3.1 Methods of water quality assessment

The collection, preservation and analysis of physico-chemical characteristics of water and wastewater samples were carried out as per the standard procedure described in American Public Health Association (APHA, 2012).

3.1.1 Collection and preservation of water samples

The collection and preservation of water samples were done as per APHA methods, mentioned in Section 1060. The equipment and containers used for sample collection were thoroughly cleaned before sampling. Samples for physico-chemical analysis were collected in one litre plastic containers. The containers were of good quality, free of analytes of interest and were rinsed out, two or three times with the sample to be examined. Sterilized containers were used to collect the samples for bacteriological analysis. All the sample containers were labelled properly with information like unique sample code, sample type, date and time of sampling. Accurate sampling location were recorded for further reference,
using global positioning system (GARMIN GPS map model 76CS). Available details of the sampling locations and peculiar characteristics of the samples, if any, were also noted during sample collection. The in-situ parameters such as pH and electrical conductivity, required immediate analysis in the field. The physico-chemical and bacteriological changes of the sample was prevented by appropriate preservation techniques. Samples were kept in cool condition without freezing, to minimize the potential for volatilization or biodegradation between sampling and analysis. For the metal analysis, samples were collected in a separate clean bottle and acidified with HNO₃ to a pH below 2 to minimize precipitation and adsorption on container walls. To get more reliable analytical results, shorter the time that elapses between sampling and analysis.

3.1.2 Analytical methods of water and wastewater

3.1.2.1 pH (APHA, 2012 Section 4500 - H⁺ B)

pH of the water samples was determined by Electrometric method using a multi-parameter PCSTestr35 pH meter. National Institute of Standards and Technology (NIST) buffers having the pH value of 4,7 and 10 were used to calibrate the pH determining instrument.

3.1.2.2 Electrical Conductivity (APHA, 2012 Section 2510 B)

Electrical Conductivity (EC) is directly proportional to its dissolved mineral matter content and is expressed as µS/cm. The electrical conductivity was measured using multi-parameter PCSTestr35 instrument and was calibrated using standard reference solution (0.01M KCl) has a conductivity of 1412 µS/cm at 25°C.
3.1.2.3 Total Dissolved Solids (APHA, 2012 Section 2540 C)

Gravimetric technique was utilized to determine the Total Dissolved Solids. A measured volume of sample was filtered through a standard glass-fiber filter (Whatman grade 934AH) with applied vacuum and the filtrate was transferred to a weighed evaporating dish and evaporated to dryness until a constant weight in an oven at 180 ± 2°C. The increased dish weight denotes total dissolved solids.

\[
\text{Total Dissolved Solids, mg/L} = \frac{(A - B) \times 1000}{\text{Sample volume (mL)}}
\]

where

\[ A = \text{weight of dried residue and dish, mg} \]
\[ B = \text{weight of dish, mg} \]

3.1.2.4 Colour (APHA, 2012 Section 2120)

Colour was analysed spectrophotometrically by UV-Visible spectrometer, Thermo Scientific- Evolution 201 at a wavelength between 450 and 465nm using platinum - cobalt standard solutions. Colour of samples as well as platinum - cobalt standards obeys Beer’s law.

3.1.2.5 Turbidity (APHA, 2012 Section 2130 B)

The determination of turbidity was done by Nephelometric method using the instrument Systronics Digital Nephelocom-Turbidity meter 132. Formazin polymer was considered as the primary standard reference suspension and the instrument was calibrated by using different standard turbidity solutions. Turbidity was measured based on the intensity of scattered light.
To measure turbidity, well mixed samples were taken into measuring cell and the instrument displayed the turbidity value directly.

3.1.2.6 Chloride (APHA, 2012 Section 4500-Cl B)

Argentometric method was adopted for the determination of chloride in water. AgNO₃ reacts with chloride ions in water to form silver chloride. The end point was determined by the formation of silver chromate from excess silver nitrate. 1 mL of potassium chromate indicator was added to the sample and titrated against 0.0141N standard silver nitrate solution. The change in colour from yellow to reddish orange was observed at the end of the reaction.

\[
\text{Chloride, mg/L} = \frac{\text{Vol. of AgNO}_3 \times N \times 35.45 \times 1000}{\text{Sample volume (mL)}}
\]

\[N = \text{Normality of AgNO}_3.\]

