CHAPTER 3
MATERIAL & METHODS
STUDY AREA
3. MATERIAL AND METHODS

3.1 STUDY AREA

In the current study, two distinct and contrasting wetlands of Central Gujarat, Pariej and Malwar wetlands were selected to determine their environmental and pollution status. Pariej wetland is a community reservoir which supplies drinking water to numerous villages of Saurashtra region. Pariej wetland has already been recognized as “Wetland of National Importance” by MoEF (2005). Malwar is a natural wetland which collects runoff water from the nearby villages. The wetland is distinct in its character because of the disposal of ceramics tiles and switches and fuse from the nearby located factory. This renders distinguishing hydro and geochemical properties to the wetland.

3.1.1 Pariej Wetland

Pariej Reservoir is a perennial water storage reservoir for the district. It is situated nearly 45km from Anand, Gujarat. It is located at 22° 33’ N latitude and 72° 38’ East longitude, at an altitude between 13 to 14m above the mean sea level. The geographical location of the wetland is shown in Fig. 3.1. It lies in a natural depression; covering an area of 445 ha, surrounded by embankment with a circumference of about 9 km. It falls under 4- B Gujarat Rajwara region of Central Gujarat and is an important Water Storage Reservoir. The climate of the region is a dry tropical monsoon type, with an average annual rainfall of about 800 mm concentrated in July, August and September. This wetland is surrounded by seven villages viz. Pariej, Viroja, Sayla, Shekhupur, Bahamangam, Indervala and Daloli. The wetland is a source of drinking water supply to nearly 52 villages in Saurashtra region of Gujarat.

The wetland harbors an abundant growth of aquatic vegetation, e.g. Vallisneria spiralis, Ipomoea aquatica, Hydrilla verticillata, Nymphaea stellata, Pogonatum and Typha angustata. The wetland is lined by a thick growth of T. angustata along the periphery. Pariej is also a host to many resident and migratory waterbirds. The resident birds consist of different varieties of egrets, moorhens, ibises, stilts, herons, lapwing and grebe. The migratory birds comprise sandpipers, coots, teals and red crested pochards. It is an important breeding ground for Sarus cranes. The population estimation of
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waterfowls of this wetland clearly signifies it as “Internationally Important Wetland” (Koning and Koning-Raat, 1975; Scott, 1989) and the “Wetlands of National Importance” (MoEF, 2005). Besides, it has already been recognized as Community Reserves by the Gujarat State Forest Department (GSFD, 2005). Commercial fishing is practiced in this reservoir. Lately, an aquaculture pond for fish and prawn culture has been maintained at Pariej. The surrounding area is mostly saline and as a result no crops are grown.

Fig. 3.1 Geographical location of Pariej Wetland

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Due to water seepage from the reservoir, the whole area is waterlogged and behaves as a permanent marsh with a heavy growth of \textit{T. angustata} and other aquatic vegetation. The villagers of the surrounding villages are dependent on this wetland for food, fodder and economy for their livelihood. The villagers are employed in fishing in the wetland. The fishes commonly taken as catch include \textit{rahu, nagri, cholt, khursa}. The removal of macrophytes like \textit{T. aungstata} and \textit{I. aquatic} is extensively carried out for use as feed for the cattle and as thatching material for roofs. Flowers of \textit{Nelumbo} also fetch meager income on sale. In the Pariej wetland two sites were recognized as the sampling points. The two sites are placed opposite to each other and differ in terms of vegetation and other abiotic and biotic components thus rendering them distinct from each other.

**Site 1:** It is located at the northeastern end of the wetland [Fig. 3.2]. The site is flanked by the fields of the villages of Viroja and Sayla on the western side. This site as the whole wetland is lined by an earthen bund. This site is approached by a tarred road upto the far southern point and thereafter is connected by an earthen path and can be assessed by light vehicles only. \textit{T. aungstata} is found abundantly at the site. The area is the point for fishery for the small fishermen residing in the villages nearby. The fishermen of the village Sayla are engaged in fishing in this area. The fishermen employ the rafts prepared from \textit{T. aungstata} and fishing nets for fish catch near this site. The major fishes found in the area are \textit{rahu, nagri, cholt} and \textit{khursa}. The site and the area around it is a significant breeding ground for Sarus crane. The observations of the Sarus crane couple with their young ones have been made at this site. Copious growth of \textit{E. crassipes} was observed at the extreme northern part of the site along the wetland boundary.
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Fig. 3.2 Site 1 of Pariej wetland with profuse growth of *T. angustata*.

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Site 2: The second site is located at the southwestern end of the wetland [Fig. 3.3]. The pumping station of the reservoir is located close to this site towards the western side. The site is bordered by an earthen bund which continues throughout the periphery of the wetland. The site is approached by tarred road. A few human settlements are placed near to this site towards the far northern end of this site. The people draw water from this area by the means of motor pumps to irrigate fields around these settlements. The site is marked by profuse growth of *T. angustata* and *N. stellata*. Pariej village is located near this site of the wetland towards the southern end, within a distance of half km. The fishermen community employed under the fishing contract in the wetland resides by this site occasionally, during the peak catch season. An overgrowth of submerged macrophytes like *Hydrilla* sp. and *Najas* sp. is observed at this site. The site is an important nesting and roosting locale for egrets and cormorants. An aquaculture pond is constructed opposite to this site across the road.
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Fig. 3.3 Site 2 of Pariej wetland.

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3.1.2 Malwar Wetland

Malwar is a natural wetland situated near Kanjari- Boriyavi railway station, about 10 kms from Anand, Central Gujarat, India. The wetland is located between 22°36'55.78" N and 72°54'58.01" E (Fig. 3.4). The wetland covers an area of 30 ha and is 5ft deep. It collects the runoff water from the nearby village and the hamlets during monsoon.

