Chapter III

MATERIALS AND METHODS
CHAPTER- III: MATERIALS AND METHODS

3.0. Materials and methods

The study was executed from January 2011 to December 2013 for a period of three years in the major wetlands of Barpeta district, Assam to reach to the expected goal.

3.1. Study area

a) Geographical location

The district Barpeta is located in Lower Brahmaputra valley of Assam between 26°5'N and 26°51'N latitudes and 90°38'E and 91°20'E longitudes. The Barpeta district covers an area of 320704 hectares, which is 4.21% of the total area of the state of Assam and 16.05% of the Lower Brahmaputra Valley Zone. The District is surrounded by the Bhutan Hills and Bagsa District in the North, Nalbari District in the East, Kamrup and Goalpara district in the South and Bongaigaon and Chirang District in the west.

b) Physical features

(i) Geology

Geological formation consists of Recent and Sub-Recent alluvial deposits and a thin strip of Upper Tertiary sandstone belonging to the siwalik group associated with clay alternations which occur all along the Bhutan
foothills. The sandstones are light grey with whitish grey, medium grained micaceous and with pebbles at the top.

Recent and Sub-Recent alluvial deposits can be divided into older alluvium and newer alluvium. The older alluvium consists of reddish to brownish impure sands and irregularly distributed pockets of unasserted pebbles covering a considerable area in the northern part of the region. The newer alluvium consists of sands, slits and clays covering the alluvial plains along the Brahmaputra valley.

(ii) Relief

The district has almost plain topography with gentle sloping from north to south. The average elevation of the district varies from 200 m above m.s.l. in north, to 18 m above m.s.l. in south. There are three pre-cambrian hillocks named Baghbar, Phulara and Chatala in the south-western part of the district. These are geologically detached parts of the Meghalaya plateau. The remaining areas of the region are covered by extensive plains and active flood plains of the Brahmaputra and its tributaries.

(iii) Drainage system

The district is drained by the river Brahmaputra and its tributaries. The river Brahmaputra flows from east to west across the Southern part of the district. The tributaries that traverses the district from north to south and then to southwest to merge with the river Brahmaputra are Tihu, Kaldia, Pahumara, Pallah, Beki and Bhalukadoba which originate from Bhutan hills and are perennial in nature. Besides these, there are a number of small streams viz.
Naljhara, Hakua, Bhelengi, Nakhandha, Choulkhowa; abandoned channels and marshy lands.

(iv) **Water bodies**

There are numerous water bodies in the form of wetlands, swamps and marshes in the low-lying areas. Those water bodies were formed due to the southward shifting of the river Brahmaputra. Some of the important wetlands are Kapla, Baria, Finguaparua, Bahuatabha, sagmara, singra, sorbhog, salmara etc. Total wetland area in the district is 59038 ha that includes 195 small wetlands (<2.25 ha). River/stream occupies 93.22% of wetlands. The other major wetland type is Lake/pond (4.48%) and Waterlogged (1.57%). There are 37 Lake/pond (locally called as Beels) with 2644 ha area. Ox-bow lakes occupied 235 ha area (0.4%) (Space Applications Centre Indian Space Research Organisation, 2010).

(v) **Soil**

There are three types of soil in the Barpeta district, viz. New- alluvium, Old -alluvium, and Hill soil. The texture of new alluvium varies mostly from clayey to sandy loam and slightly acidic in reaction. It is deficient in phosphoric acid, nitrogen and humus but rich in lime and potash. In the river banks, the new alluvium is less acidic, sometimes neutral or slightly alkaline. But in the built-up areas it is relatively more acidic. There are narrow patches of the old alluvium along the northern margin of the middle plains of the region. This old alluvium is more clayey and dark coloured with kankery composition and the acidity is relatively higher than the compact new alluvium of the middle plains. The soil of the submontane tract is found in the extreme northern foothill zone. This zone is
subdivided into a narrow Bhabar zone (composed of unassorted detritus), along the piedmont of the Lesser Himalayas and the flat Tarai belt south of the former, spreading to the middle plain of the region. In the Tarai belt water seeps out from the Bhabar zone and hence the zone is full of tall grasses with damp ground.

c) Physiographic Division

The district can be physiographically divided into four regions-the foothill zone, the high plain in the northern part, the built-up mid-plain in the middle part and the low-lying plain in the southern part.

d) Land use and Land cover

The Barpeta district is one of the leading districts in terms of agriculture production. The principal crop of the district is paddy which is grown in almost three seasons with varying degrees, followed by mustard oil seeds, potato, wheat, jute, pulses, vegetables, fruits etc. Almost all seasonal vegetable crops are grown in the district. In the homestead areas bamboo is extensively grown along with areca nut, lemon, jackfruit, mangoes etc. The District has attained a remarkable position in production of vegetables of different varieties under Rabi crops.

e) Vegetation

Different types and sub-types of vegetations ranging from mixed deciduous and semi-evergreen trees to Savannah are found in this region. The
important tree species grown in the district are Bonsum (*Phoebe goalparensis*), Sal (*Shorea robusta*), Teak (*Tectona grandis*), Gomari (*Gmelina arborea*), Nahar (*Mesua ferrea*), Titasopa (*Michelia champaca*), Koroi (*Albizzia procera*), Neem (*Azadirachta indica*) etc. Other species found in the area are *Ficus* sp., *Ziziphus* sp. *Artocarpus* sp. etc. Tall grass species belonging to the genera *Saccharum* sp., *Phramitus* sp., *Arundo* sp. and *Erinthus* sp. are also available. A large number of herbaceous plants are found in cultivated fields, roadsides and waste-land. Some of these plants are *Lantana camara*, *Imperata cylindrica*, *Mimosa pudica*, *Eupatorium odoratum*, *Cynodon dactylon* etc.

