# CHAPTER 2

# EXPERIMENTAL

## 2.0. Study area

The sites identified for sampling of sediments along the Mahi river were Rayka, Jaspur, Dodka and Mujpur and the map showing the sampling sites are shown in Figure 2.1. A few sites were also identified from Nandesari-Ranoli industrial area where several chemical and dye manufacturing units are functioning and along the Mini river (Chamunda Nagar, Angad and Amrapura) for collecting sediment and water samples. The sampling sites along the Mini river is given in Figure 2.2.

## 2.1. Geology of study area

The Mahi river basin is located in the Gujarat State, India and the river originates from the Mahi kanta hills in the Vindhyachal range of Madhya Pradesh state, entering the sea at the Gulf of Cambay. The lithology comprises the alluvial deposits of Pleistocene and Holocene age. The main channel is bounded by cliffs rising about 30-40 m from the river level. Sub humid to semi arid climate is prevailing in the Mahi river basin and the area receives the monsoonal rain fall. The Mini river passes through the Nandesari industrial area and merges with Mahi river. This channel receives effluents from the surrounding industries and is highly polluted.

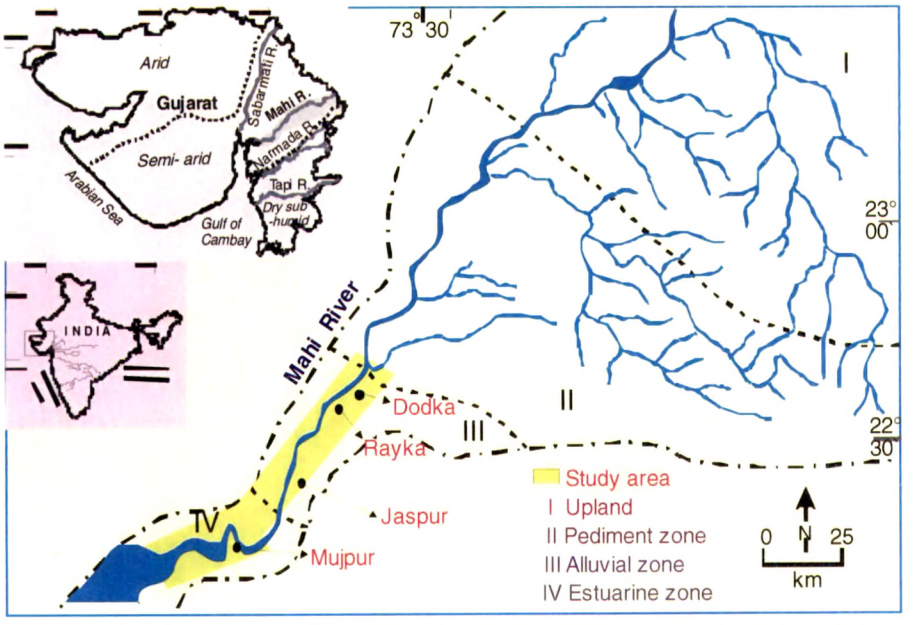



**Figure 2.1.** Map of the study area and the sampling sites along the Mahi river

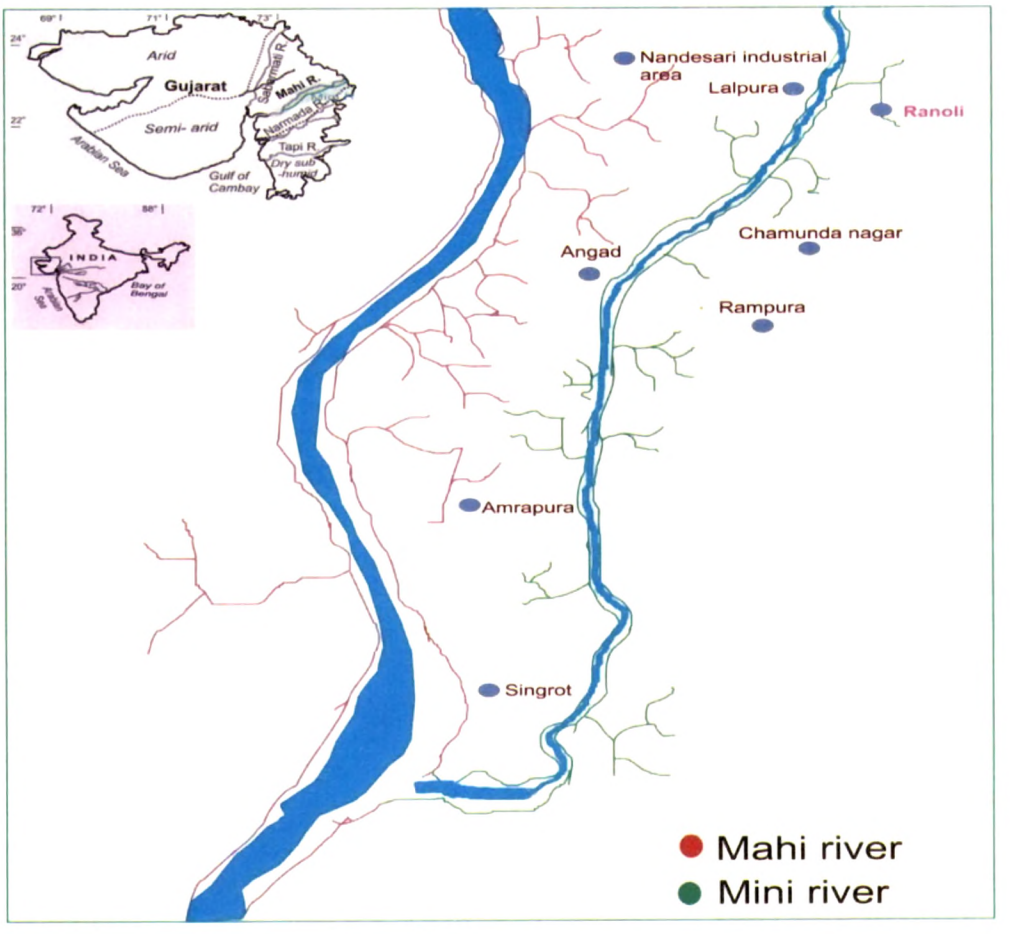



**Figure 2.2.** Map of the study area and the sampling sites along the Mini river

## 2.2. Experimental

### 2.2.1. Sample collection

Sediment samples were collected along the Mahi river bed at four different sites namely Rayka, Jaspur, Dodka and Mujpur and from three sites along Mini river bed namely Chamunda nagar, Angad and Amrapura. Samples were collected at 0.25m and 0.1m interval from the cliffs of Mahi and Mini river bed respectively. The samples were collected in thick polyethylene bags and transported to laboratory for drying. The samples were then thoroughly mixed to generate a homogenous mixture and used for further analysis.

Water samples were also collected at these sites for further analysis and the sites are shown in Figure 2.2. The ground water level (m) is given in brackets.

GW- Ground water, RW- River water
GW 1: Chamunda nagar (14m)
GW 2: Rampura (09m)
GW 3: Angad (16 m)
GW 4: Lalpura (12 m)
GW 5: Amrapura (10m)

RW 1: Chamunda nagar
RW 2: Chamunda nagar
RW 3: Angad
RW 4: Amrapura
RW 5: Singrot

