3. MATERIAL AND METHODS

Methodologies used for elaborating biodiversity (alpha and beta diversity), allied diversity measures, vegetation studies, physico-chemical analysis of soils and plants material are described in this chapter.

As specific purpose of the present investigation was to examine occurrence and distribution of plant species, their diversity and its related allied diversity measure in relation with habitats levels in upper half of Gujarat coast from Kachchh to Diu, benchmark and well-known field procedures (Kent and Coker, 1994; Smith and Smith, 2001) were followed. In the same way, to make observations more authentic and reasonable, statistically valid sampling designs were adopted and which were subjected to rigorous statistical analysis (Gurumani, 2003; Zar, 2003; Khan and Khanum, 2004).

3.1 FIELD STUDIES

Nine locations, details of which are fully described in section 4.1.1, were selected in Gujarat coast from Kachchh to Diu.

Design of Twin Belt Transect

![Diagram of Twin Belt Transect](image)

Fig. 1
Two twin belts transect (5 m x 50 m) (Figs.1) were laid down near to the creek or seacoast and away from the same at all selected locations. Plant species were counted in five alternative segments (5 m x 5 m) of either of the belts. Thus, ten sample units (5 m x 5 m) – quadrats – from each of twin belt transect as shown in the Fig. 1-3 and thereby total of 20 sample units (from 2 twin belt transects) admeasuring 500 m² at all sites (Figs. 2,3) were consider for computation of the floristic data. Preliminary observations were recorded in data sheets for further evaluation of biodiversity and allied diversity measures.

**Data sheet use for recording floristic observations.**

<table>
<thead>
<tr>
<th>Halophytes</th>
<th>Twin belt transect</th>
<th>Number of individuals of species in alternative units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Q1</td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Continued*

<table>
<thead>
<tr>
<th>Halophytes</th>
<th>Twin belt transect</th>
<th>Number of individuals of species in alternative units</th>
<th>Sub Total (A+B)</th>
<th>Total T1+T2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Q2</td>
<td>Q4</td>
<td>Q6</td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Q = Quadrat*
3.2 DIVERSITY

α diversity measures viz., Shannon index (\(H'\)), Simpson’s reciprocal index (1/D) and Pielou’s evenness index (\(J\)) of halophytic plants were worked out, as they provide vital information about degree of uncertainty/diversity, species richness and species evenness at particular site. Similarity coefficients (Jaccard’s-SCj, Sorensen’s-SCs and Morisita Horn-Cmh indices) comparing community structure and comparative species richness for two habitats were used as measures of β diversity.

Following measures (indices) were used for studying diversity of plants:

### 3.2.1 α-DIVERSITY

**Shannon Index for Diversity** *(Smith and Smith, 2001):*

\[
\text{Diversity } H' = - \sum_{i=1}^{S} p_i \log_{10} p_i
\]

Where, 
- \(S\) = Number of species
- \(p_i\) = Proportion of individuals of the abundance of the \(i^{th}\) species expressed as a proportion of total cover.
- \(\log_{10}\) = log base\(_{10}\)

**Simpson’s Reciprocal Index** for species richness *(Smith and Smith, 2001):*
\[ 1/D = \frac{1}{\sum (ni/N)^2} \]

Where, \( D \) = Simpson’s index
\( ni \) = the total number of individuals of \( i^{th} \) species.
\( N \) = the total number of individuals of all species.

**Pielou’s Index for species evenness (Smith and Smith, 2001):**

\[
J = \frac{H'}{H'_{max}} = \frac{-\sum_{i=1}^{S} pi \log_{10} pi}{\log_{10} S}
\]

Where, \( S \) = the number of species
\( pi \) = the proportion of individuals of the \( i^{th} \) species or
the abundance of the \( i^{th} \) species expressed as a
proportion of total cover.
\( \log_{10} = \log \text{ base } 10 \)

**3.2.2 \( \beta \) - DIVERSITY**

**Jaccard’s Index (SCj) (Smith and Smith, 2001):**

\[
SCj = \frac{C}{A + B - C} \times 100
\]
Where, \[ C = \text{total number of species common at both the sites.} \]
\[ A = \text{total number of species in stand A} \]
\[ B = \text{total number of species in stand B.} \]

The coefficient expresses the **ratio of common species to all species** found in vegetational groups at 2 locations.

