CHAPTER – THREE

MATERIALS AND METHODS

3.1 STUDY AREA

The present study has conducted in Urpod beel of Goalpara district of Assam during the period from February, 2014 to March, 2016. The study site (Urpod beel) is a natural lake situated at Agia in the Goalpara district of Assam, located approximately 25° 33' to 26° 12' N latitude and 90 ° 7' to 91° 5' E longitude respectively (Fig. 1.1). It is one of the largest beels in lower Assam with an area of 649.38 ha with a depth of 1.0 m – 5.3 m. and had been incorporated in the Asian wetland Directory Scot, 1988 (Kalita and Goswami, 2008).

The Urpod beel is connected to the river Brahmaputra through two of its tributaries namely the Jinziram and Jinari. In monsoon period, the beel received rain water from the nearby plains and the hilly areas through a network of stream and in this period the size of the beel is increased by about 20% of the actual size. The beel is surrounded by 10 villages and some of the villagers earned their livelihood by fishing in the beel. After 1980's due to rapid encroaching of the surrounding areas, perhaps due to anthropogenic interference, there has been drastic shrinkage of the water body of the beel is resulted (Boruah *et al.*, 2008).

For collection of water samples and other studies, the whole beel was divided into five collection sites (S-1, S-2, S-3, S- 4 and S-5) (Fig.1.2). The depth and geographical coordination of the collection sites are given in tabular form (Table 1.1).

Table 1.1: - Different collection sites with geographical location and depth

Collection Site	Name of Location	Altitude	Latitude	Longitude
Site No. 1	Chamaguri	125 ft ASL	26.099722	90.578056
Site No. 2	Garokhota	121 ft ASL	26.103611	90.601667
Site No. 3	Khagrabari	126 ft ASL	26.091667	90.600833
Site No. 4	Kalapani Chandmari	123 ft ASL	26.088889	90.591389
Site No. 5	Agia	131 ft ASL	26.090278	90.5775