### 2.2.2. Characterization of soil/sediment

**(i) Sediment composition**

The pipette method was used which is based on the fact that particles having a greater settling velocity than the fraction sought would settle below the point of withdrawl after certain time [3].

(a) Sample treatment and preparation of sample

The soil sample was treated with hydrogen peroxide, washed, filtered and dispersed. The 300 mesh sieve was used to separate the sand from silt and clay. The sediment sample was air dried and mixed thoroughly. The sample was then passed through the 2 mm sieve to separate the coarse aggregates.

(b) Removal of organic matter

A 10 gm of air dry soil sample containing particles less than 2 mm was taken in a 250 mL beaker. About 50 mL of distilled water was added into the beaker followed by 5 mL of 30 % $H_2O_2$. The beaker was then heated to about 90°C for about 30 minutes. $H_2O_2$ was added in small quantities of 5 mL during the course of heating to ensure the complete removal of organic matter.

(c) Removal of dissolved mineral matter

About 200 mL of water was added to the hydrogen peroxide treated sample and stirred well followed by filtration. The sample was then dried in an oven at 110°C., cooled in a desiccator and then weighed.

(d) Dispersion

About 10 mL of sodium hexameta phosphate as a dispersing agent was added to the oven dry sample and the sample transferred to the flask. The sample volume was made upto 100 mL followed by overnight stirring to disperse the aggregates.

(e) Separation of sands from silt and clay

The dispersed sample was washed on a 300 mesh sieve with the silt and clay components passing through the sieve. These silt and clay components were collected in a graduated cylinder. The sieve was then washed with distilled water till the volume in the cylinder reach 800 mL. The sieve with sand content was then dried in an oven at 110-120°C. The material on the sieve was collected in a porcelain dish and further dried, for about 1 hour. The silt and clay suspension in the cylinder was made up to 1 liter with distilled water and kept for pipetting.

About 25 mL of the 20 μm fraction is pipetted at a 10 cm depth after a predetermined settling time of about 4 min and the 25 mL of 2 μm fraction is pipetted after a

predetermined settling time of about 6 hr 15 min at 10 cm depth. Before the pipetting the suspension was stirred well for 5 minutes. The pipette sample was then dried in an oven at 95°C and then cooled in desiccators & weighed.

