelling of the sites. After bringing to the laboratory they were preserved in 4% formaldehyde solution. The camera lucida sketches of almost all possible taxa were made and they were identified using standard monographs and published literature (Fritch 1935, Smith, 1950, Desikachary, 1959, Prescott, 1962, Philipose, 1967, Iyengar and Desikachary, 1981, Adoni, 1985, Patel, 1989).

Pollution Indices

1. Trophic State Indices

Nygaard's (1949) trophic state indices were determined to find the trophic status of all the rivers. For calculation of the indices the number of genera in each group of algae were determined for all the sites. The members of Cyanophyceae, Chlorococcales, Desmids, Euglenophyceae and Bacillariophyceae were
scored to determine the trophic state indices. The oligotrophic or eutrophic state was ascertained as per the original ratio provided in the table (Nygaard, 1949).

The ratio of the groups were calculated for all the sites and were used as biological indices of water quality. From the values, the degree of eutrophication was recorded for all the rivers at different sites. Comparison of indices for different sites were also made.

**Nygaard's trophic state indices (Nygaard, 1949)**

<table>
<thead>
<tr>
<th>Index</th>
<th>Calculation</th>
<th>Oligotrophic</th>
<th>Eutrophic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myxophycean</td>
<td>Myxophyceae</td>
<td>0.0-0.4</td>
<td>0.1-3.0</td>
</tr>
<tr>
<td></td>
<td>Desmidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophycean</td>
<td>Chlorococcales</td>
<td>0.0-0.7</td>
<td>0.2-9.0</td>
</tr>
<tr>
<td></td>
<td>Desmidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diatom</td>
<td>Centric diatoms</td>
<td>0.0-0.3</td>
<td>0.0-1.75</td>
</tr>
<tr>
<td></td>
<td>Pennate diatoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euglenophyte</td>
<td>Euglenophyta</td>
<td>0.0-0.2</td>
<td>0.0-1.0</td>
</tr>
<tr>
<td></td>
<td>Myxophyceae +</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chlorococcales</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Myxophyceae +</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chlorococcales +</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Centric diatoms +</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Euglenophyta</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Desmidae</td>
<td>0.0-1.0</td>
<td>1.2-2.5</td>
</tr>
<tr>
<td>Compound</td>
<td>Desmidae</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2. Palmer's Algal Pollution Index

Palmer's pollution index (1969) based on genera and species were used for rating water samples for high or low organic pollution. Two indices were given — one for algal genera and other for algal species. About 20 most frequent and pollution tolerant genera and species were taken into account. He assigned each genus or species a pollution index value from 1 to 6. The value is assigned to each of them depending on their relative tolerance. The number scored by each genus or species is totalled to get the value of the pollution indices. A score of 20 or more for a sample is the confirmation of high organic pollution, while a score of 15-19 is taken as possible evidence of high organic pollution a score of 10-14 indicates moderate pollution and lower values indicates the lack of organic pollution.

For rating the samples, as high or low organically polluted, observations were made according to Palmer (1969) from all the sites of the different rivers. The most pollution tolerant genera and species of algae were listed from all the sites. Comparison of scores was made for all the sites.

C. Statistical Synopsis

A multifactorial correlation of the data for the year 1991-92 was made to study the inter-relationship of the various
physico-chemical parameters of water. The mean and standard deviation were also calculated for showing general statistical relations (Misra and Misra, 1983).

D. Toxicity testing of Khari River sample

Among the various rivers studied river Khari is highly polluted. This river carries the wastes from various industries of Vatva G.I.D.C., Ahmedabad. The toxic effect of this river water sample was studied on various test organisms in laboratory conditions.

Fresh Khari river water was collected in 10 litre plastic cans, and transported to the laboratory. To study its toxic effect a serial dilution of the water was prepared by diluting the sample water with distilled water. The various dilutions prepared were as follows.

<table>
<thead>
<tr>
<th>% Dilutions</th>
<th>Water (ml)</th>
<th>Khari River sample (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>15</td>
<td>85</td>
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</tr>
<tr>
<td>20</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>25</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>75</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>100</td>
<td>00</td>
<td>100</td>
</tr>
</tbody>
</table>
To test the toxicity of Khari river water, organisms from different trophic levels were selected. The following test organisms were used.

1. Cyanobacteria (*Anacystis nidulans*)
2. Seedlings of the higher plants (*Bajra, Radish and Mustard*)
3. Zooplankton (*Daphnia*)
4. Fish (*Puntius sps.*)

An isolate of Cyanobacteria (Blue-green algae), *Anacystis nidulans* was used B.G.-11 media (Stainer et al., 1971) was used for the growth and maintenance of the alga. The glassware and culture media were sterilized in autoclave at 15-lb pressure for 15 minutes. Inoculation and transfer of the cultures were carried out under aseptic conditions on laminar flow (Klenzoids). The cultures were grown in culture room under 3000 lux light intensity provided by day light fluorescent tube for 12 hours a day at 28±2°C.

A serial dilution of the sample with culture medium and distilled water were also made. The growth was measured by cell counts at an interval of 3 days for a period of 12-days. Haemocytometer was used for counting the number of cells.

A homogenous inoculum of exponentially growing algal cells from the stock culture was inoculated in the serial
dilutions. The total volume of the medium in each tube was kept 20 ml. All the treatments were done in triplicates and the average of the three values were taken into consideration.