![Geographical location of Malwar Wetland](image.png)

**Fig. 3.4** Geographical location of Malwar Wetland
The wetland is dominated by aquatic species *Potomogeton malaianus* Miquel, *Eichhornia crassipes* (Martius) Solms-Laubach and *Azolla pinnata* R. Brown. The wetland also is host to varied avifauna like purple moorhens, white and black ibises; cormorants, pheasant tailed jacanas, painted storks and openbills. The main source of pollution in the wetland is waste from the villages surrounding it namely Kanjari, Boriyavi and Narsanda. An important fuse and ceramic tile factory, Ravikiran Ceramics Pvt. Ltd. is located near the wetland, which dumps its waste into the wetland. This renders interesting hydro and geochemical properties to the wetland which include highly elevated levels of chloride and magnesium hardness. A temple is also located at the outskirts of the wetland which further adds to the anthropogenic activities in the wetland. The wetland is approachable by road. The area of the wetland was increased in the recent times by excavation. Off the late commercial fishing is being practiced in the wetland as a regular phenomenon.

**Site 1:** The site is situated towards the eastern end of the wetland and is in proximity to the ceramic factory [Fig. 3.5]. The site is closer to the Boriyavi village and is connected by tarred road. This site is the chief dumping spot for the factory and is thronged with waste from the industry which includes various fuses and switches used. The site is infested with prolific growth of *Eichhornia crassipes*. The wetland at this site is very shallow retaining little water in summer months. A temple is located at the vicinity of this site. The site is separated from the rest of the wetland by the means of an earthen lane. The site is devoid of human settlement, however, due to the absence of proper civic amenities and sanitation, open defecation is observed at this site.
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Fig. 3.5 Site 1 showing the electric fuse factory in the backdrop and fuse waste dumped in the wetland

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Site 2: The second site is placed exactly opposite to the first site across the wetland [Fig. 3.6]. This site is near to the residing alley precisely the settlement, surrounding the wetland. The wetland at this site is deeper compared to the first site. The people residing in the area dump household and other waste at this site. Cattle wading and open defecation is observed at this site also. Due to excessive anthropogenic pressure and regular disturbances at this site, macrophyte vegetation is observed to be sparse here. The site is used by the inhabitants of the nearby settlement area for washing and carrying out other domestic chores. The wetland at this site is used for fishing as it is deeper than the site 1.
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Fig. 3.6 The site 2 of Malwar wetland
METHODOLOGY
3.2 ABIOTIC COMPONENTS

3.2.1 Hydrochemical properties

Sample Collection

Hydrochemical monitoring in the wetlands consisted of monthly sampling of water from two predetermined sites, in the 2nd week of every month from September, 2007 to August 2009. Surface water samples were collected in acid-cleaned, two-liter polythene bottles, transported in ice to the laboratory and analyzed for physico-chemical properties. Physical parameters like pH, temperature, transparency and chemical parameter viz. dissolved oxygen (DO) were performed in situ. The other parameters like total solids (TS), total dissolved solids (TDS), free carbon dioxide, alkalinity, total hardness, calcium hardness, magnesium hardness, chloride, phosphate, sulphate, nitrate, sodium and potassium were analyzed in the laboratory according to the methods described by Trivedy et al. (1987) and APHA (1998) guidelines. Total and fecal coliform were analyzed in Malwar wetland. In the first year of the study, the site 1 of the Malwar wetland dried up in April and May 2008 due to which the samples were not collected in these two months.

Laboratory Analysis

The following methodology for hydrochemical parameters were analyzed and adopted for the water samples collected from the two wetlands:

3.2.1.1 Temperature

The measurement of temperature in water is important basically for its effects on the chemistry and biochemical reactions in the organism. It is also important in the determination of pH, conductivity and saturation level of gases in water.

Procedure: Temperature was measured in situ using a mercury-filled Celsius thermometer.

3.2.1.2 pH

Principle: The basic principle of electronic pH measurement is the determination of activity of hydrogen ions by potentiometric measurement using a standard sensing electrode (glass electrode) and a reference electrode (calomel electrode).

Procedure: The pH of the water was analyzed using Systronics pH system 361 in situ.
3.2.1.3 Transparency

Transparency is a water-quality characteristic of lakes and reservoirs. The transparency in a water body depends largely on the optical properties which are influenced by the particulate impurities present in it. This characteristic varies with the combined effect of colour and turbidity. Transparency was measured by the use of Secchi disc.

Procedure: The Secchi disc was lowered in the water and the depth at which the disc just disappeared from the view was noted. Then the disc was uplifted again and the depth at which the disc again reappeared was noted down. Transparency was calculated as follows:

\[
\text{Secchi disc transparency} = \frac{A + B}{2}
\]

Where, 
A = Depth at which Secchi disc disappears
B = Depth at which Secchi disc reappears

3.2.1.4 Dissolved Oxygen (DO)

Principle: The azide modification of Winkler method was used to determine the concentration of dissolved oxygen in the water samples. Oxygen present in the sample oxidizes the divalent manganese to its higher valency, which precipitates as brown-hydrated oxides after the addition of NaOH and KI. Upon acidification, manganese reverts to divalent state and liberates KI equivalent to DO content in the sample. The liberated iodine is titrated against sodium thiosulphate using starch as an indicator. If no oxygen is present, pure white precipitates of Mn(OH)₂ are formed when MnSO₄ and alkali-iodide reagents (NaOH + KI) are added to the sample.

Reagents

(a) Manganous sulphate solution: 36.4g of monohydrate manganous sulphate (MnSO₄·H₂O) was dissolved in distilled water and diluted to 100mL. The solution was then filtered.
(b) Alkaline-iodide-azide solution: 50g of NaOH and 15g of KI were dissolved in 90mL distilled water. 1g of sodium azide was dissolved in 5mL distilled water and then
added to the previous solution. The solution was then cooled, and the volume was made upto 100mL. The solution was stored in a dark, rubber-stoppered bottle.

(c) Conc. H$_2$SO$_4$

(d) Starch indicator: 0.5g of soluble starch was added to approximately 80mL boiling distilled water with stirring. The solution was then diluted to 100mL and boiled for a few more minutes and then left overnight. The clear supernatant was then used as the indicator.

(e) Standard sodium thiosulphate solution (0.025 N): 0.6025g of Na$_2$S$_2$O$_3$.5H$_2$O was dissolved in previously boiled and cooled 100mL distilled water. Further, 0.04g NaOH was added to it and the solution was stored in a brown color bottle.