Sand fractions:

Weight in grams of fraction on sieve
$\times$ 100/(Weight of organic free oven dry sample) = % of fraction

Silt content is obtained by subtracting the sum of the percentages of sand and clay from 100.

### (ii) Organic carbon

The soil organic carbon content was determined by wet oxidation method [146]. A 0.5 g of soil sample was treated with 10 mL of standard 1N $K_2Cr_2O_7$ solution in the presence of 20 mL conc. $H_2SO_4$. The sample was kept as such for about half an hour and then 200 mL of distilled water was added in a flask. The excess $K_2Cr_2O_7$ which is not reduced by organic matter was titrated back against a standard solution of 0.5 N Ferrous Ammonium Sulphate in the presence of 0.2g of sodium fluoride and 10 mL of Phosphoric acid as flocculating agents with diphenylamine as an indicator. The change in colour from violet to light green indicates the end point of the titration. Simultaneously, a blank experiment was carried out without soil. The amount of carbon present in the soil is calculated with the Equation 1

$$\text{Amount of carbon present in 1 g soil} = \frac{0.003 \times 10\,(B-C)}{B \times S} - - - - (1)$$

Where, Weight of soil= S g, Volume of Ferrous ammonium sulphate used in blank titration= B mL, Volume of Ferrous ammonium sulphate used against soil sample= C mL

### (iii) Cation Exchange Capacity

The cation exchange capacity of soil was determined by column technique [147]. A chromatographic glass column having an internal diameter of 1cm and length 30 cm was filled with 5 gm of soil. Then, 250 mL of 0.25 M $Na_2SO_4$ solution was allowed to drip into the column at a rate of about 2 mL/min. The collected effluent was titrated

with standard 0.1 M NaOH using Phenolphthalein as an indicator. The cation exchange capacity of soil was calculated from Equation 2

$$aV/W - - - -(2)$$

Where, a- Molarity of sodium hydroxide, V- Volume in mL, W- weight of the soil

**(iv) Bulk density**

The soil sample was dried in an oven at 105°C until constant weight was obtained. The dried soil was then taken in a measuring cylinder and the volume recorded. Then the weight of this volume of soil was measured on a balance & bulk density was calculated by the following equation & expressed in $g/m^3$

Bulk density = Weight of soil (g)/ Volume of soil ($cm^3$)

**(v) Pore volume**

The pore volume is defined as the volume of water needed to completely saturate a sediment/soil sample. Initially the soil sample was dried in an oven at 105°C until constant weight was obtained. This soil sample was then firmly packed in a glass column followed by saturation with water. The volume of water added to completely saturate the column is the total pore volume.

**(vi) Porosity**

The porosity of a porous medium is the ratio of the pore volume to the container volume. The soil system consists of three phases ie solid, liquid and gas. Thus

Total volume of the soil sample $V_t = V_s + V_l + V_g$ and the Pore volume $V_p = V_l + V_g$

So the porosity of the soil sample is calculated as follows

$$P_t = \frac{V_p}{V_t} = \frac{V_l + V_g}{V_s + V_l + V_g} - - - -(3)$$

Where $P_t$ is the total porosity of the soil sample, $V_s$ is the volume of the solid phase, $V_l$ and $V_g$ are the volume of the liquid and gaseous phase respectively.

### 2.2.3. Metal analysis of sediments

The total heavy metal concentration was determined by digestion of <63μm sediment fraction (1±0.05g) with 1M $HNO_3$ at 90°C for 1 hr, making final volume to 10 mL and the samples were centrifuged at 1500 RPM for 15 minutes and the dissolved metals in the supernatant were analyzed by atomic absorption spectrophotometer.

For the estimation of anthropogenic effect, the enrichment factor (EF) was calculated using the following equation.

$$EF = \frac{\text{Value of given metal concentration found on sediment surface}}{\text{Natural background concentration of the metal}}$$

Enrichment factor values of around 1.0 indicate the lithogenic source of pollutant whereas values exceeding one indicate anthropogenic origin of pollutant.

Similarly extent of metal contamination was evaluated using the geoaccumulation index, $I_{geo}$. This is used to assess the impact of anthropogenic activites. $I_{geo}$ gives the assessment of the extent of heavy metal contamination with respect to global standards. Geo-accumulation index ($I_{geo}$) was calculated with the formula given below.

$$I_{geo} = \log_2 C_n/1.5\ B_n$$

where $C_n$ is the concentration of element in the sediment, $B_n$ is the background value of the element and 1.5 is the factor compensating background data. The $I_{geo}$ value zero indicates the absence of sediment contamination, the value greater than five indicate the very high pollution of sediments. The classification of sediment contamination is given in Table 2.1.

