CHAPTER III
EXPERIMENTAL
COLLECTION AND ANALYSIS OF WATER SAMPLES -

The prime objective in any analytical monitoring programme is to obtain analytical data which clearly depicts the system being studied. Thus, the basic objective of analysis of various types of water samples collected is to assess the potential in a region taking into consideration both quality and pollution aspects. This chapter deals with the series of selected methods (conventional and instrumental) for determining major and minor constituents of great interest and also covers general discussion of sampling and pretreatment with all possible information on methodology.

The analysis of water samples starts from the point of its collection and accuracy of the analysis data depends to a considerable extent upon the precautions taken at the time of collection of water samples. Accordingly before proceeding with discussions of methods for analysis, a brief reference is made to the method adopted for collection of water samples and changes taking place in water samples during storage and transport.

[1] LOCATION OF SAMPLING POINTS

In order to investigate water quality and water pollution in the river 'Pandu' water samples were collected from shallow, medium and deep aquifers where the chances of pollution are maximum.

Water samples were also collected from the river 'Pandu' at different points each at approximate
distance of 2.0 to 4.0 Kms. Water samples were taken from the mid stream as far as possible. In a few cases, however, it was not possible to go to mid stream and in such cases, samples were collected a few meters away from the bank of the river to minimise the effect of any local pollution.

[II] COLLECTION OF WATER SAMPLES

Water samples were collected and analysed to ascertain characteristic of a body or mass of water. The sample is usually only a small part of the total volume and is, therefore, representation of the total mass only to the degree that uniformity of chemical composition exists within the total mass. In their natural state, surface and ground water are subjected to forces that promote mixing and homogeneity. The fact that such tendencies exist, however, is not sufficient for assuming that a body of water is so well mixed that no attention to sampling technique is required. Often, because of local conditions, the body of water may not have uniform composition.

The composition of water is also subject to change with the passage of time. The chemical quality of surface water is the resultant of the geologic, hydrologic, biologic and cultural environment of the water and varies from time to time as well as from place to place. Generally, changes in the quality of surface water are more pronounced and rapid than in ground water.

The type of investigation, purpose of the study and anticipated variations in chemical quality determine to a large degree the locations of the surface or ground water sampling site and the frequency of sample collection.

Care is taken to obtain a sample that is truly representative of existing conditions and to handle it in such a way that it does not deteriorate or become contaminated before it reaches the laboratory. The details of collection vary so much with local conditions that no specific recommendation would be universally applicable.

Sampling at the first stage was performed to study the variability in the physical constants and chemical characteristics used as a measure of water quality. Collection of water samples was made for the period 1990 to 1992 in different seasons. Grab surface water samples were taken at the depth of six inches below the surface of the stream, near the left bank of the river.

Separate samples from each sampling station (shown in Fig. 1 and Table 3) were collected initially for the measurement of pH, temperature, electrical conductance, dissolved oxygen, five days biochemical
oxygen demand (BOD at 20°C), chemical oxygen demand, ammonical - nitrogen, nitrate- and nitrite-nitrogen organic nitrogen, total free available chlorine, sulphide, cyanide and oil and grease.

Sampling was usually carried out once in a month. Samples from the waste-water outfalls joining the river, were taken simultaneously once in a month at the source and just before joining the river. Discharge rate of the waste-water outfalls was subsequently measured at the time of sampling.

A sample volume of 300 ml for DO, 500 ml each for residual chlorine, ammonical-nitrogen, metals, cyanide and phenol, 1000 ml each for BOD, oil and grease, 100 ml each for COD, nitrate- nitrogen, nitrite-nitrogen and sulphide estimation was collected.

Narrow mouthed ground stoppered glass bottles of 300 ml were employed for dissolved oxygen measurement. Samples for heavy metal estimation were taken in polythene container and/or glass bottles prewashed with 1:1 HNO3. Amber coloured glass bottles were used for the residual chlorine.

Water samples were collected by simply dipping up the container below the surface of the stream (at the depth of 6" to 8" cms.) and then destoppering the same, when the bottles got completely filled against the direction of the flow of the water, the bottles were stoppered and taken out from the stream.