Procedure: The sample was collected in a BOD bottle (300 mL capacity) by avoiding bubbling. 1 mL of manganous sulphate solution was added to the sample followed by 1 mL of alkaline-iodide-azide solution. The stopper was then placed carefully to exclude air bubbles and mixed by inverting the bottle repeatedly for at least 15 minutes. The precipitates were then allowed to settle leaving about 150 mL of clear supernatant. The stopper was then carefully removed and immediately 1 mL of conc. H$_2$SO$_4$ was added and the bottle closed and mixed with gentle inversion until the precipitates were completely dissolved. A part of the content (50mL) was then titrated against0.025 N sodium thiosulphate solution using starch as an indicator until the color changed from blue to colourless (end point). DO was calculated as:

$$DO \text{ (mg/L)} = \frac{mL \text{ of titrant} \times N \times 8 \times 1000}{V_2(V-v)/V_1}$$

Where, $N=$ normality of sodium thiosulphate

$V_1=$ Volume of BOD bottle, mL

$V_2=$ Volume of the contents titrated, mL

$v=$ Volume of MnSO$_4$ and iodide azide added, i.e. 1+1= 2mL
3.2.1.5 Total Solids (TS)

**Principle:** TS is the measure of all kinds of solids i.e. suspended, dissolved and volatile solids. TS can be determined as the residue left after evaporation at 103 to 105°C of the unfiltered sample.

**Procedure:** A porcelain evaporating dish of 100mL capacity was dried at 103 to 105°C for 1hr. The dish was then cooled in a desiccator and was weighed for the initial weight (Wi) in mg. 50mL well mixed unfiltered sample was poured into it and kept in a hot air oven at 103 to 105°C for 2hr up to dryness. The dish was then cooled in the desiccator and the final weight (Wf) in mg was noted down. The calculation was done as follows:

\[ \text{Total Solids mg/L} = \frac{(W_f - W_i) \times 1000}{\text{Volume of sample (50mL)}} \]

3.2.1.6 Total Dissolved Solids (TDS)

**Principle:** A large number of solids are found dissolved in natural water, the common ones are carbonates, bicarbonates, chlorides, sulphates, phosphates, and nitrates of calcium, magnesium, sodium, potassium, iron, magnesium, etc. TDS, thus is the sum of all cations and anions concentration expressed in mg/L. A well mixed, measured portion of sample is filtered through a standard glass-fibre filter and the filtrate portion is evaporated to dryness at 180 ± 2°C and that gives the amount of total dissolved solids.

**Procedure:** An evaporating dish of 100mL capacity was dried at 103 to 105°C for 1hr. The dish was then cooled in a desiccator and was weighed for the initial weight (Wi) in mg. 50mL sample was filtered through Whatman No. 42 and the filtrate was poured into it and kept in a hot air oven at 180 ± 2°C up to dryness. The dish was then cooled in the desiccator and the final weight (Wf) in mg was noted down. The calculation was done as follows:

\[ \text{Total Dissolved Solids (TDS) mg/L} = \frac{(W_f - W_i) \times 1000}{\text{Volume of sample (50mL)}} \]

3.2.1.7 Total Suspended Solids (TSS)

**Principle:** The term TSS applies to the dry weight of the material that is removed from a measured volume of water sample by filtration through a standard filter.
Total suspended solids can be obtained as a difference between TS and TSS.

\[ \text{TSS, mg/L} = \text{TS} - \text{TDS} \]

3.2.1.8 Free CO\(_2\)

**Principle:** Free CO\(_2\) reacts with sodium hydroxide to form sodium carbonate. Completion of reaction is indicated by the development of the pink color characteristic of phenolphthalein indicator at the equivalence pH of 8.3.

**Reagents**

(a) Standard sodium hydroxide titrant (0.05N): A stock solution of 1N NaOH was prepared by adding 4g NaOH to 100mL distilled water. This solution was then diluted twenty times to prepare 0.05N NaOH at the time of titration.

(b) Phenolphthalein indicator: 0.5g phenolphthalein was dissolved in 50mL of 95% ethyl alcohol and 50mL was added. 0.05N NaOH solution was added to it until the solution turned faint pink.

**Procedure:** 50 mL of the sample was taken in a conical flask and few drops of phenolphthalein indicator were added. The absence of free CO\(_2\) was indicated by a color change to pink. When the sample remained colourless, it was titrated with 0.05N NaOH. At the end point, pink color appeared in the solution. Free CO\(_2\) was calculated as:

\[ \text{Free CO}_2 \text{ (mg/L)} = A \times N \times \frac{44 \times 100}{\text{mL of sample}} \]

Where, \( A = \text{mL of titrant used} \)

\( N = \text{Normality of NaOH (0.05)} \)

3.2.1.9 Alkalinity

**Principle:** The alkalinity of water is a measure of its capacity to neutralize acids. Hydroxyl ions present in a sample as a result of dissociation or hydrolysis of solutes react with an addition of standard acid. Alkalinity of a sample can be estimated by titrating with standard sulphuric acid. Titration to pH 8.3 or de-colorization of phenolphthalein indicator indicates complete neutralization of OH\(^-\) and \(\frac{1}{2}\) of CO\(_3\) while to pH 4.5 or sharp
change from yellow to pink of methyl orange indicator indicates total alkalinity (complete neutralization of OH\textsuperscript{−}, CO\textsubscript{3}\textsuperscript{2−}, and HCO\textsubscript{3}).

Reagents
(a) Standard H\textsubscript{2}SO\textsubscript{4} solution (0.02N): 0.1N H\textsubscript{2}SO\textsubscript{4} was prepared by diluting 3mL conc. H\textsubscript{2}SO\textsubscript{4} to 1000mL. From this solution, 100mL volume was then diluted to 1000mL to obtain 0.02N H\textsubscript{2}SO\textsubscript{4}.
(b) Phenolphthalein indicator: 0.5g phenolphthalein was dissolved in 50mL of 95% ethyl alcohol and 50mL was added. 0.05N NaOH solution was added to it until the solution turned faint pink.
(c) Methyl orange indicator: 0.5g methyl orange was dissolved in 100mL of distilled water.