**Table 2.1.** Classification of sediment contamination

| $I_{geo}$ | $I_{geo}$ class | Sediment quality |
|---|---|---|
| 0-0 | 0 | Unpolluted |
| 0-1 | 1 | Unpolluted to moderately polluted |
| 1-2 | 2 | Moderately polluted |
| 2-3 | 3 | Moderately to highly polluted |
| 3-4 | 4 | Highly polluted |
| 4-5 | 5 | Highly to very highly polluted |
| 5-6 | >5 | Very highly polluted |

### 2.2.4. Colloid separation

Colloidal particles were separated from the sediment samples by mixing 200 g of sediment with 500 mL double distilled water in a beaker. The above mixture was stirred overnight using a mechanical stirrer. This mixture was centrifuged for 15 minutes at 750 rpm. The relative centrifugal force (RCF) was calculated by using the formula given below [148] and is an indication of the efficiency of separation ie the separation of the non colloidal particulates.

$$\mathbf{RCF} = \frac{\mathbf{G}}{\mathbf{g}} = \frac{\mathbf{4\pi^2\,(rpm)^2\,r}}{\mathbf{3600 \times 981}} \quad ----(4)$$

Where $\pi$ = 3.14, rotations per minute (rpm) = 750 & radius (r) = 4 cm. The RCF value was 25.12 and sufficiently high to separate out all suspended matter except the colloidal dimensions.

The supernatant solution was separated by decantation and the colloidal particles present in the supernatant solution were separated by flocculation with 0.5 M $CaCl_2$ solution. These aggregated colloids were dried and used for further analysis.

## 2.3. Characterization of colloids

### 2.3.1. X- ray diffraction

The mineralogical composition of soil colloids was determined with X-ray diffraction analysis (Panalytical, X'per PRO, Cu K, 2.2KW Max). X-ray diffraction analysis can be used for analyzing crystalline materials such as ceramics, metals, insulaters, polymers, thin films and organic materials among others. If single crystal diffractometers are used for the study of molecular structure, powder diffractometers are used for the analysis of phases, but this is also being used to get the molecular information.

### 2.3.2. Static light scattering

Scattered light from colloidal particles can give information about the particle size & its distribution. This is based on the theory that the intensity of light scattering at different angles differs and is referred to as Rayleigh scattering.

The angular dependent light scattering Rayleigh ratio ($R_\theta$) is defined as

$$R_\theta = \frac{I_\theta \, r^2}{I_0 \, V} ----(5)$$

where $I_\theta$ is the intensity of scattered light, $I_0$ is the intensity of incident light, V is the volume of the scattering suspension and r is the distance between the scattering volume and the detector. The excess Rayleigh ratio, $R_\theta$ of light scattered from a small volume V of colloidal suspension into a detector set at an angle θ with respect to the direction of the incident light, compared to that of solvent alone can be represented as

$$R_\theta = \frac{(I_\theta - I_{\theta\,solvent})\, r^2}{I_0 V} ----(6)$$

Where $I_\theta$ is the intensity of scattered light measured from suspension, $I_\theta$ solvent is the scattered intensity of the solvent. The angular dependent scattering light intensity P(θ) is defined as

$$P(\theta) = 1 - \frac{16\,\pi^2\, n_0^2}{3\lambda^2} \sin^2\frac{\theta}{2}\, r_g ----(7)$$

This factor P(θ) depends on the size of the particle, wavelength of the light and the scattering angle θ. Therefore, this enables to measure the size of the particle from the angular dependence scattering light intensity alone.

The Rayleigh-Gans-Debye approximation could be used if the following requirement is fulfilled,

$$|m-1| \ll 1 \text{ and } 2Ka|m-1| \ll 1$$

Where 2a is a characteristic diameter of the molecule, m is the ratio of the refractive index of the particle suspension to the refractive index of the solvent, and $K = 2\Pi n_{0/\lambda 0}$.

Where $n_0$ is the refractive index of the solvent and $\lambda_0$ is the wavelength of the incident light.

All scattering experiments were carried out on a Wyatt static light scattering instrument at 690 nm. Before the measurements were started, the flow cell was cleaned thoroughly with HPLC grade water. The glass containers for electrolyte and colloid suspension were cleaned with a chromic acid solution followed by thorough washing with distilled water. The use of detergent based materials to clean the cell was avoided as it may lead to the aggregation of particles in the flow cell. The room temperature of 25° C was maintained for all measurements. Initially, solvent (water HPLC grade) was injected followed by colloidal suspension and then solvent again. The increase in detector voltage value was observed for the colloidal suspension as compared to solvent alone. These detector voltage values are converted into scattered intensity. The difference between the scattered intensity of sample and solvent gives the excess Rayleigh ratio [149].

### 2.3.3. pH

The pH of the sediments was measured by using a glass electrode (Cyberscan 510) in 1:8 sediment-water suspension.