Important factors affecting the results are the presence of turbidity, the physical and chemical changes from the about by storage or aeration prior to analysis and the method used for its removal. Temperature, pH and electrical conductivity changes significantly in a matter of minutes, dissolved gases may be lost or gained. Therefore, these determinations were carried out at the spot.

[III] PRESERVATION OF SAMPLES

Immediately after the collection of the samples, it was made possible to measure the water quality non-specific characteristics such as pH, temperature and electrical conductivity. Measurement of other characteristics subject to the chemical or physical change, was subsequently followed. In fact it was not possible to carry out the analytical estimation of all the parameters at a time. Therefore, standard procedures (physical and chemical) were employed to preserve the samples at least for 24 to 36 hours. The analysis was completed without further delay.

Details of the maximum storage time and methods used for the preservation of the samples, wherever
necessary, are given in Table 4. In case of waste-water samples it was considered necessary to prevent the possible physico-chemical changes. Accordingly samples were kept in frozen state (0-4°C) to inhibit such changes.

Table 4

Preservation of the water samples.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Measurement</th>
<th>Storage and Preservation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>pH, temp., Electrical conductance and total Hardness</td>
<td>Measured immediately</td>
</tr>
<tr>
<td>2.</td>
<td>Dissolved oxygen</td>
<td>Measured as soon as possible</td>
</tr>
<tr>
<td>3.</td>
<td>BOD at 20°</td>
<td>6 hrs at 4°C</td>
</tr>
<tr>
<td>4.</td>
<td>COD</td>
<td>Additional at H₂SO₄ to pH 2</td>
</tr>
<tr>
<td>5.</td>
<td>Total residual chlorine</td>
<td>Analysed immediately</td>
</tr>
<tr>
<td>6.</td>
<td>Ammonia – Nitrogen</td>
<td>Addition of 0.8 ml Conc. H₂SO₄/lit. of sample store at 4-10°C.</td>
</tr>
<tr>
<td>7.</td>
<td>Nitrite – Nitrogen</td>
<td>Stored at 20 to 25°C</td>
</tr>
<tr>
<td>8.</td>
<td>Nitrate – Nitrogen</td>
<td>Addition of Mercuric chloride as 40 mg./lit. of sample.</td>
</tr>
<tr>
<td>9.</td>
<td>Sulphide</td>
<td>Addition of few drops of 2.0 M Zn (CH₃COO)₂/100 ml of sample.</td>
</tr>
<tr>
<td>10.</td>
<td>Phenol</td>
<td>24 hrs., addition of H₃PO₄ to pH ≤ 4.00 and 1.0 g. of CuSO₄. 5H₂O/lit. of sample, stored at 20°C.</td>
</tr>
<tr>
<td>11.</td>
<td>Heavy Metals</td>
<td>Samples were filtered immediately after the collection for measurement of dissolved portion and then preserved by the addition of 5 ml. of concentration HNOP₃/lit. of sample.</td>
</tr>
<tr>
<td>12.</td>
<td>Cyanide</td>
<td>24 hrs., addition of NaOH to pH 12 to check hydrolysis kept at 10 to 15°C.</td>
</tr>
<tr>
<td>13.</td>
<td>Oil and Grease</td>
<td>Addition of hydrochloric acid to pH ≤ 2.0.</td>
</tr>
</tbody>
</table>
In case of waste-water samples it was considered necessary to prevent the possible physico-chemical changes such as absorption of dissolved oxygen, hydrolysis of urea to ammonia, oxidation of ammonia to nitrite and nitrate and reduction of nitrate to gaseous nitrogen. Accordingly samples were kept in frozen state (0-4°C) to inhibit such changes.

[IV] MEASUREMENTS OF PHYSICAL CHARACTERISTICS

Analytical procedures mentioned in standard methods for the examination of water and waste-water were followed for the measurement and estimation of various parameters reported in this thesis. All the reagents and colorimetric solutions were prepared and purified according to these procedures. Reagents were prepared by using A.R. or G.R. grade chemicals.

The methods adopted are presented in Table 5 and their necessary details are briefed here.