Procedure: 50 mL sample was taken in a 250 mL conical flask and 2 to 3 drops of phenolphthalein indicator was added. In the absence of color, the phenolphthalein alkalinity was recorded as zero. In the presence of pink color, the sample was titrated against 0.02N H\textsubscript{2}SO\textsubscript{4} till the color disappeared and the volume of H\textsubscript{2}SO\textsubscript{4} required was noted (A). Next, 2 to 3 drops of methyl orange were added to the same flask, and titration was continued till orange color changed to pink and again the volume of H\textsubscript{2}SO\textsubscript{4} required was noted (B). Total (T), phenolphthalein (P) and methyl orange (MO) alkalinity were calculated as follows and expressed in mg/L as CaC\textsubscript{0}3:

\[
\text{Phenolphthalein alkalinity (mg/L as CaC03)} = \frac{A}{1000} \\
\text{Methyl orange alkalinity (mg/L as CaC03)} = \frac{B}{1000} \\
\text{Total alkalinity (mg/L as CaC03)} = \frac{(A-B)}{1000}
\]

3.2.1.10 Carbonates and Bicarbonates
Concentrations of carbonates and bicarbonates can be determined from the result of alkalinity titration as follows:
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<table>
<thead>
<tr>
<th>Result of titration</th>
<th>CO\textsubscript{3} alkalinity as CaCO\textsubscript{3}</th>
<th>HCO\textsubscript{3} alkalinity as CaCO\textsubscript{3}</th>
</tr>
</thead>
<tbody>
<tr>
<td>P = 0</td>
<td>0</td>
<td>T</td>
</tr>
<tr>
<td>P &lt; (\frac{1}{2} T)</td>
<td>2P</td>
<td>T−2P</td>
</tr>
<tr>
<td>P = (\frac{1}{2} T)</td>
<td>2P</td>
<td>0</td>
</tr>
<tr>
<td>P &gt; (\frac{1}{2} T)</td>
<td>2 (T−P)</td>
<td>0</td>
</tr>
<tr>
<td>P = T</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Where, P = Phenolphthalein alkalinity and T = Total alkalinity

### 3.2.1.11 Chloride

**Principle:** In a neutral or slightly alkaline solution, potassium chromate indicates the end point of the silver nitrate titration of chloride. Silver chloride is precipitated quantitatively before red silver chromate is formed.

**Reagents**

(a) Standard silver nitrate solution (0.02N): 3.4g of AgNO\textsubscript{3} was dissolved in 1000mL distilled water and was stored in a dark glass bottle.

(b) Potassium chromate indicator solution: 5g of K\textsubscript{2}CrO\textsubscript{4} was dissolved in 100mL distilled water and stored.

**Procedure:** 50 mL sample was taken in a conical flask and 1 mL K\textsubscript{2}CrO\textsubscript{4} indicator solution was added. The contents were titrated against 0.02N AgNO\textsubscript{3} solution until a persistent reddish brown tinge appeared. Titration was also performed for blank with distilled water. The chloride concentration was calculated as:

\[
\text{Chloride, mg/L} = \frac{(A-B) \times N \times 35450}{\text{mL sample}}
\]

Where, A = mL titration for sample

B = mL titration for blank, and

N = normality of AgNO\textsubscript{3}
3.2.1.12 Total Hardness

**Principle:** In alkaline condition ethylene-diamine-tetra-acetic (EDTA) acid or its sodium salt (Na₂EDTA) reacts with Ca and Mg to form a soluble chelated complex. Ca and Mg ions develop wine red colour when small amount of dye such as Eriochrome Black T is added in alkaline condition. When EDTA is added as titrant, the Ca and Mg is complexed with EDTA resulting in sharp change from wine red to blue, which indicates the endpoint of titration. Hardness is normally expressed as CaCO₃.

**Reagents**

(a) Buffer solution (pH 10): 16.9g ammonium chloride was dissolved in 143mL conc. ammonium hydroxide. To this, 1.179g Na₂EDTA plus 0.780g MgSO₄.7H₂O was added and diluted to 250mL. The buffer was stored in tightly stoppered glass bottle.

(b) Inhibitor: 1.5g hydroxylamine hydrochloride was dissolved in 100mL 95% ethyl alcohol.

(c) Standard EDTA solution (0.01M): 3.723g EDTA-disodium salt was dissolved in distilled water and diluted to 1000mL.

(d) Eriochrome black T indicator: 0.5g dye was mixed with 100g NaCl to get dry powder.

**Procedure:** 50mL sample was taken in a conical flask. 2mL buffer solution was added followed by 1mL inhibitor. A pinch of Eriochrome Black T was subsequently added and titrated with standard EDTA (0.01M) till wine red color changed to blue. The volume of EDTA consumed was recorded (A). A reagent blank was run with distilled water and the volume of EDTA was noted (B). The volume of EDTA required by sample was calculated as C = (A-B) and total hardness was calculated as:

\[
\text{Total hardness (as CaCO}_3 \text{ mg/L)} = \frac{\text{ml EDTA used (C) } \times 1000}{\text{mL of sample}}
\]

3.2.1.13 Calcium Hardness

**Principle:** When EDTA is added to water containing both Ca and Mg, it combines first with Ca. Calcium can be determined directly by EDTA, when the pH is made sufficiently high so that Mg is largely precipitated as the hydroxide and an indicator is used that combines with Ca only.

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**Reagents**

(a) Sodium hydroxide solution, 8%: The solution was prepared by dissolving 8g NaOH in 100mL distilled water.

(b) Murexide (ammonium purpurate) indicator: 0.2g of dye was added to 100g NaCl and mixed well.

(c) Standard EDTA solution (0.01M): 3.723g EDTA-disodium salt was dissolved in distilled water and diluted to 1000mL.

