CHAPTER- II

MATERIALS AND METHODS
2. STUDY AREA

The Nagaon and Morigaon districts of Assam lying between 25°45' N and 26° 45' N Latitude and 91° 51' E and 93° 20' E Longitude. The two districts cover an area of 5,561 sq. km and occupy the central part of extensive south bank plain of the river Brahmaputra. The river Brahmaputra flows in the East-West direction along the Northern boundary of both the district. Almost all the rivers and their tributaries flow across the extensive plain of the districts changing their courses frequently. The tributaries namely- Killing, Kolong and Kapili rivers flow through the Southern part of the district. Killing meets the Kapili at the Matiparbat where from Kapili moves westward. Kolong joins Kapili at the Jagi Dui Khuti Mukh and from here they jointly fall into the Brahmaputra. The occurrence of beels and swamps are largely controlled by the geological formation and physiography of the area. As many as 449 beel and 162 swamps are identified within the geographical boundary of the two districts and more than 87 percent of the beels are associated with the rivers and out of them around 100 beels are associated with the Kolong River alone. The frequency of occurrence of beels decreases as their distance from rivers increases. More than 30 percent of beels are found to occur within 400 meters from the rivers. Land use pattern in Nagaon and Morigaon district (Fig.2) can be broadly categorized as agricultural land (79.09%), forest land (11.71%), wasteland (3.06%), water bodies (2.82%), built-up areas (0.15%) and 3.16% of grassland with grazing reserves (Sharma and Goswami, 1993).

2.1. SONDOWA

Sondoba is lying between 26° 21' 50" N Latitude and 92° 15' 08" E Longitude at an elevation of 189 ft in the district of Morigaon. The location of the beel is at a distance of about 20 km towards the North-west direction from the district head quarter, under Laharighat revenue circle in between two PWD road connecting Bhuragaon and Laharighat with Morigaon town. One can approach the beel site either from Kushtoli via Morigaon Laharighat road or from Gerua via Morigaon Bhuragaon road (Fig.2.1).

Sondoba is a tectonic lake and once directly connected with the river Brahmaputra. On the course of time the direct link was failed to continue but during rainy season it still comes in contact with its original stream by flood water.
Originally the beel was registered as a part jointly named as Sondoba-Pabhakati. At present the Pabhakati part is on the verge of extinction due to siltation and excessive human interference. Sondoba part are still in a good condition with an area of 164 ha. The average calculated depth of the beel water was ranged between 3 and 5.5 meter (FSL) during monsoon period and 1.5 and 3.5 meter (DSL) during winter season. The soil type is sandy-loamy. On both side of the beel, human settlements growing on there resulting in increasing pressure on the beel water and its inhabitants. Use of beel water for irrigation is a regular feature in the beel. Presence
of varieties small as well as large trees like Simalu (*Salmalia malabarica*), Ahant (*Ficus religiosa*), Sonaru (*Cassia fistula*), Aam (*Mangifera indica*), Modar (*Erythrina indica*) and Gomari (*Gmelina arborea*) etc., is a characteristic feature of the beel.

![Map showing the position of Sondoba beel in the District map of Morigaon.](image)

Fig. 2.1. Map showing the position of Sondoba beel in the District map of Morigaon.

Like other registered beels in Assam, Sondoba also given on lease to some mohalder for a stipulated period by the state revenue department. Since 1977, a substantial number of beels have been handed over to the Assam Fisheries Development Corporation (AFDC) for maintenance. Sondoba was also one of them from. But AFDC withdraws their authority from the beel and again handed it over to the revenue department. As per record, during this period AFDC was able to
enhance the fish production rate of the beel with the help of their policies and control over the lessees. One of the reasons behind the withdrawal of AFDC may be the seasonal flood occurred in the areas which perhaps make them unable to follow proper policies for the development of the beel. For the present piece of work three site were selected in the beel for collection various samples of soil and water, plankton and macrophyte etc.

1. Kushtoli bridge (site-1) - This is the major fish landing site of the beel, with moderate depth, maximum open area, less infested with aquatic weeds.

2. Kur (site-2) - This site is about at the center of the beel. Devastating flood of 1980’s after demolishing an embankment in near near Tengaguri in the Laharighat flowed across the beel and created this site of maximum depth locally named as “Kur”. The site remains almost covered by water hyacinth leaving little or no space for other aquatic plants, especially for the submersed and emergent plants. This is a fish landing site especially for the varieties of cat fishes.

3. Mohkhuti (site-3) - This Station is at the western part of the beel characterized by comparatively shallow water, moderately covered with all categories of macrophyte and a heavily disturbed one because of human settlement on both side and use the beel water for day to day uses.

2.2. DEOBALI JALAH

Deobali jalah is lying between 26° 13' 15" N Latitude and 92° 32' 51" E Longitude at an elevation of 196 ft in the district of Nagaon. The location of the beel is at a distance of about 22 km towards the south west direction form the district head quarter under Raha and Kampur revenue circle in between NH 37 and the Chaparmukh- Lumding-Tinsukia railway road. One can approach the beel site either from Phulaguri, Dimow or Bebejia.

Deobali is last remaining grassland wetland in Central Assam. At present it has a total geographical area of 1600 ha. Out of these, 333.56 ha area is covered by 40 numbers of small and large interlinked waterbodies ranging from 0.5 to 95 ha scattered over the area. According to a survey report (1998) of the District Fisheries Department, Nagaon these beels are Baralimari (2.5ha), Bharidhuwa (1.2ha), Borduba (5ha), Borthal (19.7ha), Borunguri (33.33ha), Burhamikir (13ha), Dabalu
Fig. 2.2. Map showing the position of Deobali jalah in the District map of Nagaon.