### 2.3.4. Electrical conductivity

The electrical conductivity of the colloidal suspensions were measured on conductivity meter (Elico CM 180) at room temperature (30±2°C). The instrument was calibrated with 0.01 M KCl before making measurements.

### 2.3.5. Colloid concentration

Colloidal concentration in suspension was determined by drying the solution at 100°C following the method reported in literature [150].

### 2.4. Colloid adsorption

**(i) Organic dyes**

The colloids isolated from soil samples (as mentioned in section 2.2.4) were used for adsorption experiments. Two types of adsorbate were used. They are (i) Organic dyes (ii) Metal ions

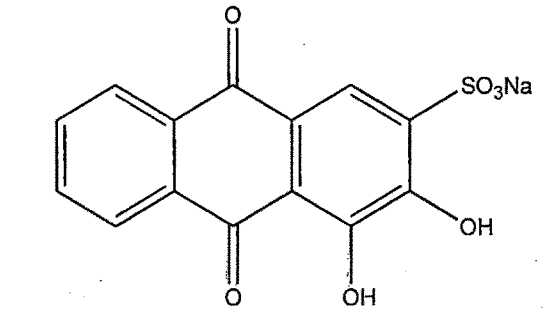
(i) The dyes used in the adsorption experiments were alizarin red and methylene blue and were of high purity and commercially available. The details of these two dyes are given in Table 2.2.

**Table 2.2.** Properties of dyes

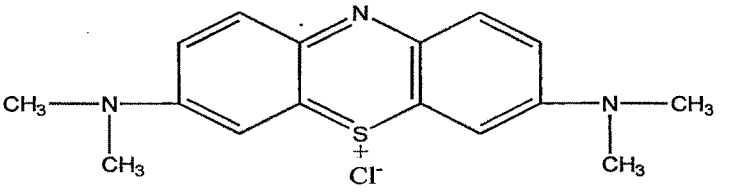
| Property | Alizarin red | Methylene blue |
|---|---|---|
| Colour Index (C.I.) | 58005 | 52015 |
| Molecular formula | $C_{14}H_6Na_2O_7S$ | $C_{16}H_{18}Cl\,N_3S$ |
| Molecular weight (g/mol) | 364.24 | 319.86 |
| $\lambda_{max}$ (nm) | 520 | 665 |
| Solubility in water (g/L) | 1.0 | 50.0 |

Alizarin red dye is an anthraquinone derivative obtained from the root of the madder plant. It is commercially available and is used in textile dyeing industries. Methylene blue is a thiazine dye, which is widely used in colouring paper, dyeing of cotton and wools and hair dyes among others. The structure of these two organic dyes is given in Figure 2.3.

**Structure of dyes**




(A)




(B)

**Figure 2.3.** Structures of dyes used in the adsorption experiments (A) Alizarin red (B) methylene blue

Adsorption of dye from its aqueous solutions by native colloidal particles was measured by placing 50 mL of dye solution in contact with 20 mg of native colloidal aggregates in a series of beakers. Samples were stirred by using magnetic stirrer with constant speed to maintain the contents completely mixed for about 6h. After equilibration, samples were centrifuged at 3000 rpm for 15 minutes. The concentration of the supernatant solution was measured with UV-Visible spectrophotometer (UV 2450, Shimadzu) at the wavelength of 520 nm and 665 nm for alizarin red and methylene blue respectively. The quantity of dye adsorbed on the colloidal particles was calculated from the difference between initial concentration and concentration at equilibrium. The batch experiments were carried out to determine the effect of initial dye concentration, pH and adsorbent mass for the adsorption of dyes on the colloids.

**(ii) Metal ions**

The metal ions used in this study were Cr(VI), Pb(II), Ni(II) & Cu(II). The choice of these metals was based on the fact that all these are toxic metals and are known to be harmful for the environments. Solution of Chromium(VI)oxide, Nickel Chloride

hexahydrate ($NiCl_2.6H_2O$), Copper(II) nitrate trihydrate $Cu(NO_3)_2.3H_2O$ and Lead(II) Nitrate $Pb(NO_3)_2$ (Merck chemicals) were prepared and used in the experiments. The concentration of Chromium was measured by UV-Visible spectrophotometer (UV 2450, Shimadzu) at a wavelength of 372 nm. A calibration curve was constructed by measuring the absorbance of known concentration of chromium solution. From this plot, the concentration of unknown chromium samples was determined from its absorbance.

The effect of pH on the adsorption of chromium on colloids was investigated with a initial solution concentration of 15 mg/L at a pH of 2.75 to 9.75. The experimental investigations involving the effect of initial solution concentration on the adsorption of Cr(VI) were carried out with a initial solution concentration of 10-50 mg/L at a pH of 2.75.