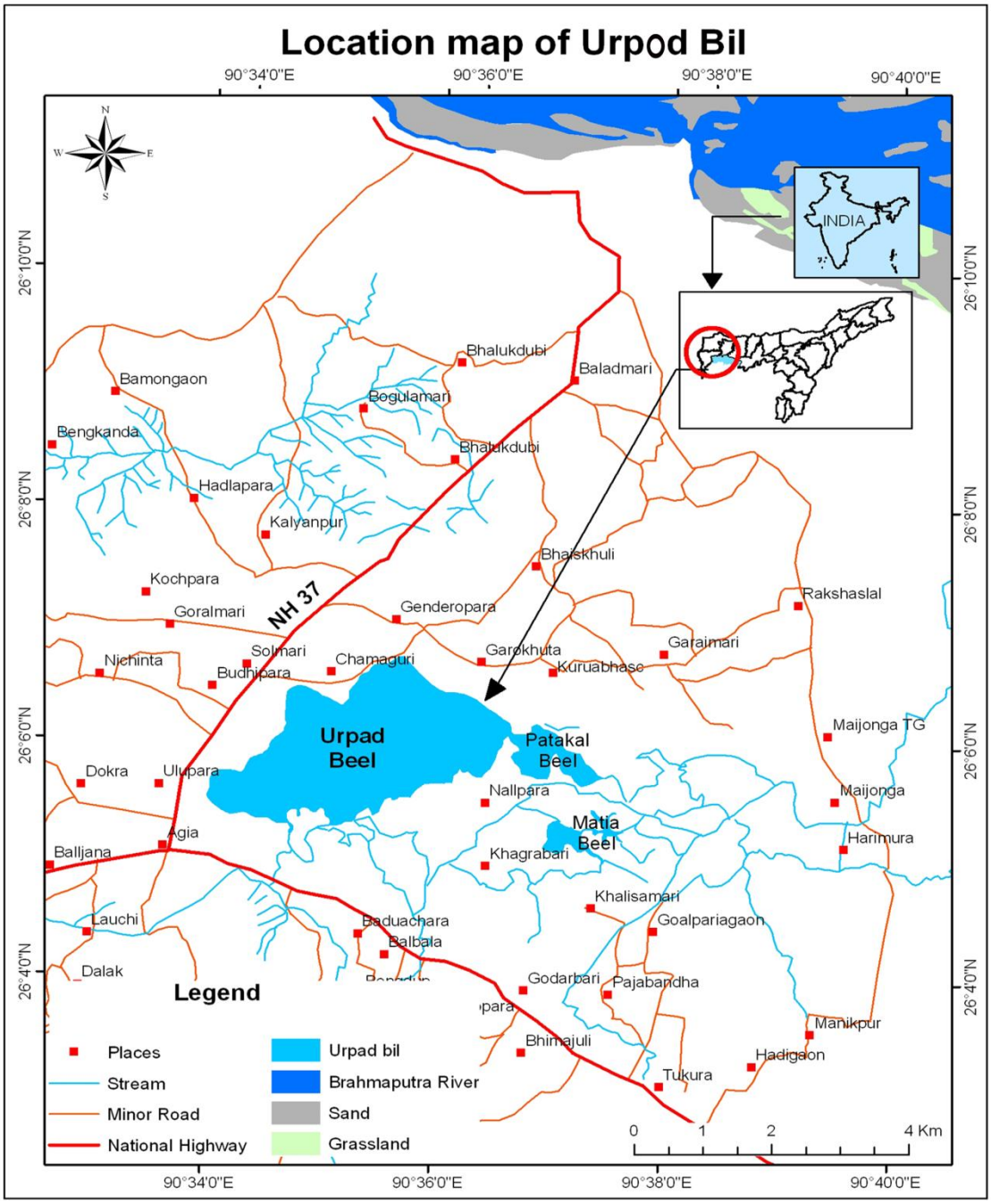

Fig. 1.1: - Location Map of Urpod Beel (Source: ARSAC, ASTEC, Guwahati, Assam)