Jakarowa (10.8ha), Kachadhara (1.5ha), Kamarjan (0.5ha), Kandhulimari (4.3ha), Magurmani (5.6ha), Mohaldarbeel (1.5ha), Moh-era (3.12ha), Moraharia (11ha), Morasalbee (16.61ha), Nadanimari (2.5ha), Nalduba (1ha), Noltoli (4.9ha), Pahupuiakahiti(a) (7.2ha), Pahupurikhaiti(b) (1.3ha), Ponadhara (1.3ha), Pukhuria (1.55ha), Putakolong (95ha), Rangagarah (1ha), Rowmari (6ha), Sepakhaity (0.5ha),
Singimari (3.6ha), Singoritoli (2.25ha), Sorabeel (12 ha), Tapatkorai (6.8ha) and Tubuki bordoba (15ha). During monsoon, rains as well as the water from the Kapili River via Haria bring all the beels under the same water cover till winter when only their individual identity can registered. Therefore, it should better to consider Deobali jalah as a cluster of beels with varied morphometric characteristics. The water level in the beel area is totally dependent on the precipitation in the hills of Karbi Anglong and Nagaland that brings all the downstream through Kapili before discharged to the Brahmaputra at Tnisutimukh near Chandrapur in Kamrup District. Torrential rains in the hilly area express by the devastating nature of Kapili in its lower reaches. Results of the fluctuations in the flood pulse can be well documented in the floral and faunal diversity of the beel area. Luxuriant growth of varieties of marshy vegetation is the characteristic feature of the beel area which supports thousands of grassland birds. Except floating group especially the water hyacinth all other macrophytes are seen flourished suitably in the beel. As a flood prone and low lying area large tress are scarce except a number of Simalu (Salmalia malabarica) trees. But, Hijal (Baringtonia acutangulla) trees are recorded in plenty of numbers. Limited permanent settlement are growing on the fringes of the wetlands, but seasonal intruder (Pam dweller) are common during the winter seasons, who erect temporary shelter in convenient places for collection of reeds and also for paddy cultivation. Record (Islam and Rahmani, 2004) reveals that the area now enlisted as an important bird area by BNHS under Important Bird Area Conservation Network in 2004. Due to vastness of the area it creates problem in selection of sites for study (Fig. 2.2).

Keeping the views on easy accessibility, three sites were selected as representative of the beel cluster for the present study. For this size of the beel, its depth as well as richness in marshy vegetation was also taken for consideration. These sites are-

1. Tapatkorai (site-1): Area is about 6.8ha. Water depth ranged between 1.5mtr (DSL) and 2.5mtr (FSL)
2. Magurmari (site -2): Area about 5.6ha. Water depth ranged between 1.2mtr (DSL) and 2.1mtr (FSL)
3. Baralimari (site -3): Area about 2.5ha. Water depth ranged between 1mtr (DSL) and 2mtr (FSL)
2.3. COLLECTION AND STORAGE OF SAMPLES

Analysis of water and soil samples provides information on the nature and quantity of constituents present in the water column whereas its living components indicate general long term trends in water quality; water samples give a precise picture of instantaneous conditions (EPA Report, 1982). Water and soil samples were collected for this investigation following the procedure outlined by Goltterman et al., (1978), EPA Report (1982), Trivedy et al., (1987), APHA (1992) and Rao (1983).

Different sampling procedures were used for different parameters. In each set, samples were collected from the same location of the station as far as practicable.

2.4. PHYSICO-CHEMICAL PARAMETERS (WATER)

2.4.01(A) Physical Parameters

[A] Water temperature: directly measured in the site itself by dipping the reversible thermometer in to water (0.5 m depth)

[B] For pH, water samples were collected from 0.5 m depth and measured by dipping the pH stick into a beaker on the spot and the same was verified with the help of digital pH meter in the laboratory.

2.4.01(B) Chemical Parameters

[A] For dissolved oxygen (DO) and Free carbon dioxide (FCO₂): water samples were collected in BOD bottles of 300 ml capacity with tapped and pointed ground-glass stopper and flared mouths. Collecting BOD bottles were pre-cleaned with detergent and tape water followed by rinsing with chromic acid and distilled water. Collections were made from the surface area avoiding bubbling or any agitation.

[B] For other chemical parameters like Alkalinity, Total Hardness, Chloride etc. samples were collected in pre-cleaned polyethylene gallon of 5 liters capacity. Samples were collected from the surface.

2.4.02. Physico-Chemical Parameters (SOIL)

Soil samples from three sampling sites were collected with the help of Ekman dradge and transported to the laboratory for their analysis. Soil temperature was measured at the site while pH, Ammonical nitrogen were analysed immediately in the laboratory using fresh samples. For analysis of total potassium, Available phosphorus, Organic carbon and Organic matter content the soil samples were air
dried. Analyses were done by following standard methods of Jackson (1962), Mishra (1968), Michael (1986), Trivedy et al., (1987).

2.4.02(A) Physical Parameters

[A] Soil temperature: It was measured with the help of soil thermometer. In low water zone, the cone of the thermometer was inserted directly into the soil and reading was noted while in deep water zone the temperature of samples collected with the help of Ekman dradge was measured immediately.

[B] For pH: For analyses of pH, 1:5 soil suspensions were prepared. For this, 20 g of soil was mixed with 100 ml of distilled water and the suspension was shake mechanically for about an hour. The pH of this unfiltered solution was observed with the help of a digital pH meter.