Batch experiments were performed to determine the adsorption of hexavalent chromium on colloids. The quantity of the colloid adsorbent was 10 mg and the adsorbate Cr(VI) taken was 50 mL. Initial Cr(VI) concentration range was 10- 50 mg/L. The samples were stirred for six hours to ensure that the aggregated colloidal contents get completely mixed with the solution. After equilibration, samples were centrifuged at 600 rpm for 5 minutes, subsequently the supernatant solution was filtered through Whatmann 0.2 μm filter and analyzed for chromium. The change in chromium concentration from the initial chromium concentration indicated the extent of adsorption. The same procedure was also adopted for the kinetic runs where the samples were withdrawn at definite time intervals of 30 min and the chromium concentration was maintained at 15 mg/L and the pH of solution was varied from 2.75 to 9.75 using 0.5 M NaOH.

The adsorption of Pb(II), Ni(II) and Cu(II) on colloids was determined as was done previously for chromium and the concentration was determined with Atomic absorption spectrometer (Electronic corporation of India Ltd. AAS 4141).

### 2.4.1. Adsorption Kinetics

In the case of kinetic experiments the same procedure described earlier was performed with initial concentrations ranging from 50- 200 mg/L. The volume of dye solution and the quantity of adsorbent taken was 50 mL and 20 mg respectively. The samples

were withdrawn from the beaker at definite time intervals and analyzed for dye concentration on a UV-Visible spectrophotometer. The amount of dye adsorbed at definite time intervals was determined using the formula given below

$$Q_t = (C_0 - C_t)V/W$$

where $C_0$ and $C_t$ are the initial and concentration of dye in the liquid phase at time t (mg/L) respectively. V is the volume of the dye solution (in liters) and W mass of the adsorbent (g).

**2.5. Colloid mobilization**

Experiments were conducted to investigate the mobility of colloidal particles from sediments packed in glass columns using distilled water as eluent. Initially, water was allowed to flow into the column at a constant rate of 0.3 mL/min using a dropping funnel. The total volume of water that was allowed to flow into the column was 100 mL. Water samples were collected at different pore volumes to get the colloid concentration. It has been reported that colloid concentration in suspensions can be determined by dry weight, after oven drying at $100^0$C. This process was adopted until a constant low concentration of colloids was obtained in the effluent.

**(i) Dye transport**

A chromatographic glass column with an internal diameter of 4 cm and length 50 cm was used to study the transport behavior of the colloidal particles. The bottom of the column has a porous disk to contain the sediment material. The average packed height of the glass column was 15 cm. Typical pore volumes were 35 mL whereas the porosity and bulk density was 0.18 and 1.7 kg/L respectively. After packing the column with sediments, the column was gently tapped with a mechanical vibrator for five minutes to ensure a uniform packing of the column. The schematic diagram of the column is shown in Figure 2.4.

After packing, the soil column was preconditioned with about 50 mL of 0.5 M $CaCl_2$ solution. Then the porous medium was saturated with 0.5 M NaCl solution. Afterwhich, 20 mL of the alizarin dye solution of 50 mg/L concentration was allowed to flow through it. This step ensured that the soil column gets contaminated with the dye so that the released colloidal particles would also carry the contaminant.

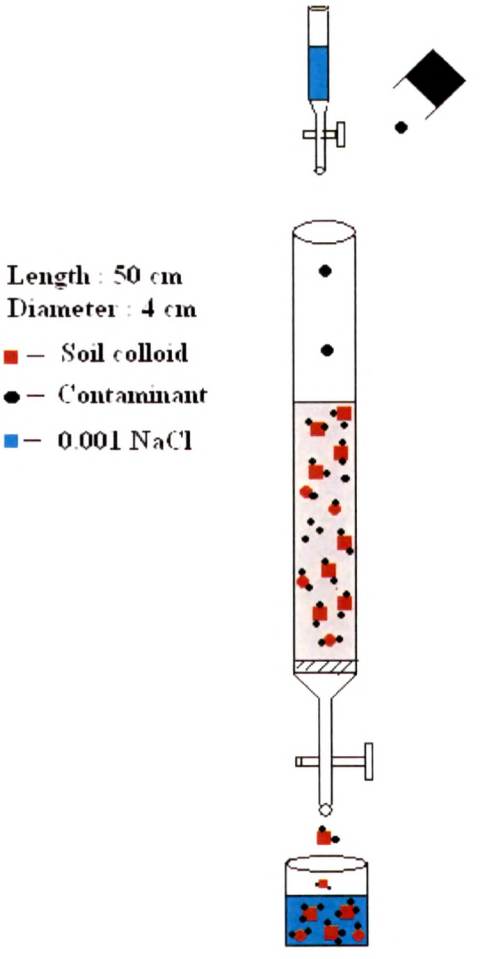



**Figure 2.4.** Schematic diagram showing the model column used for the transport experiments

After this sorption step, columns were leached with 0.01 M NaCl and 0.001 M NaCl solution at constant flow rate of 0.3 mL/min. This ionic strength reduction results in the release of colloidal particles initially present in the porous medium with adsorbed dye contaminant. The total contaminant concentrations (dissolved and colloid associated) were determined using a visible spectrophotometer (Mini spec SL 171, Elico Ltd. India) at a wavelength of 520 nm. Since the solubility of the dye in water is fixed, the total absorbance will depend only on the colloid adsorbed dye concentration.