3.1.2.7 Sulphate (APHA, 2012 Section 4500-SO}_4^{2-} E)

Turbidimetric Method was followed to estimate the sulphate concentration in the samples using Systronics Digital Nephelo - Turbidity meter 132. Sulphate ion was precipitated as barium sulphate crystals of uniform size, in an acetic acid medium with BaCl₂ and the light absorbance of the BaSO₄ suspension was measured by Nephelometer. The instrument was calibrated using 0 to 40 mg/L of standard sulphate solutions and the SO₄²⁻ concentration in samples was determined by comparison of the reading with a calibration curve.
3.1.2.8 Nitrate-N (APHA, 2012 Section 4500-NO₃⁻ E)

Cadmium reduction method was adopted to estimate Nitrate-N. In this method, when the water sample was passed through a column packed with amalgamated cadmium (commercially available Cd granules treated with CuSO₄ and packed inside a glass column) nitrate (NO₃⁻) was reduced almost quantitatively to nitrite (NO₂⁻). The NO₂⁻ produced thus was determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly coloured azo dye. The intensity of the azo dye developed was proportional to the concentration of Nitrate – N and was determined at 543 nm using UV-Visible spectrophotometer (Thermo Scientific- Evolution 201). A correction was made for any NO₂⁻ present in the sample by analysing without the reduction step.

3.1.2.9 Phosphate- P (APHA, 2012 Section 4500-P)

The estimation of Phosphate-P was carried out by Stannous Chloride method. Phosphate present in samples reacts with ammonium molybdate to form molybdophosphoric acid under acid conditions and reduced by stannous chloride to intensely coloured molybdenum blue. After 10 min, but before 12 min, intensity of colour was measured by UV-Visible spectrophotometer (Thermo Scientific - Evolution 201) at 690nm and compared with a calibration curve.

3.1.2.10 Fluoride (APHA, 2012 Section 4500-F⁻ C)

Ion-Selective Electrode Method was followed to determine fluoride in water samples. The fluoride electrode is an ion-selective
sensor. The main element in the fluoride electrode was the laser-type doped lanthanum fluoride crystal across which a potential was established by fluoride solutions of different concentrations. Ion selective electrode measured the fluoride ion activity in the solution rather than concentration and it depends on the solution total ionic strength and pH, and on fluoride complexing species. An appropriate buffer solution was added to provide a nearly uniform ionic strength background, adjusts pH, and breaks up complexes so that, in effect, the electrode measures concentration. Ion selective electrode (Thermo Scientific Orion 9609BNWP, Made in US) was used to measure fluoride ion concentration and total ionic strength adjustment buffer II (TISAB II) solution was used to maintain pH 5 - 5.5. The manufacturer’s instructions were followed to use a selective-ion meter. Ion-selective electrode was frequently recalibrated by checking potential reading of the 1.00 mg F⁻/L standard.

3.1.2.11 Total Alkalinity (APHA, 2012 Section 2320 B)

The alkalinity is the measure of water to neutralize acid. Total alkalinity was measured by titrating the water sample against standard sulphuric acid (0.02 N) using methyl orange as indicator. The end point was indicated by a colour change from pale yellow to orange red.

\[
\text{Alkalinity, mg/L as CaCO}_3 = \frac{\text{Vol. of H}_2\text{SO}_4 \times N \times 50 \times 1000}{\text{Sample volume (mL)}}
\]

\[
N = \text{Normality of standard H}_2\text{SO}_4 \text{ solution.}
\]
3.1.2.12 **Hardness (APHA, 2012 Section 2340 C)**

Hardness is expressed in terms of mg/L of calcium carbonate and was determined by the EDTA titrimetric method. To estimate total hardness, water sample was titrated against standard EDTA solution (0.01M) using Eriochrome Black T as indicator in the presence of NH₄Cl-NH₄OH buffer of pH 10. Colour change from wine red to blue was considered as end point.

\[
\text{Total Hardness, mg/L as CaCO}_3 = \frac{\text{M of EDTA x Vol. of EDTA x 100 x 1000}}{\text{Sample volume (mL)}}
\]

3.1.2.13 **Calcium (APHA, 2012 Section 3500-Ca B) & Magnesium (APHA, 2012 Section 3500-Mg B)**

Calcium hardness was also determined by EDTA titrimetric method. NaOH solution was added to the sample to attain a pH of 12 to 13. After adding murexide indicator, the sample was titrated against standard EDTA solution (0.01M) with continuous stirring, till the colour of the solution changed from pink to purple. Magnesium was estimated using calculation method. Following equations are used to determine the concentration of calcium and magnesium.