The streams in the monsoon period inundate the mixed deciduous riverine vegetation found along the alluvial tracts of the sub-Tarai zone. The primary tree species of this type of area are Kadam (*Anthocephalus cadamba*), Udal (*Sterculia villosa*) Simul (*Bombax malabaricum*), Khair (*Acacia catechu*), Sisoo (*Dalbegia sisoo*). Bamboos (*Bambusa sp.*) are common species found in the low-lying and built-up mid-plain zones. Reeds and grasses are observed in the low-lying regions.

**f) Climate**

The climate of the district is monsoon type. It is characterized by the relative coolness, extreme humidity and heavy summer rainfall and winter drought. The factors controlling the climate of the region are subtropical location, foothills on the north, the river Brahmaputra in the south with hills of south kamrup and Meghalaya adjacent to the southern border and open plains in the west and east. The northern foothill ranges protect the district from cold air
mass of Bhutan and Tibet in winter. The Himalayas provide the conducive orographic condition for relief rainfall in the plains.

(i) Temperature

The average temperature during the months of December to February is about 18.8°C with average diurnal range remaining within 5.5°C. The average temperature during the months of March to May is about 23°C with average diurnal range of about 6.1°C. The average temperature during the months of June to September is about 27.17°C with average diurnal range of about 6°C. The average temperature during the months of October to December is about 20°C with average diurnal range of about 2.8°C to 5.6°C.

(ii) Rainfall

Month of June, July and August experience 70% of total annual rainfall and Month of January and February experience least rainfall of the year. From the later part of month of November to the mid-February prolonged drought like situation prevail. The annual rainfall of the district ranges from 2150 mm. to 2350 mm. The area receives an average rainfall of 1409 mm. During the study period the rainfall pattern of the Barpeta district was recorded from rain gauge station of lower Assam investigation division (under department of water resource, Government of Assam) situated at Barpeta-road. (Table 3.1 and Fig. 3.1)
Table 3.1: Rainfall Data in monthly average (mm) from 2011-2013

<table>
<thead>
<tr>
<th>Months</th>
<th>Year-2011</th>
<th>Year-2012</th>
<th>Year-2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan.</td>
<td>0.2</td>
<td>0.655</td>
<td>0</td>
</tr>
<tr>
<td>Feb.</td>
<td>0</td>
<td>0</td>
<td>0.45</td>
</tr>
<tr>
<td>Mar.</td>
<td>8.6</td>
<td>0.04</td>
<td>0.49</td>
</tr>
<tr>
<td>Apr.</td>
<td>7.28</td>
<td>15.49</td>
<td>6.03</td>
</tr>
<tr>
<td>May.</td>
<td>20.32</td>
<td>13.56</td>
<td>20.69</td>
</tr>
<tr>
<td>Jun.</td>
<td>18.37</td>
<td>53.38</td>
<td>17.66</td>
</tr>
<tr>
<td>July.</td>
<td>22.123</td>
<td>26.39</td>
<td>17.08</td>
</tr>
<tr>
<td>Aug.</td>
<td>41.214</td>
<td>9.38</td>
<td>11.88</td>
</tr>
<tr>
<td>Sept.</td>
<td>15.494</td>
<td>16.09</td>
<td>16.55</td>
</tr>
<tr>
<td>Oct.</td>
<td>4.465</td>
<td>6.36</td>
<td>4.42</td>
</tr>
<tr>
<td>Nov.</td>
<td>4.529</td>
<td>0</td>
<td>0.93</td>
</tr>
<tr>
<td>Dec.</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig 3.1: Monthly avg. Rainfall from year 2011 to 2013
3.2. Study design

In order to collect data on Physico-chemical parameters, major cations, major anions, nutrients, micronutrients, major contaminant, trace metals and toxic metals of water and sediment analysis for estimation of micronutrients, trace metals and toxic metals the study was designed in the following manner to fulfill the purpose of the study.

a. The entire period of data collection included three consecutive years; starting from the month of January, 2011 to December, 2013. The data were collected in Dry (January-March) and Wet (June-September) seasons of each year. Data collection process for all water parameters (except chlorophyll) covers a total period of three years. But, the data on parameters under sediment analysis segment covers a total period of two years starting from January 2012 to December, 2013 and chlorophyll analysis of water was done for only one year i.e. during the year 2013.

b. For accurate characterization of water quality and to give a proper representation of all wetlands present in the Barpeta district, Assam a total of eleven major wetlands were identified. The following criteria were framed for the identification of the wetlands.
   (i) The variety in the surrounding landscape,
   (ii) Dissimilar land use practices of the adjacent areas ,
   (iii) Difference in the total land area covered by the wetlands,
   (iv) Variation in the water covered area during dry and wet seasons,
   (v) Wetlands having recognition and registration under government agencies,
Differences in the potentialities of the aquatic resources and

Importance of public concern from degradation point of view.

The major wetlands covered in the present study are as follows (Table 3.2 and Fig: 3.2):

1) Baria Beel (s1): The wetland is situated in Bamuna, kaiakuchi, and Sundardia area of Barpeta district Assam. The Wetland is registered under Assam Fisheries Development Corporation (AFDC), Government of Assam. The approximate land area covered under this wetland is 56 hectares. The wetland is partially perennial in nature in terms of water spread area and almost dries up during dry season with limited water cover area. The wetland is surrounded by residential area and agricultural fields.

2) Finguaparua Beel (s2): The wetland is situated in Patbaushi area of Barpeta district Assam. It is about three KM away from the district headquarter, Barpeta town. The adjacent areas of the wetland are occupied by residential houses, paddy fields, fishery ponds, brick industry and Kirtan Ghar & Nam Ghar of Patbaushi Satra of famous Assamese Vaishnava saint Srimanta Sankardeva. The Wetland is registered under Assam Fisheries Development Corporation (AFDC), Government of Assam. The approximate land area covered under this wetland is 64 hectares. The wetland is perennial in nature in terms of water spread area. The wetland has witnessed sever encroachment from the local public. Many houses have been constructed in the wetland proper.
3) No.1 Choulkhowa Beel (s3): The wetland is situated in Sarukhetri area of Barpeta Sub-Division. The approximate land area covered under this wetland is 1000 Bighas. Wide spread cultivation of paddy, jute and vegetable are common agricultural practice of the inhabitants in the surrounding areas of the wetlands. The wetland is perennial in nature in terms of water spread area. The Wetland is registered under the office of the Deputy Commissioner, Barpeta, Department of revenue, Government of Assam.