Table - 5

Methods adopted for the analytical estimation of different species.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Determination</th>
<th>Method and limit of determination, if any</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>pH, electrical conductance</td>
<td>Electronic measurement</td>
</tr>
<tr>
<td>2.</td>
<td>DO</td>
<td>Winkler method (iodometric)</td>
</tr>
<tr>
<td>3.</td>
<td>BOD</td>
<td>By difference in DO level before and after incubation.</td>
</tr>
<tr>
<td></td>
<td>COD</td>
<td>Refluxing with K2Cr2O7 and subsequent titration with ferrous ammonium sulphate</td>
</tr>
<tr>
<td>3.</td>
<td>Ammonical Nitrogen</td>
<td>(i) Titration (5 mg. lit) (ii) Nesslerization (mg. lit)</td>
</tr>
<tr>
<td>5.</td>
<td>Total residual Chlorine</td>
<td>Iodometric</td>
</tr>
<tr>
<td>7.</td>
<td>Metals</td>
<td>Colorimetric</td>
</tr>
<tr>
<td>8.</td>
<td>Cyanide</td>
<td>Colorimetric</td>
</tr>
<tr>
<td>9.</td>
<td>Oil and Grease</td>
<td>Solvent extraction</td>
</tr>
<tr>
<td>10.</td>
<td>Total hardness</td>
<td>E.D.T.A. Titration</td>
</tr>
<tr>
<td>10.</td>
<td>Phenol</td>
<td>Colorimetric</td>
</tr>
</tbody>
</table>
Physico-chemical analysis of water -

(a) Analysis at the source:

(i) Water and air temperatures were measured with a mercury thermometer graduated upto 100°C. Water temperature was noted at about 2-3 inches below the surface water at the time of collection.

(ii) Transparency of water was measured with a Sacchi disc of 20 cms. as recommended by Welch (1948) and Misra (1964). The result was expressed in terms of depth in centimeters.

(iii) The hydrogen ion concentration of water was determined with BDH narrow range universal indicator papers.

(iv) The dissolved oxygen was fixed separately in a special narrow neck stoppered BOD bottle of about 300 ml. capacity by Alsterberg (Sodium Azide) modification method. Care was taken not to aerate the sample through bubbling both at the time of its sampling and fixation of oxygen. Its determination was completed immediately after reaching to the laboratory.

(b) Analysis in the laboratory:

A number of parameters were investigated according to the methods given below:

Turbidity of the water was measured with Toshniwal Turbidity meter.

Dissolved oxygen (DO) was estimated both in water and waste-water samples by following winkler method and its azide modification to minimize the interference due to nitrite-nitrogen and iron etc. The method is based on the principle that dissolved oxygen forms the precipitate of polymerichydroxides of manganese with mangenese sulphate and potassium iodide-sodium hydroxide solutions, which on acidification gets dissolved and liberates free iodine. The iodine is proportional to the DO level and is measured titrimetrically.

Five days biochemical oxygen demand (BOD) measured at 20°C, was determined by the difference in the DO level, before and after the incubation of the samples. BOD incubator (Tempo-automatic, Bombay) was used for the incubation purpose. Fully saturated oxygen demand values were determined
by making the appropriate dilutions of the samples. BOD dilutions were made with properly seeded standard dilution water. Activity of nitrifying bacteria was checked by the addition of mercuric chloride solution to minimise the nitrogenous BOD contribution. Permanganate modification of the above procedure was used to remove the interference by ferrous iron whenever required. Samples with high alkalinity or acidity were preneutralized to pH 7.0. Residual chlorine, possibly present in the samples was destroyed by adding sodium thiosulphate.

Following formula was used for BOD and IOD measurements:

BOD, where seeding was not done

\[
\text{mg/lit. BOD} = \frac{D_1 - D_2}{P}
\]

and with seeded dilution water

\[
\text{mg/lit. BOD} = \frac{(D_1 - D_2) - (B_1 - B_2) f}{P}
\]

Where,

- \(D_1\) = Initial DO of diluted sample
- \(D_2\) = DO of diluted sample after 5 days incubation
- \(B_1\) = DO of dilution of seed control before incubation
- \(B_2\) = DO of dilution of seed control after incubation
- \(P\) = Fraction of the sample
- \(f\) = Ratio of seed in sample to seed in control

Seed correction = \((B_1-B_2) f = \frac{\%\text{ seed in } D_1}{\%\text{ seed in } D_2}\)

Chemical oxygen demand (COD) estimation was made by refluxing the water and waste-water samples with standard potassium dichromate solution in acid medium. Ammonia free or released from organic compounds such as urea and proteins etc. is not oxidised by this method. In fact all the carbonaceous portion of the nitrogenous compounds is oxidised by such treatment. Thus, addition of silver sulphate was required as a catalyst in order to oxidise the aliphatic and aromatic hydrocarbons and pyridine etc. which might be present in the samples. Interference due to chloride was removed by the addition of mercuric sulphate and that due to nitrites was removed by the addition of sulphanilic acid.