PHOTO PLATE - 1

A & B - Views of Urpod Beel

C & D - Views of sample Collection

D & E - Views of fishing in the Beel

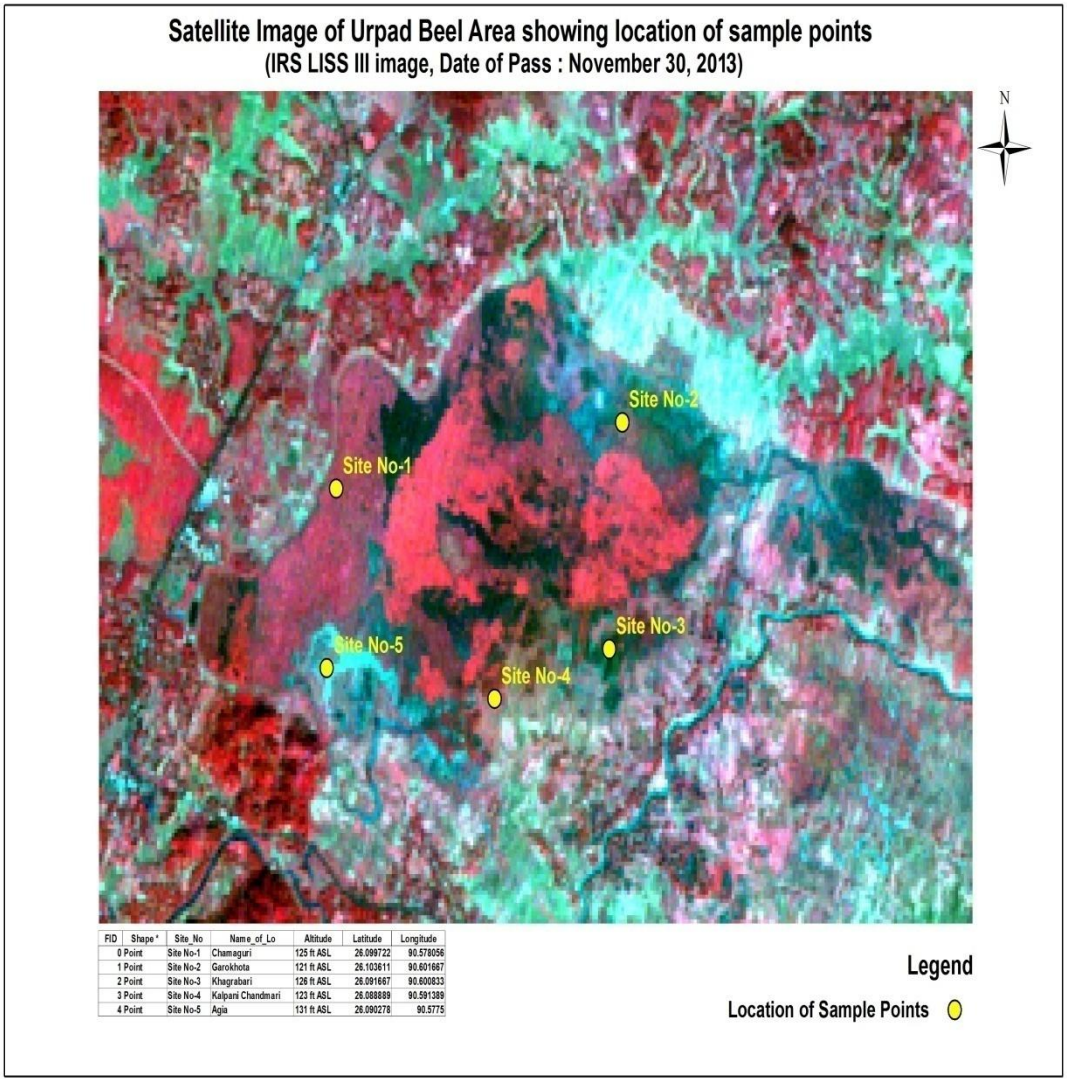

Fig. 1.2: - Satellite Image of Urpod Beel Area showing location of sampling points (Source: ARSAC, ASTEC, Guwahati, Assam)

3.2 CLIMATIC CONDITION

The climate of the district is hot in summer and dry and cool in winter. It enjoys heavy summer rainfall, winter drought, high humidity and relatively low temperature in a given year. The climate of the districts of North Eastern India normally experiences four climatic seasons (Barthakur, 1986). The seasons are (i) Pre-monsoon, (ii) Monsoon, (iii) Post -monsoon (iv) Winter which is described as follows:

A) Pre-monsoon

Pre-monsoon begins in the early part of March and continues up to the end of May. Temperature starts rising gradually from the beginning of the season onward. Pleasant morning, hot afternoons and the occasional showers with thunder and lightning are some of the important characteristics of the season. In this season marked atmospheric instability develops and severe thunderstorms occur, sometimes preceded by dust raising squalls. Rainfall increases both in amount and frequency in this season.

B) Monsoon

The monsoon sets in the last week of May or in early June and it lasts up to September or the first part of October. It is the rainy season when the state receives spells of continuous and moderate to heavy rains. June, July and August are the rainiest months when more than 70 percent of the total annual rainfall occurs.

C) Post–monsoon

The south west monsoon withdraws sometimes in between the last part of September and the first part of October. Consequently, the intensity of rainfall and the number of rainy days go on decreasing. This season continues up to the middle of November, when fogs commonly occur.

D) Winter

The winter season begins in the middle of November and continues up to the end of February. This season is characterised by low temperatures, regular morning fogs and very little amount of rainfall. December and January are the driest months and generally between which Januarys happens to be the coldest.