**Procedure:** 50 mL water sample was taken in a conical flask and 1 mL of NaOH was added to it to raise pH to 12.0, followed by a pinch of Murexide indicator (0.1 to 0.2 mg). The sample was then titrated immediately against EDTA solution till the pink colour changed to purple and the volume of the EDTA used was recorded. The calculation was made as:

\[
\text{Calcium hardness as } \text{CaCO}_3 \text{ (mg/L)} = \frac{T \times 1000 \times 1.05}{V}
\]

Where, \( T = \) Volume of titrant (EDTA), mL
\( V = \) Volume of sample, mL

3.2.1.14 Magnesium Hardness

Magnesium can be determined by calculating the difference between the total hardness and the calcium hardness of the sample. This yields the value of magnesium hardness as mg/L CaCO₃.

\[
\text{Mg hardness as } \text{CaCO}_3 = \text{Total hardness as } \text{CaCO}_3 - \text{Ca hardness as } \text{CaCO}_3
\]

3.2.1.15 Phosphate

**Principle:** In acidic condition, orthophosphate reacts with ammonium molybdate to form molybdophosphoric acid. It is reduced by stannous chloride to form a blue colored complex. The intensity of the blue color is measured, which is directly proportional to the concentration of phosphate.

**Reagents:**

(a) Stock phosphate solution: 0.3632g anhydrous KH₂PO₄ was dissolved in 500mL distilled water to obtain a solution of concentration 500mg/L.

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(b) Standard phosphate solution: Appropriate dilutions were made from the stock solution to obtain series from a range of 0.1 to 5mg/L.

(c) Ammonium molybdate solution: 12.5g ammonium molybdate was dissolved in 87.5mL distilled water and kept separately. Then, 140ml conc. H₂SO₄ was added to 200mL distilled water and cooled. Both the solutions were then mixed, and the volume made upto 500mL.

(d) Stannous chloride solution: 2.5g SnCl₂ was dissolved in 100mL glycerol by slightly heating on a waterbath.

**Procedure:** 50mL of filtered sample (free from colour and turbidity) was taken in a conical flask. 4 mL of ammonium molybdate reagent was added and mixed thoroughly. Subsequently, 2-3 drops of stannous chloride reagent were added. The optical density of the blue colored solution was measured after 10 min but before 12 min, using the same specific interval for all the subsequent determinations spectrophotometrically at 690nm using distilled water blank. The standard series was prepared in the range of 0.1 to 5.0 mg/L of PO₄-P and the color was developed and measured in the same way as for sample. The concentration of phosphate in the samples was determined with the help of standard curve.

3.2.1.16 Sulphate

**Principle:** Sulphate ion (SO₄²⁻) is precipitated in an acetic acid medium with barium chloride (BaCl₂) so as to form barium sulphate (BaSO₄) crystals of uniform size. The crystal formation is enhanced in the presence of an acetic acid buffer solution containing magnesium chloride, potassium nitrate, sodium acetate and acetic acid. Light absorbance of the BaSO₄ suspension is measured by a photometer and the SO₄²⁻ concentration is determined by comparison of the reading with a standard curve.

**Reagents**

(a) Standard sulphate solution: A standard of 100mg/L SO₄²⁻ concentration was prepared by dissolving 0.1479g anhydrous Na₂SO₄ in 1000mL distilled water.

(b) Buffer solution: The solution was prepared by dissolving 30g MgCl₂.6H₂O, 5g sodium acetate, 1g potassium nitrate and 20mL acetic acid in 500mL distilled water. The volume was then made upto 1000mL.

(c) Barium chloride crystals

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**Procedure:** 100mL volume of filtered sample was taken in the conical flask. 20mL buffer solution was added and stirred. A spoonful of BaCl₂ crystals was then added, and the solution was again stirred for 1min at a constant speed. The optical density reading was taken on a spectrophotometer at 420nm after 5min. The standard series was prepared from a range of 0 to 50mg/L at the interval of 5mg/L. The concentration of sulphate in the samples was determined with the help of a standard curve.

3.2.1.17 Nitrate

**Principle:** Nitrate reacts with phenyl disulphonic acid and produces a nitro derivative which in alkaline solution develops yellow color due to rearrangement of its structure. The colour formed follows Beer’s law and is proportional to the concentration of the nitrate present in the sample.

**Reagents**

(a) Standard silver sulphate solution: 4.4g Ag₂SO₄ was dissolved in distilled water and diluted to 1000mL.

(b) Phenol disulphonic acid (PDA) solution: 25g white phenol was dissolved in 150mL conc. H₂SO₄. Further, 85mL conc.H₂SO₄ was added to it, mixed well and was heated for 2hr.

(c) Stock nitrate solution: 721.8mg anhydrous potassium nitrate was dissolved in distilled water and the volume made up to 1000mL (1mL = 100µg NO₃⁻).

(d) Standard nitrate solution: 50mL of stock nitrate solution was evaporated to dryness on water-bath. The residue was then dissolved in 2mL PDA reagent and diluted to 500mL (1mL = 10µg N).

(e) Ammonium hydroxide: concentrated.

**Procedure:**

(a) Chloride removal: To remove the interference of chloride, an equivalent amount of silver sulphate was added (at the rate of 1mL to remove 1mg of chloride) to the sample, the solution was then filtered and the chloride was precipitated out as AgCl.

(b) Colour development: 50ml of the filtrate was evaporated to dryness in a porcelain basin by keeping on a water bath. The residue was then dissolved in 2mL phenol disulphonic acid reagent and the content was diluted to the original volume (50mL). This was followed by the addition of 5ml of ammonia and the development of yellow color,
the intensity of which was measured spectrometrically at 410nm. The standard series was prepared in a similar way from 0 to 1mg/L. The sample was compared with a standard calibration curve using distilled water blank.

3.2.1.18 Sodium

**Principle:** The estimation of sodium is based on the emission spectroscopy which deals with the excitation of electrons from the ground state to higher energy state and coming back to its original state with the emission of light.

**Reagents**

(a) Standard stock sodium solution (1000ppm): 1.271g of sodium chloride was dissolved in distilled water and the solution was diluted to 500mL to get 1000ppm stock solution.

**Procedure:** The water samples were filtered to remove any suspended particles. Sodium concentration in the water sample was determined by Flame photometer using Na-filter after 1:5 dilution of the filtrate. The standard series was prepared by diluting the standard solution to get a range from 25-100ppm Na. The standard curve was plotted and the sample was compared with standard calibration curve.