**(ii) Sorensen Index (SCs) (Smith and Smith, 2001)**:

\[
SCs = \frac{C}{\frac{1}{2} (A + B)} \times 100
\]

Where, \[ A = \text{total number of species in community A.} \]
\[ B = \text{total number of species in community B.} \]
\[ C = \text{number of species common at both the sites.} \]

The coefficient expresses the **ratio of common species to the average number of species** in vegetational groups at 2 different locations.

**Morisita - Horn Index (Magurran, 1988)**

\[
Cmh = \frac{2 \sum_{i=1}^{S} [(a_{ni})(b_{ni})]}{(da + db)(aN)(bN)} \times 100
\]

Where, \[ S = \text{total number of species at both sites.} \]
\[ aN = \text{the total number of individuals of all species at site A.} \]
\[ bN = \text{the total number of individuals of all species at site B.} \]
\[ ani = \text{the number of individuals of the } i^{th} \text{ species at site A.} \]
\[ bni = \text{the number of individuals of the } i^{th} \text{ species at site B.} \]
and in the denominator, there are two terms summed that are defined as:

\[ da = \frac{\sum_{i=1}^{s} an_i^2}{aN^2} \]

\[ db = \frac{\sum_{i=1}^{s} bn_i^2}{bN^2} \]

### 3.3 ALLIED DIVERSITY MEASURES

Though diversity indices elaborate whole and integrated picture of plant communities constituting vegetation, they do not quantify specific role of individual species. Therefore, parameters like frequency, density and abundance were used as supporting or allied diversity measures for examining composition of halophyte vegetation at half of Gujarat coast from Kachchh to Diu. Following formulae were used for calculating these parameters:

\[ \text{Frequency} = \frac{\text{Number of units in which the species occurred}}{\text{Number of units studied}} \times 100 \]

\[ \text{Density} = \frac{\text{Total number of individuals of a species in all the sample units}}{\text{Total number of sample units studied}} \]
Abundance = Number of individual in all the sample units

Number of sample units in which species occurred

Mean value of 20 sample units – quadrats – belonging to twin belt transects (T1 and T2) were considered and tabulated as under for evaluating all allied diversity measures.

<table>
<thead>
<tr>
<th>Halophytes</th>
<th>Twin belt transect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Freq. %</td>
</tr>
<tr>
<td></td>
<td>T1</td>
</tr>
</tbody>
</table>

3.4 SOIL ANALYSIS

Soil samples (0-15 cm) collected from 1st, 5th and 10th quadrats in single belt transect supporting vegetation, were taken sun dried powder and passed through 20-mesh sieve before analysis.

3.4.1 CHEMICAL ANALYSIS

100 g of soil sample was taken in a conical flask and 200 ml of distilled water was added to prepare (1:2) soil: water suspension (Chopra and Kanwar, 2007) by thorough shaking for 12 hr. on a shaker. The suspension was filtered and the filtrate was made up to 250 ml for further analysis.

Electrical conductivity of 1:2 extracts was measured on EC meter
(model ELICO EC-TDS ANALYSER CM-183), and pH on pH meter (Model ELICO, pH ANALYSER LI-614). Flame Photometer (Elico, model-128) was used for the estimation of sodium (Na\(^+\)) and potassium (K\(^+\)).

Calcium (Ca\(^{2+}\)) and magnesium (Mg\(^{2+}\)) were estimated by EDTA (ethylene diaminotetra acetic acid) method (Vogel, 1978). Preparations of standard calcium and buffer solutions as well as the standardization of EDTA solutions were carried out as under:

**Calcium solution** – 0.5004 g of calcium carbonate was dried at 120 \(^0\)C and dissolved in minimum quantity of diluted hydrochloric acid and final volume was made up to 1 L (1 ml of standard calcium solution = 0.0002004 g Ca\(^{2+}\)).