During COD determination the volumes of the water of waste-water samples and that of the reagents were taken so as to maintain a definite ratio between them. This is evident from the following table.
After the sample treatment as above, the excess dichromate was titrated against standard ferrous ammonium sulphate and COD values were calculated by the formula—

\[
\text{mg/l. COD} = \frac{(a - b \times N \times 8000)}{\text{ml of the sample}}
\]

\[
a = \text{ml of Fe. amm. sulphate used for blank}
\]
\[
b = \text{ml of Fe. amm. sulphate used for sample}
\]
\[
N = \text{Normality of Fe. amm. sulphate}
\]

### [V] MEASUREMENTS OF SPECIFIC CHEMICAL CHARACTERISTICS

**Nitrogen (ammonical-, nitrite-, nitrate- and organic-) :**

Residual chlorine if present was destroyed immediately after collection of the samples. Preliminary distillation of the water and waste-water samples was done at pH 9.5 to overcome the interference due to colour, turbidity and the presence of substances such as magnesium and calcium. Subsequently, ammonia was determined in the distillate collected in boric acid solution by nesslerisation (colorimetric) and/or by titration with standard sulphuric acid titrant (0.02N) depending upon the concentration range. Colorimetric estimation was done on Beckman spectrophotometer (model DU-21) over a range of 400-500 nm.

Nitrogen as nitrite was determined spectrophotometrically through the formation of a redish purple dye produced at pH 2.0-2.5 by the coupling of diazotized sulphanilic acid with n-(1-naphthyl)-ethylenediamine dihydrochloride. Absorption Measurement was made at 545 nm.
Nitrate-nitrogen was estimated by nitrate-electrode method. Possible interference by bicarbonate and chloride was removed by adjusting the samples to pH 4.0-4.5 and adding silver sulphate, respectively. Nitrate-ion selective electrode (Orien Model-92-08) in combination with double junction reference electrode was used for the estimation.

Organic-nitrogen was obtained as the difference between the total kjeldahl nitrogen and ammonical-nitrogen determined for the samples.

**Total Residual Chlorine:**

Estimation of total residual chlorine was made separately in water and waste-water samples. Iodometric titration was performed for the determination and standard phenylarsine oxide solution was used as reducing agent. Unreacted reagent remaining in the solution was titrated amperometrically with standard potassium iodide-iodine (0.025N) reagent. Interference by manganese, iron and nitrite etc. was removed by reducing the pH of the samples between 3.5-4.3. Amperometric cell used to detect and point consisted of a non-polarizable reference electrode emmersed in salt solution and readily polarizable noble metal electrode in contact with the salt solution and the sample to be titrated. Micro-ameter was employed for the measurement purpose.

**Sulphide:**

Sulphide was estimated as total dissolved sulphide after removing the suspended solids by floculating the samples with aluminium chloride (6N) and sodium hydroxide (6N) solutions. Sulphide was precipitated as zinc sulphide and it was then oxidised to sulphur with standard potassium iodide-iodine solution (0.025 N). Unreacted iodine was titrated with standard thiosulphate iodometrically. Sulphide concentration is calculated as:

\[
\text{mg/lit. } S = \frac{400 (a-b)}{\text{ml sample}}
\]

where

\[
a = \text{ml 0.025 N iodide used}
\]

\[
b = \text{ml 0.025 N Na}_2\text{S}_2\text{O}_3 \text{ used.}
\]

Calculation of unionised H₂S was made with the help of logarithmic practical constants for H₂S and conductivity and pH values of the samples at room temperature.