4) No.2 Choulkhowa Beel (s4): The wetland is situated in Nagaon and Pakabetbari area of Barpeta district, Assam. The approximate land area covered under this wetland is 836 Bighas 2 katha 5 lechas. The Wetland is registered under the office of the Deputy Commissioner, Barpeta, Department of revenue, Government of Assam. The wetland is surrounded by residential area and agricultural fields.

5) Kukarjan Beel (s5): The wetland is situated in Sarukhetri, Chenga and Bagri area of Kukarpar in Barpeta district, Assam. The approximate land area covered under this wetland is 110 hectare. The Wetland is registered under Assam Fisheries Development Corporation (AFDC), Government of Assam. The wetland is perennial in nature in terms of water spread area although large scale reduction in the water spread area is noticed during dry seasons. Market, residential houses, homestead gardens and paddy fields are found in the vicinity of the wetland. Large scale use of water of the wetland has been noticed for washing clothes, utensils, bathing, washing cars, and irrigation purposes. Dumping of waste
6) Kapla Beel(s6): The wetland is situated in Barkapla and Boniakuchi area of Sarthebari revenue circle of Barpeta District, Assam. The approximate land area covered under this wetland is 91 hectares. The Wetland is registered under Assam Fisheries Development Corporation (AFDC), Government of Assam. The wetland is perennial in nature in terms of water spread area. The beel is surrounded by numerous villages such as kapla, Haldhibari, Sinadi, Baniakuchi, Helaypara, Amrikhowa and Churchuria. Some other wetlands such as Barkana beel and Salmara beel are also found at its adjoining areas located in the south eastern direction and having a direct communication especially in the monsoon seasons. A large section of the people of the surrounding villages depends directly or indirectly on the resources of the beel. The dependent people earn their livelihood by capturing fishes and cultivating paddy in the wetland proper and adjacent areas. During monsoon seasons the kapla beel get connected with Nakhanda and Chaolkhowa river which in turn connect with the river Brahmaputra. A large numbers of resident and migratory birds of different species are always witnessed in the beel area.

7) Barkana Beel(s7): The wetland is situated in the Amrikhowa, Churchuria and Sinadi area of Sarukhetri revenue Circle, Barpeta District, Assam. The approximate land area covered under this wetland is 267 hectares. The Wetland is registered under Assam Fisheries Development Corporation (AFDC), Government of Assam. The wetland is partially
perennial in nature in terms of water cover and almost dries up during dry season with limited water spread area. Numerous fishery ponds have been constructed by the local people in the peripheral areas of the wetland and paddy cultivation at its marginal areas is a common practice of the locality. During monsoon season the wetland get connected with the kapla beel due to over flooding.

8) Bahua-tabha Beel(s8): The wetland is situated in the Bamundi, Ara and Baksara gaon area of Sarukhetri revenue Circle, Barpeta District, Assam. The approximate land area covered under this wetland is 62 hectares. The Wetland is registered under Assam Fisheries Development Corporation (AFDC), Government of Assam. The wetland is partially perennial in nature in terms of water cover and almost dries up during dry season with limited water spread area. The adjacent areas of wetland are primarily used for agricultural and dwelling purposes. An ashram called “Nasatra Krishnaguru ashram” with prayer hall, school, college, guest house, hostel, market etc. are present at its vicinity and practice of dumping of waste materials of the ashram and other inhabitants of the locality is a common feature.

9) Singra Beel (s9): The wetland is situated in Gohia village of Chega revenue circle of Barpeta District, Assam. The approximate land area covered under this wetland is 53 hectares. The Wetland is registered under Assam Fisheries Development Corporation (AFDC), Government of Assam. The wetland is partially perennial in nature in terms of water cover and almost dries up during dry season with limited water spread
area. The adjacent areas of wetland are primarily used for agricultural and dwelling purposes. Large scale encroachment has gradually reduced the wetland area and practice of burning of human dead bodies in the adjacent areas of the beel is a common practice of the locality.

10) Hahchora Beel (s10): The wetland is situated in Hahchora village of Chega revenue circle of Barpeta District, Assam. The approximate land area covered under this wetland is 53 hectares. The Wetland is registered under Assam Fisheries Development Corporation (AFDC), Government of Assam. The wetland is seasonal in nature in terms of water cover and almost dries up during dry season with limited water spread area. Practice of paddy cultivation in the beel proper and adjoining areas is a common feature. Constructions of houses in the wetland area have posed threatened to the existence of the Hahchora Beel in the form of encroachment.

11) Sorbhog Beel (s11): The wetland is situated in the Puthimari village area of Barnagar revenue circle of Barpeta District, Assam. The approximate land area covered under this wetland is 60 hectares. The Wetland is registered under Assam Fisheries Development Corporation (AFDC), Government of Assam. The wetland is perennial in nature in terms of water cover. Practice of paddy cultivation in the adjoining areas is a common feature. Bamboo and Battlenut trees are the dominant plant species present in the peripheral areas of the wetland. The wetland is recognized as one of the most important wetland of Barpeta district of Assam because of its faunal composition. The wetland apart from
contributing significantly as major source of fisheries to the local areas it is a breeding and feeding ground of numerous migratory birds and source of irrigation for the nearby agricultural fields. Presently the Sorbhog Beel is under tremendous pressure from waste disposal and encroachment and thus turned into a degraded wetland.