**Buffer solution** – 67.58 g of ammonium chloride was dissolved in a mixture of 570 ml of ammonia solution (sp. gr. = 0.88) and 250 ml of distilled water. A mixture of 0.931 g EDTA and 0.616 g MgSO\(_4\).7 H\(_2\)O was dissolved in 50 ml of distilled water. These two solutions were mixed together and made up to 1 L.

**EDTA titration** – In order to evaluate the buffer action, 25 ml of distilled water and 4-5 drops of Erichrome Black-T indicator were added to 10 ml of buffer solution. The composite solution was titrated against EDTA solution until the solution colour changed from wine red to purple blue indicating the end point.

50 ml of distilled water, 10 ml buffer solution and 4-5 drops of
Erichrome Black-T were added to 25 ml of standard calcium solution and titrated against EDTA solution to determine the end point. Buffer correction was subtracted from this titre value and correct strength of EDTA was calculated.

25 ml of the 1:2 soil extract was taken, and to which, 50 ml of distilled water, 10 ml of buffer solution and 4-5 drops of Erichrome Black - T were added, and titrated against standard EDTA solution. This value indicated calcium plus magnesium content in the sample.

In another flask, 2 ml of 5N NaOH and a pinch of Patton’s and Reader’s indicator was added to 25 ml (1:2) soil extract and titrated against standard EDTA solution. This titre value indicated only calcium content. It was deducted from the readings of (Ca\(^{2+}\) + Mg\(^{2+}\)) in order to get titre value of Mg\(^{2+}\). The total content of Ca\(^{2+}\) and Mg\(^{2+}\) in (1:2) soil extract were finally calculated.

Chloride (Cl\(^{-}\)) was estimated by argentometric method (APHA, 2005). Preparation of standard sodium chloride as well as silver nitrate were carried out as under:

**Standard Sodium Chloride (0.0141N)** – 824.0 mg NaCl (dried at 140°C) was dissolved in distilled water and diluted to 1000 mL.

**Standard Silver nitrate** – 2.395 g AgNO\(_3\) was dissolved in distilled water and was made upto 1000 ml to prepare 0.0141N AgNO\(_3\).
Potassium chromate indicator – 50 g K₂CrO₄ dissolved in little distilled water. 0.0141N AgNO₃ solution was added until formation of a definite red precipitate. The solution, was allowed to stand 12 h, filtered and diluted to 1 L with distilled water.

25 ml of the 1:2 soil extract was taken, and to which, 1ml of potassium chromate indicator was added, and titrated against standard silver nitrate solution. This titre value was used to calculate total content of Cl⁻ in (1:2) soil extract.

Concentrations of Na⁺, K⁺, Ca²⁺, Mg²⁺ and Cl⁻ have been expressed as milliequivalents (meq) in (1:2) soil extracts of 100 g. dry weight of soils in the text.

3.5 PLANT ANALYSIS

Leaves or phylloclade’s of selected species namely under as category wise: succulent halophytes (Haloxylon salicornicum, Bunge., Salicornia brachiata, Roxb., Sesuvium portulacastrum, Linn., Suaeda fruticosa, (L.) Frossk., Suaeda nudiflora, (Willd.) Moq.; non-succulent halophytes (Aeluropus lagopoides, Trin. ex. Thw., Helechloa setulosa, Trin., Sporobolus madraspatanus, Bor.); shrubby halophytes (Limonium stocksii, (Boiss) Kuntze., Salvadoria persica, Linn.); facultative halophyte (Cyperus conglomeratus, Rottb.); strand species (Halopyrum mucronatum, Stapf., Ipomoea pes-caprae, (L.) R. Br., Lotus garcini, DC., Sericostoma pauciflorum, Stocks., and mangroves (Aegiceras corniculatum, (L.) Blanco., Avicennia marina (Forsk.) Vierh. var. acutissima, Stapf., Rhizophora mucronata, Poir.) were collected from
natural habitats during the year 2008 – 2009.