**Metals:**

Metals were estimated as total, total suspended and total dissolved metal concentration.
Suspended metal concentration was determined by the difference of total metal concentration (determined without filtration) and total dissolved metal concentration (determined in the filtered sample).

Polarographic technique was employed for the initial screening (semi quantitative measurement) of the samples by making use of dropping mercury electrode. Sample pretreatment to remove the interference by organic matter was performed by digesting the sample (100-1000 ml) with HNO₃ - H₂SO₄ mixture.

In case of river water samples, where it was expected that concentration level of the heavy metal may be very low, sample preconcentration was achieved by evaporation on waterbath. The concentrated samples were digested to destroy organic matter as above.

The usual procedure of the estimation of each metal was the preparation of calibration curve with the help of their standard solutions and reagents blank. All the reagents required for the estimations were prepared as specified in standard procedures. The photometric measures were carried out on Beckmann spectrophotometer model DU-21.

Table 6 presents a brief outline of the method, colorimetric reagent and wave length used for the determination of each metal.

For the estimation of total chromium, conversion of trivalent chromium to hexavalent chromium by permanganate oxidation was performed in the samples. Concentration level of chromium (III) was known by the difference of total chromium and total hexavalent chromium.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Reagent</th>
<th>Metal Extraction</th>
<th>Wave length (nm)</th>
<th>Experimental conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>1:10 Phenanthroline hydrate</td>
<td>For total iron Fe (III) was reduced to Fe (II) by boiling samples with HCl and NH₂OH solution</td>
<td>510</td>
<td>pH 3.2-3.5</td>
</tr>
<tr>
<td>Manganese</td>
<td>None</td>
<td>Manganese is oxidised to permangate by persulphate in presence of silver nitrate</td>
<td>525</td>
<td>i) Mercuric sulphate is added to overcome the interference by chloride. ii) Hydrogen peroxide is added to prevent the formation of manganiessc dioxide precipitate.</td>
</tr>
</tbody>
</table>
Table 6 — (Contd.)

<table>
<thead>
<tr>
<th>Metal</th>
<th>Reagent</th>
<th>Metal Extraction</th>
<th>Wave length (nm)</th>
<th>Experimental conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nickel</td>
<td>Heptoxime in chloroform</td>
<td>Extracted first in chloroform and then in aq. phase by HCl</td>
<td>445</td>
<td>Extraction is done in strongly basic solution</td>
</tr>
<tr>
<td>Zinc</td>
<td>Dithizone in chloroform</td>
<td>Extraction with chloroform</td>
<td>535</td>
<td>i) Solution is made neutral. ii) Bis (2-hydroxy-ethyl) dithiocarbamate &amp; cyanide solutions are added to remove the interference by Pb, Cu and Ni.</td>
</tr>
<tr>
<td>Chromium as Cr (VI)</td>
<td>Diphenyl-carbazide in acetone</td>
<td>Reaction in acidic medium at pH 2.0-3.0</td>
<td>540</td>
<td>—</td>
</tr>
<tr>
<td>Lead</td>
<td>Dithizone in carbon tetracl-ord</td>
<td>Extraction with carbon tetra chloride at pH 8.0-9.0</td>
<td>520</td>
<td>i) CCl₄ is used also as reference blank. ii) Medium is strongly basic.</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Dithizone in chloroform</td>
<td>Extraction with chloroform after overcoming the interference due to copper and nickel</td>
<td>515</td>
<td>i) Medium is kept strongly basic. ii) Zinc is removed by washing the extract with 0.5N NaOH.</td>
</tr>
<tr>
<td>Arsenic as As (III)</td>
<td>Silverdi-ethyl-di-carbamate in pyridene</td>
<td>Arsenic is reduced to AsH₃ with zinc in acid solution. AsH₃ is then passed through lead acetate solution and absorbed into silver diethyl dithiocarbamate solution</td>
<td>585</td>
<td>—</td>
</tr>
<tr>
<td>Copper</td>
<td>Neocupron in chloroform</td>
<td>Extraction with chloroform and methanol mixture</td>
<td>457</td>
<td>i) pH neutral or slightly acidic. ii) Interference due to sulphide and other ions was removed as per standard methods.</td>
</tr>
<tr>
<td>Mercury</td>
<td>Dithizone in carbon tetra chloride</td>
<td>Extraction with addition of ethylene diamine tetra acetate at pH 1.5-2.0</td>
<td>610</td>
<td>i) Excess of dithizone in carbon tetrachloride</td>
</tr>
</tbody>
</table>

Arsenic mostly present as As (III) in water and waste-water, was reduced to arsenic in a Gautzeit Generator. Arsenic was passed through a scrubber containing glass wool impregnated with lead acetate into the reagent solution contained in a glass tube. Arsenic (v) present in the samples was reduced to As (III) with SnC₁₂ in acid solution prior to the estimation of total arsenic.