2.4.02(B) Chemical Parameters

Ammonical nitrogen was analysed immediately in the laboratory using fresh soil samples. For analysis of available phosphorus, the collected soil samples were air dried in room temperature. For analyses of other parameters the samples were dried at 105°C. Analyses were done by following standard methods of Jackson (1962), Mishra (1968), Michael (1986), and Trivedy et al. (1987).

2.4.03. Biological Parameters

2.4.03(A) Plankton Population

Plankton was also collected from all the sites of the beels. Two different plankton nets were used to collect phytoplankton and zooplankton separately having mesh size of 70w and 20w respectively. The plankton nets were made of nylon clothes and the diameter of the plankton net was 25 cm. Graduated plankton tubes were used in plankton net. In each site of the beels 50 liters of water were filtered through plankton net using a mug of one liter capacity and the volume of water filtered in every site was same.

For storage of collected samples small vials made up of polyethylene were used with proper label indicating date of collection, sampling site, time of collection and quantity and quality of plankton samples. The collected plankton samples were fixed and preserved in 4% formalin solution and taken to the laboratory.
2.4.03(B) Collection and Storage of Aquatic Macrophyte

The sampling of aquatic macrophytes depends on the type of habitat, type of vegetation, variation and distribution of vegetation. In this investigation vegetation were collected by hand picking or with the help of labour. Collection was made in and around the sampling sites and during general survey in the beels. Some vegetation were identified on the spot by their vernacular name with the help of skilled local persons and others were taken to the laboratory for identification with proper label indicating date of collection, habitat of vegetation etc. and were preserved by making herbarium sheets before identification.

2.4.03(C) Collection and Storage of Fishes

Collections of fishes were done with the local fisherman from the fish landing/ collection sites and were preserved in 6-8% formalin solution in plastic containers.

2.4.03(D) Observation and Identification of Birds

Studies on birds were made in both the beels during the period of study with special emphasis on winter season. Birds were observed with naked eyes, using binocular and using country boat as and when necessary.

2.4.04 Sampling and Storage of Biological Materials

In biological parameters qualitative and quantitative analysis of plankton, quantitative estimation of macro fauna comprising fish and birds and surveys of macro vegetation were also included. For collection of the said parameters excluding birds the methods given by the EPA final report (1982), Trivedy and Goel (1986) and APHA (1992) were used.

2.5. METHODOLOGY

In the present investigation, following parameters of the three (3) categories were undertaken.

[A] Water Quality Parameters

2.5.01. Physical Parameters
[a] Temperature and [b] pH

2.5.02 Chemical Parameters

[B] Soil Quality Parameters

2.5.03. Physical Parameters
[a] Temperature and [b] pH

2.5.04. Chemical Parameters

[C] Biological Parameters


2.5.01(a) Determination of water temperature: Temperature of water was measured by reversible thermometer (readings in degree Celsius) at the time of sample collection.

2.5.01(b) Determination of pH: pH values of the sample water were recorded immediately after collection of sample by using a digital pH stick. Necessary precautions were taken to calibrate the pH meter with standard buffer of known pH before each set of measurement.

2.5.02(a) Determination of Total alkalinity: The TA of water is its capacity to accept protons; it is the quantity as well as kind of compounds present that
collectively shift the pH to the alkaline range towards neutrality. However, TA of neutral water is generally the result of bi-carbonates (expressed in terms of CaCO₃). Three kinds TA are indicated namely, OH⁻, CO₃²⁻, HCO₃⁻ and termed as total TA (Rao, 1993).

**Procedure:** 50 ml of water was taken and 4 drops of phenlopheline indicator was added to it. If pink colour appears (it indicates the presence of carbonate), it was titrated against standard H₂SO₄ (.02 N) until the colour disappears (pH = 8.3) and recorded milliliters of acid use. If there was no pink colour after addition of phenlopheline, two drops of methyle-orange indicator were added and titrated following the methods of Rao (1993).

**Calculation:**

\[
\text{Phenlopheline alkalinity as mg CaCO}_3/l = \frac{\text{ml standard acid x1000}}{\text{ml of sample.}}
\]

\[
\text{Total alkalinity as mg CaCO}_3/l = \frac{\text{Total ml standard acid x 100}}{\text{ml of sample.}}
\]

2.5.02(b) **Determination of Dissolved oxygen:** Dissolved oxygen is determined by Winkler’s Iodometric Method. The principle of this reaction of the method is that the manganous sulphates reacts with the alkali (KOH or NaOH) to form a white precipitate of manganous hydroxides which in the presence of oxygen get oxidized to a brown colour compound. In the strong acid medium manganese ions are reduced by iodide ions which get converted into iodine equivalent to the original concentration of oxygen in the sample. The iodine can be titrated against thiosulphate using starch as an indicator.

**Calculation:**

- When whole contents have been titrated-

\[
\text{DO}_2 \text{ in mg/l} = \frac{[(\text{ml x N}) \text{ of titrant x 8 x 1000}] / (V_1 - V)}
\]

- When only a part of the contents has been titrated-

\[
\text{DO}_2 \text{ in mg/l} = \frac{[(\text{ml x N}) \text{ of titrant x 8 x 1000}] / \{V_2 (V_1 - V/V_1)}
\]

Where,

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

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

\[
V = \text{Volume of the MnSO}_4 \text{ and KI added.}
\]
2.5.02(c) **Determination of Free CO\(_2\):** Carbon dioxide is a normal component of all natural waters. It may enter surface water by absorption from the atmosphere, but only when its concentration in water is less than that in equilibrium with Carbon dioxide in the atmosphere. Carbon dioxide may also be produced in waters through biological oxidation of organic matter, particularly in polluted water. Thus, it may be concluded that surface waters are constantly absorbed or giving up to maintain an equilibrium with the atmosphere.