Calcium Hardness, mg/L as CaCO₃

\[
= \frac{\text{M of EDTA x Vol. of EDTA x 40.08 x 1000}}{\text{Sample volume (mL)}}
\]

Calcium, mg/L = Calcium hardness x 0.4
Magnesium, mg/L

\[ \text{Mg/L} = (\text{Total Hardness} - \text{Calcium Hardness}) \times 0.243 \]

### 3.1.2.14 Sodium (APHA, 2012 Section 3500- Na B) & Potassium (APHA, 2012 Section 3500- K B)

Flame Emission Photometric Method was used to estimate the alkali metals such as sodium and potassium. The determination of sodium and potassium was carried out at a wavelength of 589 nm and 766.5 nm, respectively. Filtered water samples were aspirated to the instrument, Systronics Flame Photometer 128 after calibration using standard solutions. Both the concentrations of sodium and potassium were recorded from display of instrument.

### 3.1.2.15 Heavy Metals (APHA, 2012 Section 3111 B)

The heavy metals concentration in water was determined by Direct Air-Acetylene Flame Method by Atomic Absorption Spectrophotometer (AAS) (M-series, Thermo). The instrument was operated as per manufacturer’s operating manual. Atomic absorption spectrometer, burner, readout, lamps, pressure-reducing valves and vent are the main components of the instrument, involved in the metal analysis. A preliminary treatment of samples was conducted by filtration, followed by acidification of filtrate with conc. HNO₃ and stored until analyses were performed. Stock standard solutions were procured commercially from Merck India Pvt Ltd. All calibration standards and dilution water were
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Prepared using metal-free water only. The concentration of each metal ion in mg/L was estimated by referring to the appropriate calibration curve.

To assure the quality of analysis, blank analysis was conducted between sample or standard readings to verify baseline stability. Rezero was done when necessary. To check the recovery of metals, a known amount of the metal of interest was added to an analysed sample and reanalysed. Recovery of added metal was found between 85 and 115%. Analysis of an additional standard solution after every ten samples or with each batch of samples were done to confirm that the test is in control.

3.1.2.16 Microbial Analysis

Multiple tube fermentation technique was followed to conduct the standard test for coliform group. Outcomes of examination of replicate tubes and dilutions were described in terms of the Most Probable Number (MPN) of organism present in 100 mL of the sample. The MPN value of the analysing samples was obtained from the MPN index chart. The media used for total coliform, faecal coliform and *E. coli* were Mac Conkey broth, Brilliant Green Lactose Broth (BGLB) and Peptone water, respectively. For determining total coliforms, 10, 1, and 0.1 mL of samples were inoculated in to the Mac Conkey broth and incubated at 37.5°C for 48 hrs. Tubes with gas bubbles and colour change were considered as the positive tubes and count was recorded. The positive tubes were mildly shaken and transferred, three loopful of culture to a fermentation tube containing BGLB broth using a sterile loop. Then, the tubes were incubated at 44.5°C for 24 hrs. The numbers of positive tubes were recorded and the count was estimated using MPN index chart. Again,
positive tubes were gently shaken and transferred one loopful of culture to a fermentation tube containing peptone water with a sterile loop. The incubation of the inoculated tubes was conducted at 44.5°C for 24 hrs. After incubation, 2-3 drops of Kovac’s reagent were added to the tube and the presence of *Escherichia coli* was indicated by the cherry red ring formation.

### 3.2 Assessment of soil and sediment quality

The physico-chemical characteristics of the soil and sediment samples were determined using the methods reported by Hesse (1971), Black (1965) and Central Soil Analytical Laboratory (2007).