Table 3.2:- Identity of the 11 wetlands with geographical coordinate

<table>
<thead>
<tr>
<th>Sl. Nos.</th>
<th>Identity of the Sampling sites</th>
<th>Names of Wetlands</th>
<th>Geographical Locations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Latitude (North)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Degree</td>
</tr>
<tr>
<td>1</td>
<td>S1</td>
<td>Baria Beel</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>S2</td>
<td>FinguaParua Beel</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>S3</td>
<td>NO.1 Choulkhowa Beel</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>S4</td>
<td>No.2 Choulkhowa Beel</td>
<td>26</td>
</tr>
<tr>
<td>5</td>
<td>S5</td>
<td>kukarjan Beel</td>
<td>26</td>
</tr>
<tr>
<td>6</td>
<td>S6</td>
<td>Kapla Beel</td>
<td>26</td>
</tr>
<tr>
<td>7</td>
<td>S7</td>
<td>Barkana Beel</td>
<td>26</td>
</tr>
<tr>
<td>8</td>
<td>S8</td>
<td>Bahua-tabha Beel</td>
<td>26</td>
</tr>
<tr>
<td>9</td>
<td>S9</td>
<td>Singra Beel</td>
<td>26</td>
</tr>
<tr>
<td>10</td>
<td>S10</td>
<td>Hahchora Beel</td>
<td>26</td>
</tr>
<tr>
<td>11</td>
<td>S11</td>
<td>Sorbhog Beel</td>
<td>26</td>
</tr>
</tbody>
</table>
Fig 3.2: Map showing the 11 major wetlands in Barpeta district, Assam
c. For proper characterization of the water quality of the wetlands covered under the present study, water and sediment samples were collected within an interval of 20 to 30 days, during day hours, randomly from different directions of the wetland, in both the dry and wet seasons. Minimum three samples were collected in each season with a total sample of 24 for three years for water, 12 for two years for sediments and six for one year for chlorophyll of water, from each wetland. The minimum time involved in sampling of eleven wetlands was of duration 66 hours in a year.

3.3. Sample collection:

a) Water collection: Water samples were collected with specific precautions, from the surface zone at random, covering different spots of the sampling sites at the respective wetland. Collections were done in thoroughly cleaned plastic bottles. Before used, the bottles were cleaned with chromic acid solution and rinsed several times with distilled water. At the sampling site, the bottles were rinsed 3 to 4 times with sample water.

b) Sediment collection: The surface sediments were collected from different directions of the wetland bed, below the water level. The collected sediments from different sites of the wetland were mixed thoroughly to prepare a composite and representative sample of each wetland. These were dried overnight at 70°C in an air oven and were grinded in a manual mortar. The sediment mixture was sieved with a 230 mesh size strainer to obtain particles of relatively uniform size.
3.4. Sample pretreatment and storage:

(i) a). Pretreatment of water: Immediate analysis of few parameters of water is essential without involving any pretreatment and storage for accurate characterization of water sample. The parameters that need immediate analysis are temperature, pH, turbidity, alkalinity, conductivity, total suspended solids, total dissolved solids, total solids and residual chlorine. These parameters were analysed within 24 hours of sample collection. The parameters like $SO_4^{2-}$, $PO_4^{3-}$, $HCO_3^-$ were measured within one week after preservation of the samples, according to standard methods (APHA, 1995).

Since values of dissolved gas such as oxygen may get reduced due to transit, temperature change and standing for a length of time, it was fixed on the spot by following azide modification method. Adsorption or ion exchange with the wall of the glass containers leads to loss of certain cations such as aluminium, cadmium, chromium, copper, iron, lead, manganese, silver and zinc. To overcome the problem of probable loss of cations during storage, water samples were immediately acidified to a pH $< 2.0$ (APHA,1995) by adding 1.5 ml. concentrated HNO$_3$ /L or an appropriate volume required to achieve the desired pH. After acidification, the samples were stored in a refrigerator at $\sim 4^\circ$C. But, determination of some parameters needs storage before analysis. The water parameters may be affected during storage by some factors and therefore pretreatment of water is necessary for storage. The factors affecting storage of water samples are as follows:

i) Colour, odour and turbidity may get change in quality.
ii) Iron and manganese are insoluble in their higher oxidation states but readily soluble in their lower oxidation states, therefore they may precipitate or may dissolve from sediment, depending on the redox potential of the sample.

iii) The changes in the nitrate-nitrite-ammonium content results from microbiological activity and thus may lead to decrease in phenol concentration and in BOD, or for reducing sulfate to sulfide.

iv) Residual chlorine may be reduced to chloride due to over length storage.

v) As a result of oxidation, Sulfide, sulfite, ferrous iron, iodide and cyanide may get lost.

vi) Calcium carbonate may get precipitate and cause a decrease in the values for calcium and for total hardness with changes in pH-alkalinity-carbon dioxide balance.

vii) Sodium, silica and boron may leach into the water sample from the glass container.

viii) Hexavalent chromium may be reduced to chromic ion on standing.

ix) Oxidation states of some constituents may get alter due to change of biology inside the water sample.