**Procedure:** FCO\(_2\) can be determined by titrating the sample using a strong alkali to pH 8 (Ramesh and Anbu, 1996). At this pH all the FCO\(_2\) is converted into bicarbonates. In a conical flask 100ml of water sample was taken and a few drops of phenlopthalein indicator were added to it (if the colour turns to pink, free CO\(_2\) is absent). The colourless sample was titrated against 0.05 N NaOH till a pink colour appeared at the end.

**Calculation:**

\[
\text{Free CO}_2 \text{ (mg/l)} = \frac{(\text{Vol. x N}) \text{ of NaOH x 1000 x 44}}{\text{Vol. of Sample}}
\]

2.5.02(d) **Determination of Total Hardness:** In water analysis, it is often desirable to know the types amounts amounts of the various forms of alkalinity present. Water hardness is caused primarily by the presence of cations such as calcium and magnesium and anions such as carbonate, bicarbonate, chloride and sulphate in water. Carbonate and bicarbonate in water can be determined by titrating a known volume of sample against dilute sulphuric acid solution using phenlopthalein and methyl orange as indicators. When a drop of phenlopthalein is added to the water sample, pink colour develops when carbonates are presents in the sample. If pink colour does not develop, it shows the absence of carbonates in water. When the sample containing carbonate and bicarbonate is titrated against standard sulphuric acid, phenlopthalein loses its pink colour when half of the carbonate is converted into bicarbonate. So, twice this value is a measure of carbonates present in water. To this colourless solution, a few drops of methyl orange are added and titrated against sulphuric acid till straw yellow colour changes to pinkish red colour. This value gives the amount of acid required to neutralize the bicarbonate originally present, and that formed from the carbonates. By substracting the first titrate value from the second one, acid required to neutralize the bicarbonate originally present in the
water is obtained (Rao, 1993).

\[ 2Na_2CO_3 + H_2SO_4 \rightarrow 2NaHC0_3 + Na_2SO_4 \]  
(phenlopthalein)

\[ 2NaHC0_3 + H_2SO_4 \rightarrow Na_2S0_4 + 2H_2O + 2CO_2 \]  
(methyl orange)

**Procedure:** In a 250ml conical flask 20 ml water sample was taken and 2 drops of phenlopthalein indicator was added (if a pink colour develops, carbonate present). The sample is then titrated against 0.02 N H₂SO₄ till the pink colour disappears. To the sample in the flask 1 ml or 2 drops of methyl orange indicator was added. Titration process was continued till the straw yellow colour changes to pinkish red.

**Calculation:**
- Volume of water sample taken = 20 ml
- Volume of 0.02 N H₂SO₄ used for phenlopthalein end point = X ml
- Volume of 0.02 N H₂SO₄ used for methyl orange indicator = Y ml
- Volume of 0.02 N H₂SO₄ required to neutralize carbonate alone = 2X ml
- Volume of 0.02 N H₂SO₄ required to neutralize bicarbonate alone = (Y - 2X)

\[
\text{CO}_3^{2-} (\text{mg/l}) = \frac{(2X) \times 0.02 \times 30}{20} \times 1000
\]

\[
\text{HCO}_3^- (\text{mg/l}) = \frac{(Y - 2X) \times 0.02 \times 61}{20} \times 1000
\]

2.5.02(e) **Determination of Chloride:** Chloride is an anion found in variable amount in natural and waste water. The concentration of chloride in natural water generally bears a strong correlation with the sodium content and specific conductance. Chloride determinations may serve to indicate the intrusion of waters of different compositions or to trace and measure rates and volumes of water mass movements. From an environmental standpoint chloride is basically a conservative parameter and may serve as an index of pollution occurring in natural fresh water from primary sources such as industrial and municipal outlets (Mohr's titration method, Ramesh and Anbu, 1996)

**Procedure:** From a 250 ml conical flask, 20 ml of water sample is pipetted out and keep in a clean porcelain tile. 1 ml of potassium chromate (K₂CrO₄) indicator added with a 1ml pipette. 0.05 N Silver Nitrate (AgNO₃) was added drop wise swirling the liquid constantly until a red colour was formed. Titration was continued by adding...
silver Nitrate drop-wise until a faint but distinct change in colour occurs. A faint persistent reddish-brown colour is seen the end point.

**Calculation:**

\[
\text{Chloride (mg/l)} = \frac{(A-B) \times N \times 35.45}{\text{Vol. of sample.}} \times 1000
\]

Where  
A = Vol. of silver nitrate require for sample  
B = Vol. of silver nitrate required for blank  
N = Normality of AgNO₃

2.5.02 (f & g) Determination of Calcium and Magnesium: Calcium and magnesium are the most abundant elements in natural surface and ground water and exists mainly as bicarbonates and to a lesser degree in the form of sulphate and chloride. Calcium may dissolved readily from rocks or be leached from soils. In the presence of CO₂, Calcium carbonate in water dissolved, in which case the resulting buffer system is likely to maintain the Ph of most natural waters between 6 and 8. Other sources include primarily industrial and municipal discharges.

However, dissolved magnesium concentration is lower than calcium for majority of the natural waters. Because of the high solubility of Mg salts, the metals tend to remain in solution and are less readily precipitated than calcium. Typically, Ca concentration in natural freshwater falls below 10mg/l, although waters in the proximity of carbonate rocks and lime stones may contain calcium ranging from 30 to 100mg/l.