3.3 METHODOLOGY

3.3. A PHYSICO-CHEMICAL ANALYSIS OF WATER

3.3. A.1 Collection of water samples

Water samples were collected from the surface level of water body in different climatic seasons from five different collection sites of the beel (Table 1.1, Photo Plate 1). At least five water sample(s) were collected from each collection site. The samples were collected between 7 to 10 am in sterilized plastic bottles.

3.3. A.2 Analysis of water for different physico-chemical parameters

The collected samples were then taken into laboratory and water was analyzed for a number of physico-chemical characteristics employing standard method (APHA, 2012).

The physico-chemical parameter study included – Water Temperature, Transparency, p^H, Free CO_2, Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Total alkalinity (TA), Total hardness (TH), Calcium (Ca), Magnesium (Mg), Chloride (Cl), Sulphate (SO_4), Nitrate (NO_3), Phosphate (PO_4), Bicarbonate, Nitrogen (N), Total Dissolved Solid (TDS), Total Suspended Solids (TSS), Sodium (Na), Potassium (K), Zinc (Zn), Copper (Cu), Chromium (Cr) and Cadmium (Cd).

i) Water Temperature (˚C): - The Water Temperature (WT) was measured using mercury thermometer and noted in 0˚C.

ii) Transparency (cm): - In the present study water transparency was measured by a 20 cm diameter Sacchi disc fitted to a long calibrated attachment. The transparencies of the water were observed visually by immersing the disc in the water until it just disappeared and reappeared. The unit of transparency was expressed as centimetre (cm).

Calculation:-

$$\text{Transperancy (cm)} = \frac{A+B}{2}$$

Where, A = depth of disappearance, D = depth of reappearance.

iii) p^H : - p^H of water was measured with the help of a digital p^H meter at collection spot.

iv) Dissolved Oxygen (mgl^{-1}): - The estimation of dissolved oxygen was done by BOD bottles and by modified Winkler's method. The water samples were collected in BOD bottles and then fixed with 1.0 ml. $MnSO_4$ and 1.0 ml of KI before bringing the samples to the laboratory. Titration was done after the addition of 2.0 ml of H_2SO_4 for dissolving the precipitate with Sodium thio-sulphate ($Na_2\ S_2O_3$), using starch as an indicator. The amount of DO in water was expressed in mgl^{-1}.

Calculation:-

DO (mgl^{-1}) = ml. of 0.025 $Na_2S_2O_3$ used x 4.0.

v) Biochemical Oxygen Demand (BOD) (mgl^{-1}) : - For the estimation of BOD the samples were placed in a full air tight 300 ml BOD bottle and maintaining the temperature at 20^0 C and incubated for 5 days. It is represented as BOD_5 20^0 C. Depending upon the organic load of the effluent, dilution and bacterial seeding was done, dissolve oxygen was measured initially and after incubation. BOD was calculated from the difference between initial and final dissolved oxygen (DO).

Calculation: -

$$BOD_5\ mgl^{-1} = \frac{D1-D2}{P}$$

Where,

D1 = DO of freshly prepared diluted sample.

D2 = DO of diluted sample after 5 days incubation.

P = Decimal volumetric fraction of the sample.

vi) Free Carbon-dioxide (FCO_2) (mgl^{-1}): - Free Carbon dioxide reacts with the strong alkali Sodium hydroxide (NaOH) to form Sodium Carbonate. Development of

pink colour after adding 8 – 10 drops of phenolphthalein indicator indicates the completion of reaction at near to the p^H 8.3. Free CO_2 was expressed in mgl^{-1}.