The general technique of sample preservation (APHA, 2005) and the precautions taken before analysis of few specific parameters are given in Table-3.3.
Table 3.3 - Methods of sample preservation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Volume (ml)</th>
<th>Container</th>
<th>Preservation</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>--</td>
<td>Polythene, Glass</td>
<td>Analyze immediately</td>
</tr>
<tr>
<td>Turbidity</td>
<td>--</td>
<td>Polythene, Glass</td>
<td>Analyze same day; store in dark up to 24 h, refrigerate</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>200</td>
<td>Polythene, Glass</td>
<td>Storage at 4°C in dark.</td>
</tr>
<tr>
<td>Conductivity</td>
<td>500</td>
<td>Polythene, Glass</td>
<td>Refrigerate, Storage at 4°C in dark.</td>
</tr>
<tr>
<td>Hardness</td>
<td>100</td>
<td>Polythene, Glass</td>
<td>Add HNO₃ to pH &lt; 2</td>
</tr>
<tr>
<td>TDS</td>
<td>--</td>
<td>Polythene, Glass</td>
<td>Analyze immediately</td>
</tr>
<tr>
<td>Chlorine, residual</td>
<td>500</td>
<td>Polythene, Glass</td>
<td>Analysed immediately</td>
</tr>
<tr>
<td>Sulphate</td>
<td>--</td>
<td>Polythene, Glass</td>
<td>Refrigerate</td>
</tr>
<tr>
<td>Phosphate</td>
<td>100</td>
<td>Glass, rinsed with 1+1 HNO₃</td>
<td>For dissolved phosphate filter immediately; refrigerate</td>
</tr>
<tr>
<td>Nitrate</td>
<td>100</td>
<td>Polythene, Glass</td>
<td>Analyze as soon as possible or refrigerate</td>
</tr>
<tr>
<td>DO</td>
<td>300</td>
<td>DO bottle, glass</td>
<td>Fixed at the time of sample collection and titration may be delayed.</td>
</tr>
<tr>
<td>BOD</td>
<td>1000</td>
<td>Polythene, Glass</td>
<td>Refrigeration at 4°C in dark</td>
</tr>
<tr>
<td>COD</td>
<td>100</td>
<td>Polythene, Glass</td>
<td>Add HCl to pH&lt;2.0 and analyze as soon as possible</td>
</tr>
<tr>
<td>Chlorophyll</td>
<td>500</td>
<td>Polythene, Glass</td>
<td>30 days in dark</td>
</tr>
<tr>
<td>Oil and grease</td>
<td>1000</td>
<td>Glass, wide-mouth calibrated</td>
<td>Add H₂SO₄ to pH &lt;2, refrigerate</td>
</tr>
<tr>
<td>Metals</td>
<td>--</td>
<td>Polythene or Glass rinsed with 1+1 HNO₃</td>
<td>Add HNO₃ to pH &lt; 2</td>
</tr>
</tbody>
</table>

b) Pretreatment of sediment: Sediment sample of one gm weight was taken in a beaker and was mixed with the appropriate amount of triple acid [H₂SO₄ (Sp. gravity = 1.84): HCl (Sp. Gravity = 1.19): HNO₃ (Sp. Gravity =
37

1.40) = 4: 2: 1), evaporated to dryness over a heating plate at 100°C till the sediments become white after volatilization of organic compounds. The residue was put in 20 ml 1:1 HCl and was kept overnight; filtered and the volume was made up to 100 ml; this was analysed for metal content in the Atomic Absorption Spectrometer.

3.5. Methods for analysis of different parameters

In order to analyse all the parameters, standards methods (APHA, 2005) were adopted.

3.5.1 Physico-chemical parameters: The methods adopted for analysis of physicochemical parameters of water samples are as follows:

(i) Temperature: The Water temperatures were measured in the sampling site with a mercury in-glass thermometer graduated 0°C to 100°C by directly immersing it 10 cm into water.

(ii) pH: pH of water was measured at the sampling site with an electronic portable pH meter after calibrating it with phosphate buffer of known pH value. The data were further confirmed immediately with a digital pH meter (Elico pH-Meter, Model LI 120; Electrode type-CL-51B) using standard buffers for calibration purposes at the laboratory.

(iii) Bicarbonate alkalinity: Alkalinity of the water samples was determined by titrating against 0.1(N) HCl acid using methyl orange as the indicator. The carbonate alkalinity of the water sample was found to be zero as there was no change in colour with the phenolphthalein indicator. The total alkalinity of the water sample was determined as follows:
Total alkalinity (mg/L) = \[
\frac{[(V \times N) \times 1000 \times 50]}{\text{ml of water sample}}
\]

Where, \( V \) = total volume (in ml) of HCl used in the titration with methyl orange indicator, \( N \) = Normality of HCl solution.

(iv) Turbidity: Turbidity of the water samples was determined with the help of digital turbidity meter (Model- Decibel – DB-1126). The values were calibrated with respect to a set of formazine suspensions (Hydrazine sulphate, \((\text{NH}_2)_2 \cdot \text{H}_2\text{SO}_4\) and Hexamethylene tetramine, \((\text{CH}_2)_6\text{N}_4\)) of known turbidity and are expressed in nepheloturbidity units. (NTU).

(v) Electrical Conductivity: Electrical Conductivity (EC) of the water samples in mS/cm was measured with a digital conductivity bridge (Simtronics, Model-SE 976) and a conductivity cell of cell constant 1.0 was used.

(vi) Total solids (TS): 100 ml of well mixed unfiltered sample was taken in a pre-weighed Borosil beaker. The water was evaporated to dryness on a hot plate and then it was kept inside an oven at 103\(^{\circ}\)C - 105\(^{\circ}\)C. After that it was allowed to cool and then kept in a desiccator. The process was repeated till the attainment of a constant weight. The increased in the weight of the beaker was the total solids present in the sample. The following expression was used to calculate the value.

\[
\text{Total solids (mg/L)} = \frac{(A - B) \times 10^6}{V}
\]

Where \( A \) = Final weight of the beaker along with the residue in mg.

\( B \) = Initial weight of the beaker in mg.

\( V \) = Volume of the sample in ml.
(vii) Total dissolved solids (TDS): Total dissolved solids present in water sample was estimated by filtering well-mixed 100 ml sample through a standard glass filter and by evaporating the filtrate to dryness in a pre-weighed borosil beaker on a hot plate. The residue obtained in the beaker was then dried in an oven at 103\(^0\)C-105\(^0\)C till a constant weight was observed. The increase in the beaker weight represents the total dissolved solids. The values were expressed in mg l\(^{-1}\) using the following formula.

\[
TDS, (mg/L) = \frac{(A - B) \times 10^6}{V}
\]

Where,

\(A\) = Final weight of the beaker and residue in mg.

\(B\) = Initial weight of the beaker in mg.

\(V\) = Volume of the sample taken in ml.

(viii) Total suspended solids (TSS): The total suspended solid present in the water sample was determined by subtracting TDS value from TS value. The value of TSS is expressed in mg/l.

(ix) Redox potential: The redox potential of the water samples was measured with Elico pH-Meter,(Model LI 120; Electrode type-CL-51B) using standard glass electrode and calomel reference electrode.