Calcium is an essential nutritional element for animal life and aids in maintaining the structure of plant cells and soils. Magnesium poses no more concern with regard to public health or the aquatic environment, and limits of concentration set for water are based mainly on palatability, corrosion and incrustation criteria, the criteria depending on the intended use.

In general, both Ca and Mg can be determined together. The titrimatric and atomic absorption spectrophotometric methods are best suited for Ca and Mg determination. Hence, the procedures for the estimation of Ca and Mg are described together. This titrimatric method is applicable to the determination of total calcium and magnesium present in surface and ground water. Minimum detection limit is 1mg/l.
**Principle:** The titration method depends on the ability of ethylene diamine tetra acetic acid (C\textsubscript{10}H\textsubscript{16}O\textsubscript{8}N\textsubscript{2}), EDTA, or its disodium salt to form stable unionized complexes with calcium and magnesium ions. When the dye Eriochrome Black-T (C\textsubscript{2}OH\textsubscript{13}N\textsubscript{3}O\textsubscript{7}S) is added to a solution containing calcium and magnesium ions, a wine red complex is formed. When the solution is then titrated with standard EDTA solution, which removes calcium and magnesium from the dye complex, the dye is changed back to its original blue colour. In this way, Eriochrome Black-T is used to indicate the end point for the titration of calcium and magnesium together.

**Equations:**

\[
\begin{align*}
Ca^{2+} + H_2Y^{2-} & \leftrightarrow CaY^{2-} + 2H^+ \\
Mg^{2+} + H_2Y^{2-} & \leftrightarrow MgY^{2-} + 2H^+ \\
MgD^+ (\text{red}) + H_2Y^{2-} & \leftrightarrow MgY^{2-} + HD^2+ (\text{blue}) + H^+
\end{align*}
\]

**Procedure:** To determine calcium and magnesium content, 20 ml of water sample was taken in a conical flask and diluted with 25 ml of double distilled water. 2 ml of ammonium chloride-ammonia buffer solution and 30-40 mg of Eriochrome Black-T indicator was added. Then the content was titrated against 0.02 M EDTA solution until the colour changes from wine red to steel blue. Care was taken not to remain tinge of reddish hue at the equivalence point by lowering the rate of titration process.

**Calculation:**

Volume of sample taken = 20 ml.

Volume of 0.02 M EDTA used for calcium alone = A ml.

Volume of 0.02 M EDTA used for calcium and magnesium = B ml.

Volume of 0.02 M EDTA used for Magnesium alone = (B-A) ml.

**Calcium**

1 ml. of 0.02 M EDTA = 0.0004 g of Ca

A ml. of 0.02 M EDTA = 0.0004 x A) g of Ca

\[
Ca (mg/l) = \frac{0.0004 \times A \text{ ml.}}{20} \times 10^6
\]

**Magnesium**

1 ml. of 0.02 M EDTA = 0.00024 g of Mg

(B-A) ml. of 0.02 M EDTA = 0.00024 \times (B-A) g

\[
Mg (mg/l) = \frac{0.00024 \times (B-A) \text{ ml.}}{20} \times 10^6
\]
2.5.02(h) **Determination of Nitrate:** Nitrate generally occurs in trace quantities in surface water but may attain high levels in some ground water. It is an essential nutrient for many photosynthetic autotrophic organisms in it and some cases has been identified as a growth-limiting nutrient.

Nitrate may be reduced to nitrite in a column containing cadmium (Cd) which has been treated with copper. The Cd$^{2+}$ ions released in this way simultaneously form a complex with the help of EDTA-NH$_4$Cl buffer in order to prevent the interference with the efficiency of the column (Cadmium reduction method, Ramesh and Anbu, 1996). The reduction can be represented as:

$$\text{NO}_3^- + \text{Cd} + \text{H}_2\text{O} \leftrightarrow \text{NO}_2^- + \text{Cd}^{2+} + 2\text{OH}^-$$

**Procedure:** The Cadmium reduction column was rinsed with dilute EDTA-NH$_4$Cl solution and allowed it to run through, and drain the excess. Care was taken not to enter air in the column and to leave 10 ml of buffer above the cadmium fillings. Using HCl or NaOH the pH adjusted to 7 - 9. Now 75 ml of dilute EDTA-NH$_4$Cl solution mixed well with 25 ml of the sample. The mixture sample was then poured in the column and collected it at a rate of 10 ml/min. The first 10 ml of the collected sample was discarded and the rest was collected in a sample flask. Within 15 minute of collection after reduction 2 ml sulfanilamide was added to 50 ml sample. Again after 2 minute 2 ml of N ethylene diamine dihydrochloride (NED) solution was added to the mixture and mixed well. The absorbence was then measured at 543 mm between 10 min. and 2 hours.

**Calculation:**

Calculation of NO$_3^-$ - N concentration for sample made directly from standard curve.

2.5.03. **Physical Parameters (SOIL).**

2.5.03(a) **Soil Temperature:** It was measured with the help of Soil thermometer. In the area where water level was low, the cone of the thermometer was inserted directly in to the soil and the reading was noted while in deeper water area the temperature of soil sample collected with the help of Ekman dredge, was measured immediately (Trivedi *et al.* 1987).

2.5.03(b) **Hydrogen ion concentration (pH):** For pH analysis, 1:5 soil suspensions are prepared. For this, 20 g of soil was mixed with 100 ml of distilled water and the
suspension was shaken mechanically for about an hour. pH of the unfiltered solution was observed with the help of a digital pH meter.