Calculation:-

Free CO_2 = ml. of N/44 NaOH required for titration x 10.0

vii) Total Alkalinity (mg/l): - Total alkalinity was measured by titration method. To determine the total alkalinity water samples are titrated against 0.02 N H_2SO_4 using phenolphthalein and methyl orange as indicator solutions. After recording the end points of the titrant for the two indicator solution, total alkalinity was measured by using the formula as given below:

$$\text{Phenolphthalein alkalinity } (mgl^{-1}),\ A = \frac{\text{Volume of titrant (ml)} \times 1000}{\text{Volume of the sample (ml)}}$$

$$\text{Methyl orange alkalinity } (mgl^{-1}),\ B = \frac{\text{Volume of titrant (ml)} \times 1000}{\text{Volume of the sample (ml)}}$$

$$\text{Total alkalinity } (mgl^{-1}) = A + B$$

viii) Total Hardness (mgl^{-1}): - Hardness is generally due to Calcium and Magnesium ions present in the water. Total hardness was determined with EDTA (Ethylene di-amine tetra acetic acid) titrimetric method using BDH – TH indicator tablets. Total hardness is calculated using the formula as given bellow:

$$\text{Total hardness } (mgl^{-1}) = \frac{\text{Volume of titrant (ml)} \times 1000}{\text{Volume of the sample (ml)}}$$

ix) Calcium (mgl^{-1}): - Calcium was estimated by EDTA Titration method as describe bellow:

EDTA titrant is used to estimate the presence of calcium in water. EDTA solution shows reaction with Mg^{++} and Ca^{++} ions. To determine the Ca^{++} ion in water, the volume of pH must be high by using NaOH buffer. Under this condition if Mg^{++}

ion is present it shows the precipitation of magnesium hydroxide. But a specific indicator (Murexide) is used to determine calcium.

$$\text{The volume of the used titrant (ml)} = \frac{\text{Used volume of titrant} \times A \times B}{\text{Volume of the sample}}$$

$$\text{Calcium, } (mgl^{-1}) = \frac{X \times 400.8}{\text{ml .of sample}}$$

Where,

X =Volume of EDTA (titrant)

A = 0.4008 mg of Ca^{++} present when 1 ml of titrant used.

B = 1000 ml

x) Magnesium (mgl^{-1}): - Magnesium was also estimated by EDTA titration method and the value of EDTA used in calcium determination is found out. Again the value of EDTA is used in hardness ($Ca^{++} + Mg^{++}$) determination with the same volume of the sample as taken in the calcium determination.

$$Mg^{++}\ (mgl^{-1}) = \frac{Y - X \times 400.8}{\text{Volume of sample} \times 1.645S}$$

Where,

Y = EDTA used in hardness determination.

X= EDTA used in Ca determination for the same volume of the sample

xi) Chloride (mgl^{-1}): - Chloride was determined by Argentometric Titremetric method. The sample water was titrated with soluble 0.2N $AgNo_3$ using 8% K_2CrO_4 as indicator. The brick red $AgCrO_4$ was formed at the end point when all the silver ions reacted with chromate. The formula is given below:

$$Cl\ (mgl^{-1}) = \frac{\text{Volume of titrant (ml)} \times 0.2\ N \times 35.5 \times 1000}{\text{Volume of sample (ml)}}$$

Where, 0.2 = blank value attributed to K_2CrO_4

xii) Bicarbonate (mgl^{-1}): - Bicarbonates can be estimated by titrating the sample with a strong acid (HCL or H_2SO_4), first to p^H 8.3 using phenolphthalein as an indicator and then further to p^H between 4.2 and 5.4 with methyl orange or mixed indicator. In first case, the value is called as phenolphthalein Alkalinity (PA) and in second case; it is total alkalinity (TA). Values of bicarbonates can be computed from these two types of alkalinities.

xiii) Total Dissolved Solids (mgl^{-1}): - Total solids present in water samples were estimated as the residue left after evaporation of the unfiltered sample.

$$\text{Total Solids in } (mgl^{-1}) = \frac{(A-B) \times 1000}{V}$$

Where,

A = Final weight of the dish in grams.

B = Initial weight of the dish in grams.

V = Volume of sample taken in ml.