(x) Total hardness: Total hardness of the water sample is expressed as milligram calcium carbonate per litre. It is measured by complexometric titration method using EDTA as the chelating ligand and Eriochrome black T as the indicator. Disodium salt of EDTA (Ethylenediaminetetraacetic acid) forms a soluble chelated complex with certain metal cations. Eriochrome Black T dye is used as an indicator which when
added to an aqueous solution containing calcium and magnesium ions at pH of 10.0 ± 0.1, gives wine red colour to the solution. On addition of EDTA solution, calcium and magnesium will be complexed, and when they are completely complexed, the solution turns from wine red to blue indicating the end point of the titration. Magnesium ion must be present to yield a satisfactory end point. To insure this, a small amount of complexometrically neutral magnesium salt of EDTA is added to the buffer; this automatically introduces sufficient magnesium and obviates the need for a blank correction. In a separate set of experiments, calcium hardness is determined by following the same procedure as in case of total hardness but using the indicator, Murexide. In this case, the end point is determined with change of colour from purple to pink. Magnesium hardness was calculated by subtracting the value of calcium hardness from total hardness.

(a) Total hardness (as mg/L CaCO₃) = \[\text{ml of EDTA used} \times 1000 / \text{ml of sample}\]

(b) Ca hardness (as mg/L CaCO₃) = \[\text{ml of EDTA used} \times 400.8 / \text{ml of sample}\]

(c) Mg hardness (as mg/L CaCO₃) = \[\text{Total hardness} (\text{as mg/L CaCO}_3) - \text{Ca hardness (as mg/L CaCO}_3)\] x 0.243.

(xi) Dissolved oxygen (DO): Amount of DO in natural water is a measure of physical, chemical and biochemical interaction taking place in water body. As DO in water can loss easily, it should be determined at the spot or fixed at the time of collection. DO in the water samples of wetlands of
Barpeta district was determined by azide modification method. The principle of the method is based on oxidation of Mn$^{+2}$ to Mn$^{+4}$ in alkaline medium by DO in water and then oxidation of I$^{-}$ to free iodine in acidic medium by Mn$^{+4}$. The amount of iodine released was measured by titrating it against standard solution of sodium thiosulphate. The volume of sodium thiosulphate solution used up is equivalent to DO originally present in the water sample. In this method, alkaline azide is used to destroy the interference of oxidizing agents present in water.

DO was fixed in the field by adding 2 ml alkali iodide azide (700 gm. NaOH and 150 gm. KI were dissolved in distilled water which is diluted to 1 litre. To this solution 10 gm. NaN$_3$ dissolved in 40 ml. distilled water was added) and 2 ml of manganous sulphate solution (344 gm. MnSO$_4$.4H$_2$O was dissolved in distilled water, filtered and diluted to 1 litre.) to the collected water sample, in a 300 ml BOD bottle. Titration was carried out in the laboratory with 0.025(N) Na$_2$S$_2$O$_3$ after dissolving the precipitate in 2 ml of conc. H$_2$SO$_4$. During collection of the sample the bottle was filled to overflowing ensuring that no air bubbles were trapped in the bottle.

\[
\text{Dissolve Oxygen (mg l}^{-1}) = \frac{(ml \times N)\text{of titrant} \times 8 \times 1000}{V_2 \left(\frac{V_1 - v}{V_1}\right)}
\]

Where,

\[V_1 = \text{Volume of sample bottle after placing the stopper}\]

\[V_2 = \text{Volume of the part of the contents titrated}\]

\[v = \text{Volume of MnSO}_4 \text{ and NaOH + KI added}\]
(xii) Biological Oxygen Demand (BOD): It is defined as the amount of oxygen required by the microorganism for stabilizing biologically decomposable organic matter. This test is used to determine the pollution load of waste water, the degree of pollution and efficiency of wastewater treatment methods. The biochemical oxygen demand (BOD) test measures the oxygen utilization during a specified incubation period for the biochemical degradation of organic material and the oxygen used to oxidize inorganic material such as sulphides, and ferrous irons (APHA, 1989). It may also measure the oxygen used to oxidize reduced forms of nitrogen unless an inhibitor prevents their oxidation.

In this method 300 ml. of the sample was collected in the BOD bottle and incubated at $27^\circ$C (BOD incubator, NABFIT) for three days. DO was measured after incubation. BOD was computed from the difference between initial and final DO.

$$\text{BOD (mg/L)} = \text{Pre-incubation DO} - \text{Post Incubation DO}$$

Caution: In case when BOD value is expected to be higher small amount of sample should be taken and diluted in multiples of its volume by adding BOD free distilled water. Although the BOD is determined by the above process but the result should be multiplied by its dilution factor.

(xiii) Chemical Oxygen Demand (COD): It is the measure of oxygen equivalent to the organic content of the sample that is susceptible to oxidation by a strong chemical oxidant. It is measured by the open reflux method. In this method the organic matter in the sample gets oxidized completely by strong oxidizing agents such as potassium dichromate in
the presence of concentrated sulphuric acid to produce CO\(_2\) and water.
The excess potassium dichromate remaining after the reaction is titrated
with ferrous ammonium sulphate using ferroin indicator. The dichromate
consumed gives the oxygen required for the oxidation of the organic
matter.