2.5.04. Chemical Parameters (SOIL)

2.5.04(a&b) Organic carbon and Organic matter: With the help of Walkley and Black Method (Trivedi et al. 1987) these parameters were measured. For this 5mg of dried soil was taken in a conical flask and 10 ml of 1 N K$_2$Cr$_2$O$_7$, 20 ml of Conc. H$_2$SO$_4$ was mixed thoroughly with it. The flask was then allowed to stand for 30 minutes. 10 ml of phosphoric acid and 1 ml of diphenyle indicator was added to develop bluish purple colour. The content was titrated with 1 N ferrous ammonium sulphate until the colour changes to brilliant green.

$$\frac{V_1 - V_2}{S} \times 0.003 \times 100$$  
Organic Carbon (%)  
Organic Matter (%) = % Organic Carbon x 1.724  
Where  
V1 = Volume of K$_2$Cr$_2$O$_7$ (10 ml.)  
V2 = Volume of ferrous ammonium sulphate.  
S = Weight of the soil

2.5.04(c) Ammonical Nitrogen: 100 g of fresh soil sample was taken in a 500 ml. conical flask and mixed with 200 ml. of acidified NaCl solution. After 30 minutes the suspension was filtered through Whatman No. 42 filter paper. The conical flask was rinsed with 500 ml. of NaCl solution and rinsings were poured into soil.

Soil washed with additional 200 ml. of sodium chloride. The volume of lechate was raised to 500 ml. with acidified NaCl solution. Then calibration curve was prepared by placing aliquots of diluted standard (ranging between 3 to 30 ml) in a series of 100 ml volumetric flasks. 2 ml. of sodium tartrate solution and 50 ml. of acidified sodium chloride were added to it and final volume was raised to 90 ml. with distilled water. Now 5 ml. of Nessler reagent was mixed thoroughly and again volume of the content was prepared by plotting percentage transmission on a log scale against concentration on a linear scale (Trivedi et al. 1987).

Ammonical Nitrogen (mg/100g) = 10 x (mg NH$_4$ from curve).

2.5.04(d) Available Phosphorous: To estimate available phosphorous, 1g of air dried
soil was taken in a flask and 200 ml of 0.002 N NH₂SO₄ was mixed into it. The suspension was shaked for 40 minutes and it was finally filtered through Whatman No. 50 filter paper. The concentration of phosphorous of filterate was estimated spectrophotometrically (Trivedi et al. 1987).

\[
\text{Available Phosphorous (mg/g)} = \frac{\text{mgP/L IN SOIL x 1000 extract}}{50}
\]

2.5.04(e) **Total potassium:** For estimation of total potassium in soil the ammonium acetate leachate, prepared for calcium and magnesium determination was used. The level of Na and K in ammonium acetate leachate was obtained by flame photometer (Trivedi et al. 1987).

\[
\text{Total Potassium (mg/100g)} = \frac{\text{Mg Na/l of soil extract x V}}{10 \times S}
\]

Where

\[
V = \text{Total volume of soil extract prepared}
\]
\[
S = \text{Weight of soil taken (g)}.
\]

2.5.05. BIOLOGICAL PARAMETERS

2.5.05(a) **Analysis of plankton:** Planktons which are the microscopic free floating were collected by the filtering 50 liters of water in each site through plankton net of bolting silk. Filtered plankton samples were fixed and preserved in 4% formalin and studied under light microscope following the methodology of Battish (1992), Needham and Needham(1986). After collection of the sample, it was preserved in 4% formalin. In the laboratory 10 – 20 ml of the collected samples was centrifuged about 15- 20 min at 1000 rpm. in an electrical centrifuge. The supernatant sample with some water was removed from centrifugation and the volume was reduced to 8 ml. After centrifugation qualitative and quantitative estimation were done by taking samples in Sedgwick rafter counting cell.

**Calculation:** Total number of plankters present in a liter of water sample were calculated using the following formula :

\[
N = \frac{(n \times v)}{V}
\]

Where

\[
N = \text{Total number of plankton cells per liter of water filtered.}
\]
\[
n = \text{Average number of plankton cells in 1 ml of plankton}
\]
Identification of plankton was done following after Edmonson (1966), Needham and Neeham (1986), and APHA (1992).

2.5.05(b) Collection of Aquatic Macrophytes: The frequency of aquatic plants was determined by using quadrates of 1 x 1 m. Several quadrates were laid in a water body. The presence or absence of the plants was recorded in the quadrate. Alternatively, it is difficult to lay down the quadrates, anchors washrown as quadrates and pulled. The presence or absence of the species pulled with each anchor was recorded. After collection of the macrophytes they were taken to the laboratory and identification was made with the help of APHA (1992). The marshy vegetations were identified by their local names on the spot and then the records were confirmed with the help of Mondal and Mondal, (1998) and Dutta (2008). Those are out of reach were collected and identified at BSI, Shillong.

2.5.05(c) Data on fishes were collected on the spot i.e. fish collection/ landing stations and identified by using standard keys of Jhingran (1991), Jayram (1999), Nath and Dey (2000).

2.5.05(d) Data on bird population was collected following the transact methods (Point and Line transact) using binocular and country boat as and when necessary during the period from October/04 to September/07.

Studies were conducted seasonally from October (Retreating monsoon) to September (Monsoon) with special emphasis on winter season. Bird counts were done between sunrise and 12:00 h and between 15:00 h and sunset, using Nikon field binoculars (10 ×50). Surveys began near the wetland, where most of the surface area and edge was visible, and proceeded to identify and count all birds present (Bibby et al. 1993). The observer then walked around the perimeter of the wetland to flush and identify any unseen birds. Country boats are also used whenever necessary to observe birds. For study of land birds, especially the grassland bird, it is necessary to erect concealment tent. Birds were considered to be
resident (encountered daily) and migratory (encountered seasonally). Equal importance was given to observation of birds by sight and sound. Species, composition, species richness and bird abundance were determined. Observed birds were identified following the standard keys of Ali and Ripley (1983), Ali (1996), Richard et al. (2000).