Calculation of metal concentration in the samples was made with the following formulae:
(i) For digested and treated samples

\[
\text{mg/lit. total metal} = \frac{\mu g \text{ of the metal calculated from calibration curve} \times 100}{\text{ml of the original sample} \times \text{ml portion of the digested sample}}
\]

(ii) For filtered and undigested sample

\[
\text{mg/lit., total metal} = \frac{\mu g \text{ of the metal as calculated from calibration curve}}{\text{ml of the sample}}
\]

Cyanide:

Cyanide was estimated as total and free cyanide (CN\(^{-}\)). After the removal of interfering substances from the polluted river and waste-water samples by preliminary distillation, cyanide was converted to hydrogen- cyanide gas which was absorbed in caustic soda solution\(^2\). Subsequently, sodium cyanide thus formed was analysed.

For free cyanide estimation, a portion of the sample was chlorinated and then distilled. Free cyanide as CN\(^{-}\) was determined by the difference in total cyanide of unchlorinated samples and chlorinated sample.

In colorimetric procedure, cyanide as CN\(^{-}\) in the distillate from preliminary distillation was converted to CNC1 by reaction with chloramine-T at a pH 8.0 without hydrolysing to cyanate\(^3\). The concentration of CNC1 was estimated by producing the colour (red blue dye) with pyridine barbituric acid reagent and measuring the absorbance at 575 nm.

The concentration was calculated as:

\[
\text{mg/lit. cyanide} = \frac{\mu g \text{ CN obtained from calibration curve} \times \text{Total ml of absorbant used in distillation}}{\text{ml original sample} \times \text{ml of absorbant used in distillation}}
\]

Free cyanide concentration = Total cyanide in unchlorinated sample.
- Total cyanide in chlorinated sample.

Oil and Grease:

These were determined by solvent (petroleum ether 40-60\(^\circ\)C) extraction process which was carried out
in a soxhlet apparatus at a rate of 20 cycles/hr for four hours. The residue, obtained after the evaporation of the solvent, was weighed to give the concentration of oil and grease in the samples.

[VII] METAL ABATEMENT (CHROMIUM)

The results of the analysis conducted for the measurement of various characteristics are tabulated and given in next chapter.

Adsorption of Chromium (VI) with Alumina

Continuous mixed batch experiments involving the different alumina dosage and initial chromium (VI) concentration in the effluent were performed under controlled laboratory conditions to study the adsorption of chromium with alumina.

Effluent samples containing primarily hexavalent chromium were collected from the local industrial sources engaged in the production of dichromate and chromate salts of sodium. The effluent, with high concentration level of chromium (VI) was diluted with distilled water to obtain different initial chromium (VI) concentration in the samples. Estimation of initial chromium (VI) and total chromium [chromium (VI) + chromium (III)] concentration was made spectrophotometrically with diphenyl carbazide as described earlier.

Commercially available aluminium oxide (A12 O3) was obtained from the local sources. It was washed with deionized distilled water to remove dust and other fine particles and was dried in hot air oven at a temperature of 100 to 105°C. After cooling to room temperature it was powdered to desired mesh size (retained on British standard Sieve No. 170).

Equilibrium studies were performed by first preparing three litres of chromium solution at a desired pH, salt concentration, initial chromium (VI) concentration and temperature. Then 100 ml portions of this solution were taken in 150 ml wide mouth Erlenmeyer flasks each containing a measured quantity of alumina. The usual procedure was to fill 20 flasks in this manner along with four flasks containing no alumina to serve as blanks. The weight of alumina varied from 0.5 g/lit. to 20 g/lit. of effluent sample for an initial chromium (VI) concentration. Flasks were agitated using shaking incubator (SEW, India) for requisit time to attain equilibrium after maximum adsorption. The shaking speed was so controlled as to ensure the homogenous suspension of alumina. During agitation process a fraction of the samples in the flasks was analysed at definite intervals of time for chromium (VI) concentration to determine the
equilibrium time which was found to be about one and half hour. After the equilibrium was attained alumina was filtered by passing through a 0.45 μm filter.