#### 3.2.1 Collection and pre-processing of samples

Soil auger was used to collect the soil samples with minimal disturbance. Soil samples were collected at the depths of 15 - 30 cm, in polythene bottles which were labelled properly from the sampling site itself. The visible objects present on the soil surface like plant materials, roots, undecomposed organic matters were removed from before sample collection. Random samples were collected following the zig-zag movement. Soil samples were pooled to give a composite sample for that site and reduced to 1 to 2 Kg size by quartering method. Sediment samples were collected from the bottom of water bodies. Soil and sediment samples were transported to the laboratory and a portion of the samples were air-dried, sieved and stored in polythene bottles for physico-chemical analysis.
Soil/sediment - water suspension of 1:10 (w/v) was used for the determination of pH and electrical conductivity and the filtrate of the suspension was used to estimate alkalinity, sulphate and chloride. Wet digestion method or Walkley-Black method was followed for the estimation of organic carbon. For the analysis of exchangeable cations like Ca$^{2+}$, Mg$^{2+}$, Na$^+$ and K$^+$, samples were extracted with 1 M ammonium acetate solution. Extraction of samples using 1 N hydrochloric acid were conducted to determine inorganic phosphorous. Soil and sediment quality characteristics were determined by the following procedures.

3.2.2 Soil and Sediment quality characteristics

3.2.2.1 pH

10 g of the air-dried soil/sediment was taken in a beaker followed by the addition of 100 mL of deionized water to form a 1:10 ratio. The soil/sediment - water mixture was continuously stirred at least 5 times over a 30 min interval, to reach equilibrium. Allow it to stand for an hour to settle out the soil/sediment from the suspension. To measure pH, the glass pH electrode was placed deep into the clear supernatant solution for better electrical contact. The pH meter was calibrated with standard buffer solution, prior to the measurement.

3.2.2.2 Electrical Conductivity (EC)

Soil/sediment - water suspension of 1:10 (w/v) was used to determine the electrical conductivity. 10 g of air-dried soil/sediment sample was mixed with 100 mL of distilled water and the suspension was stirred for 4-5 times over a 30 minutes interval. The mixture was kept overnight to
settle and the EC of the clear supernatant solution was measured with the aid of calibrated conductivity electrode (PCSTestr35 instrument).

### 3.2.2.3 Alkalinity

10 g of air-dried sample was taken in beaker followed by the addition of 100 mL of distilled water. The suspension was filtered through Whatmann No. 40. The filtrate was titrated against standardized 0.02N sulfuric acid using methyl orange as indicator until the colour changes from golden yellow to orange red.

### 3.2.2.4 Chloride

The chloride content in the soil/sediment - water suspension was determined by argentometric titration. The suspension was filtered using Whatman No:40 and titrated against 0.0141N silver nitrate solution with potassium chromate indicator. The colour change from yellow to orange red with a curdy precipitate was considered as end point.

### 3.2.2.5 Sulphate

The same filtrate was used in the estimation of sulphate concentration by turbidimetric method using Systronics Digital Nephelo-Turbidity meter 132. The nephelometer was calibrated using appropriate sulphate standards, prior to analysis and to obtain results the reading were compared with the calibration curve.

### 3.2.2.6 Organic carbon & Organic matter

The estimation of organic carbon was done by Wet digestion method or Walkley-Black method. 0.5 g of air-dried and sieved soil/sediment
samples was used in this method. During wet combustion, soil/sediment organic matter was digested using the oxidizing agent, potassium dichromate (1N) and to convert all forms of carbon into carbon dioxide, concentrated sulphuric acid (20 mL) was added. The potassium dichromate present in excess after oxidation of carbon was titrated against ferrous ammonium sulphate solution (0.5M). To avoid interference of chloride in oxidation of the organic matter, sulphuric acid containing 1.25 % silver sulphate was used to precipitate chloride as silver chloride. To make the reaction complete, solution was kept to stand for half an hour and then the diluted solution was titrated with 0.5M Ferrous ammonium sulphate solution using diphenyl amine indicator. At the end point, the solution colour changed from yellow to red with an intermediate green colour formation. The blank determination of organic carbon content was analysed without soil/sediment.

\[
\text{Organic carbon in the soil (\%) = } \frac{(X - Y) \times N_1 \times 0.003 \times 100 \times f}{(N_2 \times S)}
\]

where,

\[X = \text{Volume of FAS used in the blank}\]
\[Y = \text{Volume of FAS used to oxidize soil/sediment organic carbon (mL)}\]
\[N_1 = \text{Normality of FAS}\]
\[N_2 = \text{Normality of potassium dichromate}\]
\[f = 1.33 \text{ Correction factor}\]
\[S = \text{Weight of soil/sediment taken (g)}\]
The organic matter of soil/sediment sample indicates the whole non-mineral fractions present. Organic matters were contributed to the physical condition of the soil/sediment by holding moisture and affecting the structure. The organic matter of soil/sediment was estimated from its organic carbon content and it was considered as 1.724 times the organic carbon.