2.6. DATA ANALYSIS

2.6(a) Measures of Diversity index

Using the pooled abundance data on plankton, macrophytes, fish and birds sampling by season and beel type for years (2004-2007) the following estimators were computed as measures of species diversity-

All of these measures are widely used in ecology literature (Krebs, 1989; Magurran, 1988). The selected diversity indices are relatively easy to interpret ecologically and less sensitive to rare species and sample size (Magurran, 1988). Diversity measures vary in the relative emphasis placed on the number of species (richness) and their relative abundances (Evenness), and each has its limitations. Some information on the applied diversity indices is given below:

A frequently used diversity measure is the Shannon-Weaner Index and it is calculated as

\[ H' = - \sum_{i=1}^{S} p_i \ln p_i \]

and \( p_i = n_i / N \)

Where \( p_i \) is the proportion of the sample represented by species \( i \), and \( \ln \) is the natural logarithm and considers the proportion of individuals found in the \( i^{th} \) species, \( p_i \). This value is estimated as \( n_i / N \) (number of individuals in the \( i^{th} \) species divided by the total number of individuals). Compared to other indices, the Shannon index is sensitive to changes in abundance of rare or intermediate abundant species. It appeared to have only moderate discriminate ability and since it is strongly influenced by changes in rare species, is sensitive to sample size. The index assumes that sampling is random and allows including all species present in the community.

A second group of diversity measures are referred to as dominance measures since they particularly consider the abundance of the most common species rather than providing a measure of species richness. One of the most used dominance
measures is the Simpson’s index, which calculates the probability that any two 
individuals drawn at random from infinitely large community belong to different 
species. It is calculated as:

\[ D = \Sigma pi \text{ (reciprocal)} \]

\[ 1/D = 1/\Sigma pi \]

and \[ pi = ni (ni -1)/N(N-1) \]

In the formula, \( pi \) is the proportion of individuals in the \( i^{th} \) species and is 
estimated from the relation of the number of individuals in the \( i^{th} \) species to the total 
number of individuals. Simpson’s index especially takes into account the abundance 
of the most common species, and is less sensitive to species richness. It has a 
moderate discriminate ability and low sensitivity to sample size, since it does not 
stress on changes in abundance of rare species, but of the most common species. 
Since diversity decreases when \( D \) increases, mostly the reciprocal form of the index 
is used \((1/D)\).

Diversity depends on the number of species (i.e. \( S \)), but also on the evenness 
(\( E \)); if all species are equally abundant (\( E \) max) then diversity is high, but if one 
species is especially abundant and the rest are rare (\( E \) min) then diversity is low.

Simpson's Inverse Index of Diversity is calculated as:

\[ D = 1/\Sigma pi^2 \]

Where \( pi \) is the proportional abundance of species \( i \), i.e. \( ni/\Sigma ni \).

So, to calculate \( D \), we have the following parameters:

- add up the species' abundances \((ni)\) to give the total \((N = \Sigma ni)\);
- divide each \( ni \) by \( N \) to give \( pi \);
- square each \( pi \), before we
- add them up to give a value for Dominance \((= \Sigma pi^2)\),
- the reciprocal of which is Simpson's Inverse Index of Diversity.

Another frequently used index is the Fisher’s alpha diversity. It is calculated as:

\[ A = N (1-x)/x \]

\[ S/N = (1-x)/x-1n (1/x) \]

and principally considers the total number of individuals \( N \) and \( x \) which is estimated 
from the iterative solution of \( S/N = (1-x)/x-1n(1/x) \). It does not take into account the 
relative abundance of the species. This means, in situations where the total number 
of species and individuals remain constant but the evenness of the community
changes, alpha will not indicate a difference. However, the index appeared to have a very good discriminate ability between different samples and is not so sensitive to sample size. This attribute of alpha is a result of its dependence on the numbers of species of intermediate abundance, it is relatively unaffected by either rare or common species abundance changes. The index is based on the log-series species abundance model developed by Fisher and is strictly speaking only appropriate when the community shows a log-series distribution. However, in practice it appeared to be a good diversity measure independent of the underlying abundance pattern.

Pielou's evenness index, $j$ (Pielou, 1975) is calculated as:

$$ j = \frac{H(s)}{H(\text{max})} $$

Where $H(s)$ = the Shannon-Wiener information function

$H(\text{max.})$ = the theoretical maximum value for $H(s)$ if all species in the sample were equally abundant.

Rarefaction diversity is a measurement for analyzing the number of species (species richness) among collections, when all collections are scaled down to the same sample size (Hurlbert, 1971). The number of species $S_n$, which can be expected from a random sample of $n$ individuals, drawn without replacement from $N$ individuals distributed among $S$ species is calculated as:

$$ E(S_n) = \sum_{i=1}^{S} \left( 1 - \left( \frac{N - N_i}{n} \right) \frac{\binom{N}{n}}{\binom{N_i}{n}} \right) $$

Where $S$ is the total number of species found in the collection, and $N_i$ is the number of individuals of the $i$th species (Hurlbert, 1971).