3.2.1.19 Potassium

**Principle:** The estimation of potassium is also based on the emission spectroscopy which deals with the excitation of electrons from the ground state to higher energy state and coming back to its original state with the emission of light.

**Reagents**

(a) Standard potassium solution (1000ppm): 0.9533g of potassium chloride was dissolved in distilled water and the solution was diluted to 500mL.

**Procedure:** The water samples were filtered to remove any suspended particles. Potassium concentration in the water sample was determined by Flame photometer using K-filter after 1:5 dilution of the filtrate. The standard series was prepared by diluting the standard solution to get a range from 25-100ppm K. The standard curve was plotted, and the sample was compared with a standard calibration curve.
3.2.2 Geochemical properties

Sample Collection

Geochemical analysis consisted of collection of sediment samples from each site at the two wetlands. The sediment samples were collected with the help of a spade and transferred in polyethylene zip bags and transported to the laboratory. pH of the sediment was first measured. The samples were thereafter air dried and sieved through a sieve of mesh size 20 μm. The samples were then analyzed for parameters like chloride, total organic matter, phosphate, sulphate, nitrate, sodium and potassium according to the standard methods described in Trivedy et al. (1987) and Maiti (2003).

Laboratory Analysis

The following geochemical parameters were analyzed from the sediment samples collected from the two wetlands:

3.2.2.1 pH

*Principle:* pH of soil is the measure of hydrogen ion activity of the soil water system and depends largely on the relative amounts of the adsorbed hydrogen and metallic ions. It is a good measure of the intensity of acidity and alkalinity of sediment-water suspension and provides a good identification of the chemical nature of the sediment. The basic principle of electronic pH measurement is the determination of activity of hydrogen ions by potentiometric measurement using a standard sensing electrode (glass electrode) and a reference electrode (calomel electrode).

*Procedure:* Sediment suspension in a ratio of 1:5 was prepared by taking 20 g of sediment and adding 100mL of distilled water. The sediment solution was stirred for about an hour at regular intervals. The pH of this suspension was then determined using Systronics µ pH system 361.

3.2.2.2 Chloride

*Principle:* Most of the chlorides are soluble in water and can be determined directly in sediment solution by titrating them with silver nitrate using potassium chromate as an indicator. In a neutral or slightly alkaline solution, potassium chromate indicates the end
point of the silver nitrate titration of chloride. Silver chloride is precipitated quantitatively before red silver chromate is formed.

**Reagents**

(a) Standard silver nitrate solution (0.02N): 3.4g of AgNO₃ was dissolved in 1000mL distilled water and was stored in a dark glass bottle.

(b) Potassium chromate indicator solution: 5g of K₂CrO₄ was dissolved in 100mL distilled water and stored.

**Procedure:** A 1:5 sediment suspension was prepared by shaking 20 g of soil in 100mL of aerated distilled water for about an hour. The suspension was then filtered through Whatman No.49 filter paper. 50mL of this filtrate was taken and analyzed for chloride as described in hydrochemical analysis.

### 3.2.2.3 Available Phosphorus

**Principle:** Phosphorus in sediment is generally determined as available phosphorus, which can be extracted from sediment with 0.002N H₂SO₄. In acidic condition, orthophosphate reacts with ammonium molybdate to form molybdophosphoric acid. It is reduced by stannous chloride to form a blue colored complex. The intensity of the blue color is measured, which is directly proportional to the concentration of phosphate.

**Reagents:**

(a) 0.002N H₂SO₄ solution

(b) Stock phosphate solution: 0.3632g anhydrous KH₂PO₄ was dissolved in 500mL distilled water to obtain a solution of concentration 500mg/L.

(c) Standard phosphate solution: Appropriate dilutions were made from the stock solution to obtain series from a range of 0.1 to 5mg/L.

(d) Ammonium molybdate solution: 12.5g ammonium molybdate was dissolved in 87.5mL distilled water and kept separately. Then, 140ml conc. H₂SO₄ was added to 200mL distilled water and cooled. Both the solutions were then mixed, and the volume made up to 500mL.

(e) Stannous chloride solution: 2.5g SnCl₂ was dissolved in 100mL glycerol by slightly heating on a waterbath.

**Procedure:** One gram of air dried sediment was taken in a 500mL conical flask and 200mL of 0.002N H₂SO₄ was added. The suspension was subjected to shake for half an
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hour and was then filtered through Whatman No.49 filter paper. 50mL of this filtrate was taken and analyzed for phosphate as described in hydrochemical analysis.

3.2.2.4 Sulphate

**Principle:** Like chlorides, most of the sulphates are soluble in water and can be directly determined in sediment solution. Sulphate ion (SO$_4^{2-}$) is precipitated in an acetic acid medium with barium chloride (BaCl$_2$) so as to form barium sulphate (BaSO$_4$) crystals of uniform size. The crystal formation is enhanced in the presence of an acetic acid buffer solution containing magnesium chloride, potassium nitrate, sodium acetate and acetic acid. Light absorbance of the BaSO$_4$ suspension is measured by a photometer and the SO$_4^{2-}$ concentration is determined by comparison of the reading with a standard curve.

**Reagents**

(a) Standard sulphate solution: A standard of 100mg/L SO$_4^{2-}$ concentration was prepared by dissolving 0.1479g anhydrous Na$_2$SO$_4$ in 1000mL distilled water.

(b) Buffer solution: The solution was prepared by dissolving 30g MgCl$_2$.6H$_2$O, 5g sodium acetate, 1g potassium nitrate and 20mL acetic acid in 500mL distilled water. The volume was then made up to 1000mL.

(c) Barium chloride crystals

**Procedure:** A 1:5 sediment suspension was prepared by shaking 20g of sediment in 100mL of aerated distilled water for about an hour. The suspension was then filtered through Whatman No.49 filter paper. 100mL of this filtrate was taken and analyzed for sulphate as described in hydrochemical analysis.

3.2.2.5 Nitrate

**Principle:** Nitrate from the soil is extracted with copper sulphate and then is determined following the phenol disulphonic acid method as described for nitrate determination in water.