In this method 10 ml of water sample is mixed with 20 ml 0.25M
K\(_2\)Cr\(_2\)O\(_7\) solution, 30 ml. conc. H\(_2\)SO\(_4\) and a pinch of silver sulphate. The
mixture is refluxed for 2 hours, cooled, 80 ml. of distilled water is added
and titrated with 0.1 (M) Ferrous-ammonium sulphate (FAS) solution
using ferroin indicator. The whole procedure is repeated with a blank
taking 10 ml of distilled water in place of sample water. COD is
computed from the following formula:

\[
COD, (mg/L) = \frac{(X_1 - X_2) \times \text{Molarity of FAS} \times 8000}{V}
\]

\(X_1\) = Volume of FAS consumed for Blank titration

\(X_2\) = Volume of FAS consumed by the sample

\(V\) = Volume of the sample taken

(xiv) Chlorophyll: Chlorophyll measurement is an indirect method of
measurement of photosynthesizing plants present in water sample. In the
water samples of lakes, algae and phytoplankton represent the
photosynthesizing plant community and estimation of chlorophyll gives
the measurement of all green pigments whether active or dead.
Chlorophyll being essentially involved in photosynthesis their concentration is considered to be a very good index of primary production. In the water samples three varieties of chlorophylls are found to be present. They are Chlorophyll a, Chlorophyll b and Chlorophyll c. Chlorophyll a is considered to be the most important phytopigment because of its occurrence in all plants. The distribution of chlorophyll a, b, c in some important algal groups is as follows: Cyanophyceae-only a; Chlorphyceae-a,b; and Bacillariophyceae-a,c. Phaeopigments are the degradation products of chlorophylls and they increased with the age of the plants. A mixture of 90% acetone and 1% MgCO₃ was used to extract chlorophyll from the water sample. The water sample with the mixture was kept in the refrigerator for six hours. The extract was taken out and centrifuged at a 3000 rpm. for about 15 minutes. The supernatant liquid was further mixed with 10ml. 90% acetone. The Optical density of the extract was recorded on a Spectrophotometer (UV-VIS spectrophotometer; Systronics, Model no-2205) at 630 nm., 645 nm., 663 nm., and chlorophyll content of the sample was estimated by using the following expression (Saxena, 2001):

\[
\text{Chlorophyll-a (\(\mu g/l\) or \(mg/m^3\))} = AX \frac{V_2}{V_1 X l} \\
\text{Chlorophyll-b (\(\mu g/l\) or \(mg/m^3\))} = BX \frac{V_2}{V_1 X l} \\
\text{Chlorophyll-c (\(\mu g/l\) or \(mg/m^3\))} = CX \frac{V_2}{V_1 X l}
\]

Where, 

\[
A = [11.64 X (OD at 663nm.) - 2.16 X (OD at 645nm.) + 0.1 X (OD at 630 nm.)]
\]

\[
B = [20.97 X (OD at 645nm.) - 3.94 X (OD at 663nm.) - 3.66 X (OD at 630 nm.)]
\]
C = [54.22 X (OD at 630nm.) - 14.81 X (OD at 645nm.) - 5.53 X (OD at 663 nm.)]

\[ V_1 = \text{Volume of the sample filtered} \]

\[ V_2 = \text{Volume of the extract} \]

\[ L = \text{Path length of the cuvette of the Spectrophotometer}. \]

3.5.2 Major Cations:

The major cations analysed in the present study includes the following:

(i) Sodium and Potassium: The quantity of sodium and potassium present in sample were estimated with the help of Flame Adsorption Photometer (Elico, Model-CL-361), using the sodium-potassium tertrate standards in five different concentrations for calibration purposes.

(ii) Calcium and Magnesium: Calcium and Magnesium were estimated by complexometric titration method using EDTA as titrant, as described earlier in the method for determination of hardness of water sample.

3.5.3 Major anions:

The major anions analysed in the present study includes the following:

(i) Carbonate/ Bicarbonate: The method for determination of Carbonate/Bicarbonate was already mentioned in the methodology for the estimation of bicarbonate alkalinity. Amount of Bicarbonate ion in the sample is determined by the following expression (Saxena, 2001):

\[ \text{HCO}_3^- \text{ (mg/l)} = \text{Bicarbonate alkalinity X 1.22 (in CaCO}_3, \text{ mg/l)} \]
(ii) Sulphate: Sulphate content of the sample was measured by Turbidimetric Method using a UV-visible spectrophotometer (UV-VIS spectrophotometer; Systronics, Model no-2205). To 50 ml. of water sample 2 ml. of conditioning reagent (75 g of NaCl, 30 ml conc. HCl, 100 ml 95 % ethyl alcohol and 300 ml distilled water mixed together) was added followed by addition of approximately 1 g of solid BaCl$_2$. The solution was continuously stirred by a magnetic stirrer and when turbidity due to precipitation of BaSO$_4$ develops, it was measured at 420 nm wavelength after 4 minutes of precipitation. Following the same procedure the concentrations of five different standard potassium sulphate solutions were measured and a calibration curve was prepared for determination of sulphate concentration in the water sample.

(iii). Chloride: Estimation of chloride content in the water was done by using argentometric method (APHA, 1995). In this method 50 ml. of the sample was taken in a conical flux and to it 2ml. of 5% of Potassium chromate solution (5% solution- 5 g potassium chromate in 100ml. of the solution in distilled water) was added. The mixture was then titrated against 0.02 N Silver nitrate solution. At the end point all chloride ion get precipitated as silver chloride and free Ag$^+$ ions react with chromate ion to form red brown precipitate of silver chromate. Chloride ion content in the sample is given by

\[
Chloride \ (mg/L) = \frac{V_1 \times N \times 1000 \times 35.5}{V_2}
\]

Where, \( V_1 \) = Volume of AgNO$_3$ used
\[ N = \text{Concentration of AgNO}_3 \text{ in normality} \]

\[ V_2 = \text{Volume of the sample taken} \]

### 3.5.4 Nutrients:

The nutrients analysed in the present study includes the following:

(i). **Nitrate**: Nitrate was determined by UV Spectrophotometric screening method (Hitachi 3210 UV-Visible spectrophotometer). By using UV absorption at 220 nm, rapid estimation of nitrate was done. Since, dissolved organic matter also absorb at 220 nm wavelength, a second measurement at 275 nm was used to correct the nitrate value. This wavelength was absorb by only organic matter but not by the nitrate. The nitrate content is determined from a standard calibration curve.