The concentration of chromium (VI) in the filtrate from each flask was determined spectrophotometrically. The amount of chromium (VI) adsorbed, S, in mg/gm of alumina, thus determined, was then plotted against C eq. (chromium concentration in sample at equilibrium) to obtain the adsorption isotherm at room temperature. Further, the final pH of each system was also measured and compared with initial pH.

In a similar way, a series of experiments were performed to study the effects of variation in pH, temperature, initial chromium (VI) concentration and that due to other anionic impurities on the adsorption of chromium with alumina. The effect of cyanide as impurity was studied by performing the experiments, optimized with reference to other conditions, in presence of its known added quantity.

The adsorption capacity of alumina was also studied after its treatment with acid and alkali separately and was subsequently compared with the results obtained with the non-treated alumina samples. For the treatment, washed and dried alumina was agitated at a proper speed in acid or alkali solution of known strength upto 48 hours. It was then filtered, dried at 110°C for 24 hours and cooled for 24 hours. Different batch experiments as described already were carried out with samples of alumina pretreated with hydrochloric acid, nitric acid and sodium hydroxide separately.

[VII] ENUMERATION OF PHYTOPLANKTON:

Quantitative enumeration of the phytoplankton was made with the help of Haemocytometer and the number of cells or organisms was expressed per ml. of the sample.

Haemocytometer is a special ruled glass slide, with a supported glass cover that holds definite volumes of fluid, allowing the exact number of organisms or cells to be determined for a given volume. The slide has a centrally located 'H' shaped groove. On either side of the horizontal groove there is a large sized ruled area of 3 mm × 3 mm. Each ruled area consists of 5 large (4 at the corners and one in the centre) special counting chambers. Each of the four large chambers at the corners encloses 16 small squares while the central one is divided into 25 small squares, which are further divided into 16 small squares.
Each counting chamber is 1 mm on a side i.e. 1 sq. mm in area and is 0.1 mm in depth under cover glass. So the volume of counting chamber,

\[
1 \text{ mm} \times 1 \text{ mm} \times 0.1 \text{ mm} = 0.1 \text{ cu. mm.}
\]

or 0.1 cm. \times 0.1 \text{ cm} \times 0.01 \text{ cm.} = 0.0001 \text{ cu. cm.} = 0.0001 \text{ c.}

As counts are recorded in numbers per cc. the volume of the counting chamber is 0.0001 cc. The number of cells or organisms arrives at this volume in the following manner:

\[
0.1 \text{ cu. mm.} = 0.0001 \text{ cu. cm.} = 1/10,000 \text{ cc.}
\]

So, \(10,000 \times 0.1 \text{ cu. mm.} = 1 \text{ cc.}\)

\[
10^4 \times 0.1 \text{ cu. mm.} = 1 \text{ cc.}
\]

Hence, \(10^4 \times \) number of cells = number of cells per cc.

40 ml. of thoroughly shaken river water sample, divided equally in four parts of 10 ml. each in graduated centrifuge tube of 15 ml. capacity were concentrated by centrifugation for two minutes at 1000 p.m. The upper supernatent water was pipetted off very carefully from each tube. The remaining concentrated sample per tube, after well shaking and ensuring uniform distribution of the plankton, a drop of it from each tube was transferred on either side of the horizontal groove by means of a wide broad graduated pipette. After one minute two separate drops from each of the two tubes were covered with the special cover glass. Each terms was recorded under low power and also under high power when needed. The counts were made as cells of each organisms. In multicellular form the individual cells were counted separately.

Twenty counts in such counting chambers per sample were made and averaged. Care was taken to count the cells only, if they are on the line forming the top or right side of the counting chamber to reduce the chances of counting the same cell twice. The average number of cells were multiplied by 10,000 and then divided by 10 to get the number of cells per cubic centimeter.
REFERENCE