**Reagents**

(a) 1 N copper sulphate: 1 N copper sulphate solution was prepared by dissolving 125g CuSO$_4$.5H$_2$O in 1 litre distilled water.

(b) 0.6% silver sulphate solution: It was prepared by dissolving 6g of Ag$_2$SO$_4$ in 1 litre of distilled water, 200ml of 1 N CuSO$_4$ solution and 1000ml of 0.6% solution of Ag$_2$SO$_4$ and then diluted to 10 litres.
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(c) All other solutions were same as in determination of nitrate in water by phenol disulphonic acid method, except silver sulphate solution.

(d) Calcium hydroxide \( \text{Ca(OH)}_2 \)

(e) Magnesium carbonate \( \text{MgCO}_3 \)

**Procedure:** 50 g of freshly collected soil was taken in a 500 ml conical flask followed by addition of 250 ml of the nitrate extraction solution. The contents of flask were mixed well for 10 minutes. 0.4 g \( \text{Ca(OH)}_2 \) was added to this and stirred for further 5 min. followed by addition of 1 g \( \text{MgCO}_3 \). These two reagents are added to precipitate Cu and Ag, and clarifying the suspension. The solution was filtered through a dry filter paper; first 20 ml of solution is decanted and the nitrate content in the soil extraction was estimated following the phenoldisulphonic method as described for determination of nitrate in water, except the removal of chlorides by addition of silver sulphate solution.

**Calculation:**

For air dried soil

\[
\% \text{ nitrate} - \text{N} = \frac{\text{NO}_3 - \text{N mg/L of soil extract} \times V}{10000 \times S}
\]

Where, \( V \) = volume of total soil extract prepared (ml)

\( S \) = weight of soil taken

### 3.2.2.6 Total Organic Matter (TOM)

**Principle:** The organic matter of the sediment was estimated by multiplication of organic carbon (OC) concentration by the ratio of TOM to OC commonly found in the sediment. The factor is 1.724. Organic carbon was estimated by Walkley and Black technique of rapid dichromate oxidation. The organic matter (humus) in the sediment gets oxidized by chromic acid (potassium dichromate plus conc. \( \text{H}_2\text{SO}_4 \)). The unreacted dichromate is determined by back titration with ferrous (ammonium) sulphate (redox titration).

**Reagents**

(a) Potassium dichromate (IN): 49.04g of \( \text{K}_2\text{Cr}_2\text{O}_7 \) was dissolved in 1L distilled water.

(b) Ferrous ammonium sulphate (0.5N): 196g hydrated crystalline \( \text{FeSO}_4\cdot(\text{NH}_4)_2\text{SO}_4\cdot6\text{H}_2\text{O} \) per litre containing 20mL of conc. \( \text{H}_2\text{SO}_4 \).

(c) Conc. \( \text{H}_2\text{SO}_4 \)
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(d) Diphenylamine indicator: The indicator was prepared by dissolving 0.5g \((C_6H_5)_2NH\) in a mixture of 20mL of water and 100mL of conc. \(H_2SO_4\).

(e) Ortho-phosphoric acid

**Procedure:** 0.5g of perfectly dried sediment was taken in a 500mL conical flask and 10mL of 1 N \(K_2Cr_2O_7\) was added to it, followed by 20mL of concentrated \(H_2SO_4\) and mixed by gentle swirling. The flask was then allowed to stand for 30 minutes and after the reaction was over, the contents were diluted to 200mL by distilled water. It was followed by the addition of 10mL phosphoric acid and 1mL diphenylamine indicator. The colour of the solution changed to bluish-purple. The contents were then titrated against ferrous ammonium sulphate till the blue colour changed to brilliant green. The % carbon and % organic matter were calculated as:

\[
\text{% Carbon} = \frac{V_1-V_2 \times 0.0C3 \times 100}{W}
\]

\[
\text{% Organic matter} = \frac{\text{% C} \times 1.724}{1}
\]

Where, \(V_1= \) Volume of \(K_2Cr_2O_7\)

\(V_2= \) Volume of the ferrous ammonium sulphate

\(W= \) Weight of the soil taken

3.2.2.7 Sodium

**Principle:** The estimation of sodium is also based on the emission spectroscopy which deals with the excitation of electrons from the ground state to higher energy state and coming back to its original state with the emission of light.

**Reagents**

(b) Ammonium acetate solution (1N): 70mL of reagent grade conc. \(NH_4OH\) was added to 57mL glacial acetic acid and diluted to 1L. The pH of the solution was adjusted to 7.

(c) Standard stock sodium solution (1000ppm): 1.271g of sodium chloride was dissolved in distilled water and the solution was diluted to 500mL to get 1000ppm stock solution.

**Procedure:** 5g of sediment was shaken with 25mL ammonium acetate solution for 5 minute and was filtered immediately through Whatman No.1 filter paper. The first few mL of the filtrate was rejected. Sodium concentration in the sample was determined by...
Flame photometer using Na-filter after 1:25 dilution of the filtrate. The standard series was prepared by diluting the standard solution to get a range from 25-100ppm Na. The standard curve was plotted, and the sample was compared with a standard calibration curve.

3.2.2.8 Potassium

**Principle:** The term available potassium incorporates both exchangeable and water soluble forms of nutrients present in the sediment. The readily exchangeable plus water soluble potassium is determined in the neutral normal ammonium acetate (1N NH₄OAc) extract of sediment. The estimation of potassium is also based on the emission spectroscopy which deals with the excitation of electrons from the ground state to higher energy state and coming back to its original state with the emission of light.

**Reagents**

(d) Ammonium acetate solution (1N): 70mL of reagent grade conc. NH₄OH was added to 57mL glacial acetic acid and diluted to 1L. The pH of the solution was adjusted to 7.

(e) Standard potassium solution (1000ppm): 0.9533g of potassium chloride was dissolved in distilled water and the solution was diluted to 500mL.

**Procedure:** 5g of sediment was shaken with 25mL ammonium acetate solution for 5 minute and was filtered immediately through Whatman No.1 filter paper. The first few mL of the filtrate was rejected. Potassium concentration in the sample was determined by Flame photometer using K-filter after 1:25 dilution of the filtrate. The standard series was prepared by diluting the standard solution to get a range from 25-100ppm K. The standard curve was plotted, and the sample was compared with a standard calibration curve.