Similar experiments were carried out to understand the effect of pH on the release of colloidal particles. After preconditioning the column with $CaCl_2$ and saturating it with 0.5 M NaCl, as was done previously, the contaminated sediments were exposed to influent solutions with ionic strength of 0.001 M NaCl at three different pH values of 7.5, 9.5 and 11.5. The collected effluent samples were analyzed for pH and total contaminant concentrations.

The procedure adopted for the alizarin red was repeated for methylene blue. In this case, the column was contaminated with 20 mL of methylene blue solution having concentration of 25 mg/L. The total dye concentrations (dissolved and colloid associated) were determined by a Uv-Visible spectrophotometer(UV 2450 Shimadzu ) at a wavelength of 665nm.

**(ii) Metal transport**

The column setup was similar to the ones used for the dye transport. A chromatographic glass column 60 cm long, having an internal diameter of 4 cm, and fitted with a porous disk at the bottom of the column, was used to study the transport behavior of the colloidal particles. Sieved sediments were dry packed in the column and uniform packing was ensured by gentle tapping with a mechanical vibrator for five minutes. The average packed height of the soil column was 30 cm and shown in Figure 2.3.

Column transport experiments were carried out with the in situ released soil colloidal particles. The chromium transport experiments were performed through the soil column preconditioned with 0.5 M NaCl solution. After this step, 50 mL of the chromium solution (15 mg/L) was allowed to flow through it. After sorption, the

influent was changed to 0.001 M NaCl. The release of colloidal particles from the soil can easily be achieved in the presence of monovalent cations, namely $Na^+$. Thus, the column was preconditioned with 0.5 M NaCl followed by flushing with low ionic strength solution of 0.001 M NaCl. This ionic strength difference initiates the release of colloidal particles with adsorbed contaminant. The total mobilized contaminant concentrations (dissolved and colloid associated) were determined by a UV-Visible spectrophotometer (UV 2450, Shimadzu) at a wavelength of 372 nm. Transport experiments were also performed with Pb(II) and Ni(II) and Cu(II). In this case the column was contaminated with 35 mL of solution having the concentration of 3 mg/L and the concentration was determined with Atomic absorption spectrometer (Electronic corporation of India Ltd. AAS 4141).

For the experiments performed to investigate the transport of chromium through uncontaminated soil zones, three layers of uncontaminated sediments having height of 5cm, 10 cm and 15cm were used. Over each of these layers 10 cm of contaminated sediment containing 0.75 mg of Cr was placed. Experiments were also conducted with 10 cm layer of contaminated soil only as a blank case. The chromium was stripped by passing 0.001 M NaCl solution at a rate of 0.3 mL/min.

### 2.6. Water analysis

Water samples collected at the sediment sites are shown in Figure 2.2.

**(i) Metals**

The following procedure was adopted for the pretreatment of water samples before the heavy metal analysis. The collected water samples were adjusted to pH 4 with concentrated nitric acid. Then 100 mL of this water sample was transferred to 250 mL conical flask. About 5 mL of conc HCl was added followed by heating on a hot plate for 20 min. Further, this sample was filtered through the sintered-glass crucible after cooling at room temperature. This filtered sample was made upto 100 mL with double distilled water used for rinsing filter flask. These samples were used for the heavy metal analysis by atomic absorption spectroscopy.

**(ii) Total dissolved solids**

The amount of dissolved solids in the water samples were determined by dry weight method. About 10 mL of sample was taken in Gooch crucible and dried in an oven at 103°C. After drying the crucible was cooled in a desiccator followed by weighing.

$$\text{Total suspended matter(mg/L)} = \frac{A \times 1000}{B}$$

Where A = mg of suspended solids and B = mL sample

**(iii) Dissolved oxygen**

**Iodometric method**

The dissolved oxygen levels in water bodies depend on the physical, chemical and biochemical activities prevailing in the water. It is key test in water pollution control activities and waste water treatment.