(ii) **Phosphate**: Phosphate content in the water was estimated by Stannous Chloride method. In this method 50 ml of the clear and colourless water sample was taken in a conical flask, followed by addition of 2 ml of ammonium molybdate solution (25g of \((\text{NH}_4)_6\text{Mo}_7\text{O}_{24}.4\text{H}_2\text{O}\) in 175 ml distilled water, mixed with 280 ml concentrated \(\text{H}_2\text{SO}_4\) and diluted to 1 liter) and 5 drops of stannous chloride reagent (2.5g \(\text{SnCl}_2.2\text{H}_2\text{O}\) in 100 mL glycerol, dissolved by heating in a water bath with constant stirring). A blue colour develops and the optical density is measured at 690 nm (Sistronics -UV-VIS-Double Beam Spectrophotometer-2205) after 5 minutes but before 12 minutes of the addition of stannous chloride reagent. The concentration of phosphate was determined with the help of
a standard curve obtained with standard phosphate solutions (KH$_2$PO$_4$) of at least five different concentrations in the equivalent range.

3.5.5 Contaminants:

The contaminants analysed in the present study include the following:

(i). Oil and Grease: Oil and grease content in the water samples were estimated by Soxhlet extraction with dichloro methane, followed by evaporation at 70°C (APHA, 1995). The following expression was used to find out the oil and grease content in the sample.

$$\text{Oil and grease (mg/L)} = \frac{(A - B) \times 1000}{V}$$

Where, $A =$ Final weight of the beaker and residue in mg.

$B =$ Initial weight of the beaker in mg.

$V =$ Volume of the sample taken in ml.

3.5.6. Trace elements:

The trace elements analysed in the present study include the following:

(a) Micronutrients: Manganese (Mn) and Iron (Fe).

(b) Trace Metals: Mercury (Hg), Copper (Cu) and Zinc (Zn).

(c) Toxic Metals: Arsenic (As), Lead (Pb), Chromium (Cr) and Nickel (Ni).

(i) Analysis of water sample for the determination of metals:
The pretreated water samples were digested for determination of metals by Nitric acid digestion technique (APHA, 1995). For digestion of water samples 50 ml. of pretreated well mixed water sample was taken in a beaker. To it 5 ml. of concentrated Nitric acid was added and the mixture was slowly evaporated on a hot plate. The volumes of the samples were reduced to about 25 ml. The digested samples were cooled to room temperature. After cooling samples were filtered through Whatman no.40 filter paper and the volume of each sample were made up to 50 ml with double distilled water and stored for analysis. The heavy metals such as Pb, As, Ni, Cr, Cu, Mn, Fe and Zn were estimated using Flame Atomic Spectrophotometer (Agilent Technologies Spectra-240AA and Perkin Elmer AAnalyst 200) with Air Acetylene Flame but for estimation of Hg, N\textsubscript{2}O-C\textsubscript{2}H\textsubscript{2} flame was used. Arsenic was estimated by hydride generation atomic absorption spectrometry (HGAAS) using Varian VGA-77 vapour generation assembly with ETC-60 temperature controller as a heat source to atomize the hydride generated with the reducing agent NaBH\textsubscript{4} (Merck) and 8M HCl acid (Table 3.4). The standard solution calibration curves were prepared separately for each metal by running suitable concentration of the standard solutions. The concentrations of the metals were determined from the calibration curves. Protocols outlined in APHA (2005) were used for all analysis.
(ii) **Analysis of sediment sample for the determination of metals:**

The metal contents in the digested sediment sample were determined by following the same methods as described for the analysis of metals present in the water samples.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Wavelength (nm)</th>
<th>Lamp current (mA)</th>
<th>Slit width (nm)</th>
<th>Optimum working range (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>193.7</td>
<td>10</td>
<td>0.5</td>
<td>3.0 – 150.0</td>
</tr>
<tr>
<td>Cr</td>
<td>429.1</td>
<td>5</td>
<td>0.5</td>
<td>0.1 – 3.0</td>
</tr>
<tr>
<td>Cu</td>
<td>324.8</td>
<td>4</td>
<td>0.5</td>
<td>0.02 – 3.0</td>
</tr>
<tr>
<td>Fe</td>
<td>248.3</td>
<td>5</td>
<td>0.2</td>
<td>0.06 – 15.0</td>
</tr>
<tr>
<td>Mn</td>
<td>279.5</td>
<td>5</td>
<td>0.2</td>
<td>0.02 – 50.0</td>
</tr>
<tr>
<td>Ni</td>
<td>232.0</td>
<td>4</td>
<td>0.5</td>
<td>0.02 – 3.0</td>
</tr>
<tr>
<td>Pb</td>
<td>217.0</td>
<td>5</td>
<td>1.0</td>
<td>0.1 – 30.0</td>
</tr>
<tr>
<td>Zn</td>
<td>213.9</td>
<td>5</td>
<td>1.0</td>
<td>0.01 – 2.0</td>
</tr>
<tr>
<td>Hg</td>
<td>253.7</td>
<td>4-8</td>
<td>0.5</td>
<td>0.02-50.0</td>
</tr>
</tbody>
</table>

### 3.6. Data analysis:

For statistical analysis of data, Microsoft Excel and SPSS statistical software, version 17.01 were used. The generated data were subjected to basic statistical analysis and bivariate Pearson correlation analysis. Bivariate Pearson correlation coefficients are calculated within the season-wise recorded values of all the parameters included in the present study in order to detect the degree of associationship among the variables. Levene test of homogeneity was performed before proceeding for ANOVA analysis. Analysis of variance (ANOVA) was carried out using one way ANOVA model which relies on the additive decomposition of the data into grand mean, main effects, possible
interactions and an error term (Gelman, 2005). One way ANOVA was performed within the explanatory variables to observe the temporal and spatial variations.

Plate 1 (a): Photograph of major wetlands in Barpeta district (s1 to s6)

Baria Beel (s1)  Finguaparua Beel (s2)

No.1 Choulkhowa Beel (s3)  No.2 Choulkhowa Beel (s4)

Kukarjan Beel (s5)  Kapla Beel (s6)
Plate 1 (b): Photograph of major wetlands in Barpeta district (s7 to s11)

Barkana Beel (s7)  Bahua-tabha Beel (s8)

Singra Beel (s9)  Hahchora Beel (s10)

Sorbhog Beel (s11)