2. Rooted Vascular Plants

Seeds of three crop plants, Bajra (Pennisetum americanum) Mustard (Brassica juncea) and Radish (Raphanus sativus) were selected for the study. Certified seeds were procured from the seed market. Seeds of average size were grown in filter paper lined petridishes. Seeds soaked in dilution series were allowed to germinate in the petridishes. The percent germination was noted every day for a period of 7 days. The emergence of radicle was taken as the criterion for germination. After 7 days of growth, the growth parameters viz. Root length, shoot length, fresh weight and dry weight were measured. The seedlings grown in distilled water was taken as control. All the treatments containing 20 seeds were given in triplicates, and the average was taken for consideration. The effect is described as LC-50 the concentration that would inhibit the growth by 50 percent.

3. Daphnia

A crustacean, the widely used Zooplankton, Daphnia, was selected for the present study. It was collected from the nearby freshwater ponds of Vallabh Vidyanagar. Daphnia of the same size
numbering ten were introduced in dilution series containing 200 ml of the test solution in each flask and the E.C.-50 was calculated. All the treatments were given in triplicate and the average value was taken into consideration.

4. Fish

4.1. Acute Toxicity Test

Puntius (sps.) a commonly available fish was selected for the present work, were procured from the local fish farm. They were maintained in large artificial pond with tap water and fed by commercially available fish food. Acute toxicity tests were conducted in glass troughs of 8 litres capacity, in laboratory, for a period of 96 hours. Five litres of the serially diluted sample was taken in two replicates. To each troughs 10-fishes averaging 6.4 cms length were introduced into it. The water was replenished everyday with freshly collected samples. Behavioural responses and L.C.-50 values were noted.

Physico-chemical properties of the raw sample and diluted sample were also carried out.

4.2. Chronic Toxicity Test

To study the chronic effects of the sample, the fish were exposed to sublethal and lethal concentrations for a period
of 30 days. The selected sample concentration being highly acidic, two series of experiments were conducted. In one series the pH of diluant around 7.0 and it was then aerated for two hours to bring the D.O. concentration around 5.0 mg/l, while in second series sample water was not adjusted. After an exposure of 30 days the fish were killed and biochemical constituents of the body muscle were estimated. The following constituents were measured.

4.2.1. Glycogen

The total glycogen was estimated following the method of Carrol et al. (1956). A known amount of the tissue was homogenised with 5% T.C.A. (Trichloroacetic acid) in a mortar and pestle and the homogenate was centrifuged. The procedure was repeated twice with the residue for complete extraction, and the final volume was made up to 10 ml with T.C.A. From this 1.0 ml was pipetted out into a test tube; 5 ml 95% ethanol was added, and mixed thoroughly. The tubes were then capped with clean rubber stoppers and placed in a water bath at 37 to 40°C for 3 hours. The glycogen was precipitated and it was then centrifuged at 3000 rpm, for 15 minutes. The residue was dissolved in 2 ml d.w. and used for the estimation. A reagent blank was prepared by pipetting 2 ml d.w. instead of sample and standard was run containing 2 ml standard glucose solution having 0.1 mg of glucose, in the tube. To each tube 10ml anthrone reagent (0.05%
anthrone and 1% thiourea in 72% sulphuric acid) was added and mixed thoroughly. The tubes were then recaped and placed in cold water bath. After cooling, tubes were transferred to a boiling water bath for 15 minutes. After cooling the readings were noted at 620 nm. The glycogen was calculated using the following formula and expressed as mg/gm fresh weight.

\[
\text{D.S.} \quad \text{Vol. of the extract} \quad \frac{\text{---} \times 0.1 \times \text{------------------} \times 0.9}{\text{D.U.} \quad \text{gm. of tissue}}
\]

where, D.U. - O.D. of the unknown sample
D.S. - O.D. of the standard

0.1 - glucose in mg. present in 2 ml of the standard solution

0.9 - Factor to convert glucose value to glycogen value

4.2.2. Protein

Total protein was estimated following the method of Lowry et al. (1951). The known amount of tissue was homogenised and precipitated with 5% T.C.A. It was then dissolved in 0.1 N NaOH and made upto a known volume. To the sample aliquots 5 ml of reagent C [(A) 4% Na₂CO₃, (B) 0.5% CuSO₄ in 1% CaH₄KNaO₆.4H₂O, Reagent C was prepared freshly by mixing A and B in the ratio 50:1] and kept for 10 minutes at room temperature. After 10 minutes 0.5 ml Folin Phenol reagent (commercially available
reagent diluted to 50% with d.w.) was added and mixed thoroughly. It was then kept 30 minutes for the development of colour. The O.D. was noted at 660 nm. The values were extrapolated to a standard curve prepared using vitamin free casein as standard and expressed as mg/gm fresh wt.

4.2.3. Lipids

Total lipid was estimated following the method of Bradgon (1951). It was extracted in 2:1 chloroform : methanol mixture. Known volume of the sample aliquots were transferred to 25 ml volumetric flask and the solvent was evaporated. 10ml dichromate reagent (2 gm K$_2$Cr$_2$O$_7$ in 100 ml con. H$_2$SO$_4$) was added to it and the flasks were stoppered and sealed with a drop of reagent. It was then kept in boiling water bath for 30 minutes. A reagent blank was prepared by pipetting same volume of chloroform-methanol mixture in place of aliquots with sample. The flasks were then cooled and the final volume was made up with d.w. at 25°C. The absorbance was recorded at 660 nm. The calculations were made from the standard curve prepared using Palmitic acid as standard and expressed as mg/gm fresh weight.