The analysis is based on the addition of divalent manganese solution followed by strong alkali to water sample. The dissolved oxygen oxidizes the equivalent amount of manganous hydroxide precipitate to hydroxides of higher valency states. The oxidized manganese reduced to divalent state in the presence of iodide ions and on acidification, with the liberation of iodine equivalent to the DO content in the water sample. The liberated iodine is then titrated with a standard solution of thiosulfate using starch indicator and the DO calculated as below.

$$\text{Amount of Dissolved oxygen} = 0.8 \times V_2 \text{ppm}$$

Where $V_2$ is the volume of the thiosulfate solution.

**(iv) Chemical oxygen demand (COD)**

The chemical oxygen demand is a measure of the oxygen equivalent of the portion of the organic matter in the water sample that can be oxidized using strong oxidant. The dichromate refluxion method was used as it has advantage over other oxidants interms of oxidizability. About 20 mL of water sample was refluxed with known amount of potassium dichromate solution and sulphuric acid for 2 hr and the excess dichromate titrated with standard ferrous ammonium sulphate solution using ferroin indicator. A sharp colour change from blue green to reddish brown indicated the end point of titration. The blank experiment was carried out with 20 mL of distilled water with the same reagents. The amount of organic matter present in the sample is equivalent to the potassium dichromate consumed and the COD calculated as shown below

$$\textbf{Chemical oxygen demand (mg/L)} = \frac{\textbf{(a - b)N} \times \textbf{8000}}{\textbf{mL sample}}$$

Where COD = Chemical oxygen demand from dichromate
a= Ferrous ammonium sulphate used for blank
b= Ferrous ammonium sulphate used for sample
N= Normality of Ferrous ammonium sulphate

**(v) Chloride content**

The Argentometic method was used to determine the chloride content. 1 mL potassium chromate was added to the 100 mL of water sample followed by titration with standard silver nitrate solution. A pinkish yellow colour indicate the end point of titration and the chloride content calculated as below :

$$\textbf{Chloride (mg/L)} = \frac{\textbf{(A - B)} \times \textbf{N} \times \textbf{35450}}{\textbf{mL sample}}$$

Where A = Volume of sample titration
B= Titration for blank
N = Normality of $AgNO_3$

**(vi) Sulphate**

The gravimetric method was used to determine the sulphate content in water samples. The sulphate was precipitated in a hydrochloric acid medium as barium sulphate with warm barium chloride solution. The precipitate was digested at 90°C for about 2 hr. Then the precipitate was filtered with ashless filter paper, dried and ignited in a silica crucible for 1 hr. The crucible was then cooled in a desiccator and residue weight taken and the sulphate content calculated as below

$$\textbf{Sulphate (mg/L)} = \frac{\textbf{mg BaSO}_4 \times \textbf{411.5}}{\textbf{sample(mL)}}$$

**(vii) Nitrate-Nitrogen ($NO_3$-N)**

The phenoldisulfonic acid method was used to determine the Nitrate- Nitrogen content in water samples. To 100 mL of sample 1 mL of sulphuric acid is added followed by $KMnO_4$ solution for conversion of nitrite to nitrate. Then the sample is evaporated to dryness in a water bath. The residue in then dissolved in a phenoldisulfonic acid reagent with mild heating on the hot water bath. About 5 mL of

KOH solution is added till maximum colour is developed. The sample is then filtered to remove flocculent hydroxides. The absorbance of this sample is determined with UV-Visible spectrophotometer at the wavelength of 403 nm. The readings also taken for blank samples. The Nitrate Nitrogen content calculated as below

$$\text{Nitrate N}\left(\frac{\text{mg}}{\text{L}}\right) = \frac{\mu\text{g nitrate N}}{\text{mL sample}}$$

$$\text{NO}_3\left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{mg}}{\text{L}}\text{nitrate N} \times 4.43$$

**(viii) Fluoride**

The Alizarin visual method was used to determine the Fluoride content. The standard solution of sodium fluoride was prepared from low to high concentration range in a volumetric flask. About 5 mL of acid-alizarin-zirconyl reagent was added in 100 mL of water samples and to the standard sample using volumetric pipette. The sample was then thoroughly mixed and compared with standard solution visually. The Fluoride content can be calculated as below

$$\text{Fluoride (mg/L)} = \frac{\text{mg Fluoride}}{\text{mL sample}}$$

**(viii) Phosphate**

The Stannous chloride method was used to determine the Phosphate content in water samples. Phophate occurs in natural waters and in waste waters in the form of various phosphate. They are classified into orthophosphates, condensed phosphates and organically bound phosphate. These forms of phosphate may occur in the soluble form, particulate form or in water bodies.

To a 100 mL of sample 4 mL of molybdate reagent and 0.5 mL of $SnCl_2$ was added. After 10 minutes, the absorbance of sample was measured with UV-Visible spectrophotometer at the wavelength of 690 nm and compared with the calibration curve of standard samples. From this the concentration of phosphate in water samples was determined.

$$\text{mg/L P} = \frac{\text{mg P} \times 1000}{\text{mL sample}}$$