The technique of rarefaction can be used to calculate species richness for sub samples consisting of fewer individuals. The following formula predicts the average species richness that would be obtained if $N$ individuals were randomly sampled instead of the total $N_{\text{tot}}$ individuals of the survey, when each species contains $N_i$ individuals in the entire survey: The species diversity, richness, evenness and rarefaction estimates were analysed separately for the study sites of Sondoba and Deobali jalah by season and year. The pooled plankton abundance data from the 3 fixed transects of Sondoba and 3 fixed transects of Deobali jalah were used in the
analysis. The software program used for the computation of the diversity indices was the 'R' package 'Vegan' version 1.8-2 (2006-06-01).

2. 6(b) Indicator Taxa

To determine the characteristic genera/species for each transect/beel type, we used the indicator (IndVal) method of Dufrene and Legendre (1997). This method calculates an indicator value (IV) for each genus in predefined clusters (like the clusters identified by a cluster analysis). It is especially suited for identifying indicator taxa independently of the animal (or plant) community as a whole (Dufrene and Legendre, 1997; McGeoch and Chown, 1998). The method gives an integrated measure for the relative mean abundance and the relative frequency of the studied genera in each cluster and is calculated as follows:

\[ A_{ij} = \frac{N \text{ individuals}}{N \text{ individuals}_i} \]

\[ B_{ij} = \frac{N \text{ locations}}{N \text{ locations}_j} \]

\[ \text{INDVAL}_{ij} = A_{ij} + B_{ij} \times 100 \]

where \( A_{ij} \) (relative mean abundance) is the mean number of individuals of genus \( i \) in cluster \( j \) divided by the mean number of individuals of genus \( i \) in cluster \( j \) plus the mean number of individuals of genus \( i \) outside cluster \( j \); \( B_{ij} \) (relative frequency) is the number of locations in cluster \( j \) where genus \( i \) is present divided by the total number of locations in cluster \( j \); \( \text{INDVAL}_{ij} \) is the relative mean abundance of genus \( i \) in cluster \( j \) multiplied by the relative frequency of genus \( i \) in cluster \( j \) multiplied by 100%. Genera that are weakly associated with a cluster because they are either not abundant or not present in all the locations within that cluster will score a low IV. Only genera that have both a high mean abundance and are present in the majority of locations of a cluster will score a high IV for that particular cluster. IVs can vary between 0% and 100%, in which 0% indicates no association with a cluster, while 100% indicates that the genus was found in all locations of that particular cluster, and was absent in all other locations outside that cluster.

To test whether the observed IV of a genus in a cluster was significantly higher than could be expected based on a random distribution of individuals over the locations, the observed IV was compared with 999 randomly generated IVs. These random IVs were generated with a random reallocation procedure in which the
number of individuals per genus per location were randomly reshuffled over the locations (Dufrene and Legendre, 1997). If the observed IV of a genus in a cluster fell within the top 5% of the random IVs (sorted in decreasing order) it was considered to deviate significantly from the expected random mean, i.e. the genus had a significantly higher IV than expected. Species with significant high IndVal values (>30%) show strong habitat specificity, so they may be considered as characteristic species of the assemblages (McGeoch and Chown, 1998). This analysis was performed in the ‘vegan’ subroutine of the program ‘R’.

2.6(c) Canonical Correspondence Analysis

We used canonical correspondence analysis (CCA) to determine the effect of environmental variables (Ter Braak, 1995) with the biotic components of the ecosystem and also the interrelations among the biotic components itself.

The CCA ordination was run using the axis scores centred and standardized to compartment variance and compartments were plotted on diagrams using linear combination scores in the statistical program ‘R 2.3.1.’ (R: A programming Environment for Data Analysis and Graphics. http://cran.r-project.org/ 2006-06-01).

Canonical Correspondence Analysis (CCA) was used for ordination of the locations, species composition and distribution on the plankton, macrophyte, fishes and birds abundance data. CCA is a ‘direct’ gradient analysis (ordination) method that places plots in species space relative to a matrix of habitat variables (Ter Braak, 1986). It is one of the most important eigenvector methods in community ordination and is based on Chi-square distances. In this method a set of species can be directly related to a set of environmental variables. The ordination axes are chosen in the light of known environmental variables. In this way community or species variation can be directly related to environmental variation. These variables may be quantitative or nominal. As many axes can be extracted as there are variables. Constrained axes are constructed to maximize the fit with linear combinations of environmental represent species and sites, and arrows represent environmental variables. Such a diagram shows the main pattern of variation in species as accounted for by the environmental variables, and remaining (unconstrained) axes represent a residual variation in the species data after extracting the constrained axes. In the resulting ordination diagram, points shows the main pattern of variation in species as accounted for by the
environmental variables and also shows in an approximate way, the distributions of species along each environmental variable (Ter Braak, 1986). The significance of the relationships between selected external variables and data structure was assessed using permutation tests: the contrasts were permuted randomly and the model was refitted. Values of external variables were randomly assigned to the samples and eigenvalues of the CCA analysis for both constrained and unconstrained axes could be performed.

The method of multivariate analysis by constrained canonical correspondence ordination (Ter Braak, 1986) was used for examining the effects of some independent, continuous environmental variables related to the properties of water and soil in different year and seasons on plankton, macrophyte and fish population and species assemblages during the three-year study period. The separating effects of season as categorical variables were examined for observing their effects on the abundance and distribution of these communities within the study area.

We used the indicator (IndVal) method of Dufrene and Legendre (1997) especially for plankton population to detect some characteristic indicator taxa within the study period (seasons) by defining the indicator values of the group and species assemblages and seasonal abundances. The analyses were done separately for the three study sites of each beel based on the pooled abundance data. All the six study sites were moderately to heavily disturb as observed from the land-use pattern and actual field observations. A total sample effort of three years (four seasons in a year) across the six study sites in a fixed date and months during all the seasons of the three-year study period.

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