The material was thoroughly washed to remove dust, mud and salts and blotted to dryness. It was dried in the oven at 75-80 °C to a constant weight. Dry material so obtained, was ground to fine powder and preserved. The material was again dried in oven before using for analysis of mineral ions.

3.5.1 MINERAL IONS

About 1 g plant material was taken into a silica crucible, incinerated and ashes in a muffle furnace at a temperature of 450 - 480 °C. To achieve complete oxidation of the organic matter, about 0.5 - 1 ml of concentrated nitric acid was added to the crucibles after cooling them to room temperature. The acid was evaporated on a water bath and the crucibles were again placed in the furnace for complete ashing.

After cooling at room temperature, 10 ml of 1:1 hydrochloric acid was added to the crucibles containing the ash and was evaporated on water bath. This was followed by addition of 20 ml distilled water and the extract was filtered through Whatman filter paper No. 42 ash less with repeated washing and final volume of 250 ml was made by adding de-ionized water. The aqueous extract was further used for the estimation of sodium (Na⁺) and potassium (K⁺) by flame photometry and calcium (Ca²⁺) and magnesium (Mg²⁺) by EDTA titration.

For estimation of chloride about 1 g of plant material was mixed with about 0.25 g of calcium oxide to make a paste with distilled water.
The moisture was first evaporated on water bath. The plant material was incinerated and ashes as mentioned earlier. Extraction of ash was made with hot deionised double distilled water and it was continued till the extract gave to no reaction with silver nitrate. The extract was further used for estimation of chlorides (APHA, 2005).

### 3.5.2 HEAVY METALS

Soil samples and plant material collected from study sites were also analysed for estimation of heavy metals *viz.*, Fe, Mn, Ni, Zn, Cu and Pb by atomic absorption spectrophotometry.

#### Soils

About 1.00 ± 0.05 g dried soil sample was taken into silica crucible, incinerated and was ignited in a muffle furnace at a temperature of 400 °C before transferring to a 100 ml Teflon beaker. 10 ml of 1:1 diluted hydrochloric acid was added to the sample and was kept on waterbath (60-80 °C for 1 hour). The supernatant was decanted, while, 10 ml of Hydrofluoric acid and 10 ml of Hydrochloric acid were added to the residue, which was evaporated to dryness on waterbath. The last step was repeated once, later on 5 ml of both these acids was added and sample was evaporated to dryness. The residue was dissolved in 10-12 ml of Hydrochloric acid and was combined with the supernatant separated earlier and final volume was made upto 250 ml with distilled water. This extract was further used for estimation of Fe, Mn, Ni, Zn, Cu, Cd and Pb by atomic absorption spectrophotometry (Perkin Elmer Analyst 200 Fig. 4.) (Perkin Elmer Analyst 200 Manual).
Fig. 4. Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 200).

Fig. 5. Gerhardt Digestion Unit (Turbotherm)
Plants:-

100 mg of ground dried plant sample was taken in a Gerhardt digestion tube (Fig. 5) and 7 ml of concentrated nitric acid (HNO₃) was added. After allowing the mixture to stand overnight, it was heated carefully at 150 – 180 °C on a Gerhardt digestion unit (5-6 hrs) until the production of red NO₂ fumes was ceased. After cooling the solution at room temperature, 3 ml of HNO₃ was added and digested second time. This was followed by addition of 4 ml of 70 % perchloric acid (HClO₄) and evaporated to a small volume. The solution was filtered through ashless Whatman No.42 and filtrate was made up to 100 ml with deionised water. The aqueous extract was further used for analysis of heavy metals (Fe, Mn, Ni, Zn, Cu, and Pb) by Atomic Absorption Spectrophotometry (Fig. 4). Since Cu and Pb were not detected in extracts of 100 mg of plant tissue, so 500 mg of plant material was digested in 5 times more volume of the acids (Perkin Elmer Analyst 200 Manual).