3.3 BIOTIC COMPONENTS

3.3.1 Phytoplankton

Phytoplankton samples were collected using plankton nylon net, with sieve size of 10 microns. The samples were preserved in 4% formalin at the field and then transported to the laboratory on ice. Plankton samples were observed under 100X lens in Labomed photo microscope. The plankton were identified according to standard monographs-
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Cyanophyta (Desikachary, 1959), Fresh-Water Biology (Edmondson, 1963) and Indian Freshwater Microalgae (Anand, 1998). Phytoplankton density was determined by Lacky's Drop method (Trivedy et al., 1987).

3.3.2 Coliforms

**Principle**: The coliform group of bacteria comprises all the aerobic and facultative anaerobic gram negative, non-spore forming rod-shaped bacteria, which ferment lactose with gas formation within 48hrs at 35°C. Coliforms are colon bacteria (*Escherichia coli*) residing normally in the human or other warm blooded animals' intestine, which are used as an indicator organism in microbiological examination of water.

**Reagents**
(a) Mac Conkey Broth (Double strength): The double strength broth was prepared by dissolving 40g media in 250mL distilled water.
(b) Mac Conkey Broth (Single strength): The single strength broth was prepared by dissolving 40g media in 500mL distilled water.
(c) Brilliant Green Lactose Bile (BGLB) Broth: The broth was prepared by dissolving 40g BGLB media in 1000mL distilled water.

**Procedure**: The water samples for coliform analysis were collected separately in clean sterile bottles. The bottles were then transported to the laboratory on ice, properly sealed so as to prevent any contamination. Counts of total and fecal coliform were determined by the standard most probable number (MPN) technique. Three sets of five test tubes each were prepared. One set consisted of five tubes with 5ml double strength Mac Conkey broth with Durham's tubes inverted in each tube. The other two sets consisted of 5mL single strength media in each tube with inverted Durham's tube. The tubes of double strength media were inoculated with 10mL of water sample each. In the second and third set with the single strength media, tubes were inoculated with 1mL and 0.1 mL water sample respectively. All the tubes were then kept for incubation at 37°C for 24hrs. The tubes with the bubble formation after 24hrs were marked as positive. For each positive tube, duplicate tubes of 2% Brilliant Green Bile Broth (BGLB) with 5mL media were prepared. From the positive tube, one drop of solution was transferred into both the respective tubes. One set of tubes was incubated at 37°C (Total coliform) and the other
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set at 44°C (Fecal coliform) for 24hrs and the number of positive tubes were recorded after 24hrs. The MPN index/100mL was calculated by referring the standard table.

3.3.3 Vegetation
For the assessment of biotic components, macrophyte study was performed using quadrats of floating type. Three quadrats of size 1 X 1 m were laid at each site in each of the two wetlands. Plant specimens were collected and were identified according to Fresh Water Biology (Edmondson, 1963) and Flora of Gujarat State (Shah, 1978) and their densities was estimated from their proportions in triplicates. The number of species and their individuals were encountered for diversity indices mentioned in page numbers 58 and 59.

3.3.4 Avifauna
Avifaunal study included waterfowl census, including diversity, density and population. The waterfowl counts were made early morning during the monthly visits to the wetlands. Birds observed were counted by motoring once around the perimeter in a small boat. Care was taken not to count twice the birds that flushed ahead of the boat. Additional visits exclusively for waterfowl counts were made in winter months when the migratory birds visited the wetlands. Waterfowls were identified with books like "The Book of Indian Birds" by Ali (1996), "Birds of India" by Samarpan (2006) and "Pocket Guide to the Birds of Indian Subcontinent" by Grimmett et al. 1999. Species richness was defined as the total number of bird species observed throughout the entire sampling period (Hoyer and Canfield, 1994).

3.4 STATISTICAL ANALYSIS
The data was subjected to various statistical analyses. Correlation coefficients matrix was calculated among the various hydro and geochemical properties. Correlation was also established between abiotic and biotic components in the two wetlands. One way ANOVA was performed to signify the variations observed in various parameters analyzed using the software KyPlot Version 2.0 beta 15 (32 bit).
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Algal pollution index according to Palmer (1969) based on genus and species was used in rating the wetland for high or low organic pollution. A pollution index factor was assigned to each genus by determining the relative number of total points scored by each alga genus. The following numerical values followed the classification of Palmer as:

- 0-10 suggests the lack of organic pollution.
- 10-15 indicates moderate pollution.
- 15-20 indicates probable high organic pollution.
- 20 or more confirms high organic pollution.

Diversity indices for different biotic components were calculated using the software BioDiversity-Pro (2000). The data of different biotic components was used for calculating various indices such as Shannon-Weiner (H') (1949), Simpson's Index (D) (1949) and Margaleff’s (M) (1968) using BDPRO (2000) statistical software.

The Shannon-Weiner (1949) diversity index, which gives the ratio between the number of species and the total number of individuals, was estimated by using:

\[ H' = \sum_{i=0}^{S} \log p_i \times \log_2 p_i, \]

where, \( p_i = S/N \)

where, \( S = \) total individual number of a species and \( N = \) total individual number of all species.

The Simpson’s Diversity Index (D) (1949) is calculated as

\[ D = \sum (n_i (n_i - 1)/N (n-1)) \]

where \( n_i = \) number of individuals of species, \( N = \) total number of species in community.

The Margaleff’s index (1968) of species richness in each of the wetland, which is a simple ratio between the total species \( S' \) and total number of individuals \( N' \) was determined by:

\[ SR = S - 1 / \ln N, \]
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Where, $S$ = number of species in each wetland and $N$ = total number of individuals in the sample.

The coefficient of similarity ($S$) was computed between sites following Jaccard (1942):

Coefficient of Similarity ($S$) = \( \frac{C}{A+B-C} \)

Where, $C$ is the no. of common species;
$A$ is the total no. of species at the site, $A$; and
$B$ is the total no. of species at the site $B$.

Material and Methods