3.2.2.7 Exchangeable Sodium & Potassium

To estimate exchangeable sodium and potassium, soil/sediment sample was extracted with ammonium acetate solution (1 M). Soluble sodium and potassium were measured by Flame Emission Photometric Method (Systronics Flame Photometer 128). The standard of sodium and potassium solution were prepared in ammonium acetate. Sodium and potassium were determined at a wavelength of 589 nm and 766.5 nm, respectively.

3.2.2.8 Exchangeable Calcium & Magnesium

To determine exchangeable calcium and magnesium, soil/sediment sample was extracted with neutral ammonium acetate solution (1 M) and both were estimated using complexometric titration with EDTA solution. The extract was titrated with 0.01 M EDTA solution in the presence of NaOH buffer and murexide indicator for the estimation of calcium. The colour change from pink to purple was considered as end point. For the determination of magnesium, ammonium acetate buffer and Erichrome Black T indicator were added to the filtrate after extraction and titrated with 0.01 M EDTA. The end point was the colour change from wine red to blue.
3.2.2.9 Inorganic Phosphorous

The inorganic phosphorous content in the soil/sediment sample was determined by measuring the orthophosphate using the UV Visible spectrophotometer (Thermo Scientific - Evolution 201). Air dried sample was extracted using 1 N hydrochloric acid and the soil/sediment - acid mixture was shaken in a shaker for a period of 16 hrs for extraction. After centrifugation at 2000 rpm for 5 min, stannous chloride and ammonium molybdate solutions were added to the solution. The intensity of the complex ‘molybdenum blue’ was measured using UV Visible spectrophotometer at 690 nm.

3.2.2.10 Heavy Metals

The heavy metals present in the soil/sediment sample was extracted using the mixture of acids, HNO₃-HClO₄ (USEPA, 1999). Hot, concentrated HClO₄ was extremely effective in decomposing organic matter and sulphides, because of its powerful oxidizing and dehydrating properties. Nitric acid dissolves the majority of the metals occurring in nature except gold and platinum Excess amount of nitric acid was added than perchloric acid, so that much of the oxidation was carried out before the action of perchloric acid was initiated. 20 mL of con. HNO₃ and 5 mL of distilled water were added to 0.2 g of air-dried soil/sediment sample. Heated on a hot plate for 2 hrs. 10 mL of HClO₄ was added after some time. The heating was continued until the white fumes come and the soil become white. Then, the filtered solution was made up to 50 mL. The analysis of heavy metals like iron, manganese, lead, cadmium, nickel, copper and zinc in the filtrate was
conducted using Atomic Absorption Spectrophotometer (AAS) (M-series, Thermo).

3.2.2.11 Soil texture

Hydrometer method based on Stokes law was followed to analyse the texture of soil/sediment. 40 g of air-dried sample was weighed in to 500 mL beaker and about 30 mL of distilled water and 5 mL of H₂O₂ were added to oxidize the organic matter. The beaker was placed on a sand bath and H₂O₂ was added until the frothing no longer continued. The soil was transferred completely to a beaker containing calgon solution (100 mL) and distilled water (300 mL) and mixed for 5 min with an electric mixer. The entire suspension was transferred to a sedimentation cylinder and made up to the mark using distilled water. The solution was mixed, properly and the readings of hydrometer and thermometer (°C) after 5 and 120 min were noted. The same procedure was also followed to determine the blank value.

3.3 Statistical analysis

The analytical results were subjected to various statistical analyses, for the easy interpretations of data. Maximum, minimum mean and standard deviation values were computed and reported. The statistical software such as SYSTAT 12 was used for the interpretation of water quality results. Pearson correlation analysis of two distinct groups of heavy metals were performed. Chemometric or multivariate analysis techniques such as Principle Component Analysis and Cluster Analysis were used to identify the heavy metal pollution sources affecting water quality. The correlation coefficient and sum of squares of the error were determined in the adsorption study to identify the best fitted kinetic model.