Total dissolved solids were determined as the residues left after evaporation of the filtered sample and the sample was filtered, to 250.0 ml. of filtrate was evaporated to dryness at 100˚C and then residue was weighted.

$$\text{Total Dissolved Solids in } (mgl^{-1}) = \frac{(A-B) \times 1000}{V}$$

Where,

A = Final weight of the dish in grams.

B = Initial weight of the dish in grams.

V = Volume of sample taken in ml.

xiv) Total Suspended Solids (mgl^{-1}): - The Total Suspended Solids (TSS) is the difference between total solids and total dissolved solids.

$$TSS = TS - TDS$$

xv) Sodium (mgl^{-1}): - Sodium was calculated by flame photometric method (APHA, 2012).

xvi) Potassium (mgl^{-1}): - Potassium was estimated by Flame Photometric method (APHA, 2012).

xvii) Sulphate (mgl^{-1}): - Sulphate was determined by *Turbidimetric method*. The principle is based on that sulphate can be precipitated by $BaCl_2$ in an acetic acid medium. Measurement of the light scattering of the resulting suspension is carried out using Nephelometer and sulphate concentration can be determined by comparing with a standard curve.

xviii) Nitrate (mgl^{-1}): - The estimation of Nitrate nitrogen was done by *Phenol disulphonic* method (APHA, 2012).

xix) Phosphate (mgl^{-1}): - Phosphate was done by *stannous chloride* method (APHA, 2012).

xx) Nitrogen (mgl^{-1}): - For determination of ammonia nitrogen, sample should be fixed with a few drops of H_2SO_4 and the estimation done at the laboratory by distillation method.

No. of ml of NH_4Cl solution x0.01×4×2 = ppm of ammonia nitrogen.

xxi) Zinc (mgl^{-1}): - Zinc estimation was done by Zincon method (APHA, 2012).

xxii) Copper (mgl^{-1}): - The estimation of Copper was done by Neocupronine method (APHA, 2012).

xxiii) Chromium (mgl^{-1}): - Total chromium can be determined by the use of colorimetric method. A compound of red violet colour is formed by the reaction of

dissolved hexavalent chromium with diphenylcarbazide in acid solution. Total (dissolved + particulate) chromium can be obtained after digestion of sample with $H_2SO_4 – HNO_3$. For determination of total desolved chromium, the trivalent chromium can be oxidized to hexavalent by K – permanganate. The excess of $KmnO_4$ is then destroyed by Na – azide (NaN_2). The formula used in the calculation of cadmium is:

$$\text{Total Cr } (mgl^{-1}) = \frac{A \times 100}{B \times C}$$

$$\text{Dissolved Cr } (mgl^{-1}) = \frac{A}{ml.\ sample}$$

Where,

A = µg Cr from the standard curve

B = ml original sample digested

C = ml portion from 100 ml total digest

xxiv) Cadmium (mgl^{-1}): - Cadmium can be determined by the use of Dithizone method. Cadmium ions react with dithizone to form a pink red colour. The colour so formed is extracted with chloroform and can be determined colorimetrically. The formula used in the calculation of cadmium is:

$$\text{Total Cd } (mgl^{-1}) = \frac{A \times 100}{B \times C}$$

$$\text{Dissolved Cd } (mgl^{-1}) = \frac{A}{ml.\ sample}$$

Where,

A = µg Cd from the standard curve

B = ml original sample digested

C = ml portion from 100 ml total digest

3.3 B. ESTIMATION OF PLANKTON DIVERSITY

3.3 B.1 Collection of Plankton samples

Sample collection were done by filtering a volume of 50 litre surface water through a plankton net No. 25 of bolting silk with mesh size 40 micron and were preserved in 5% formalin in 100 ml sterilized bottles (Edmonson 1963, Sharma, 2010, Sharma and Sharma, 2013). The samples were collected during both the study years in all the four climatic seasons from different sampling stations (Fig. 1.1 and Table 1.2). At least five samples were collected from each collection site for each analysis. The sample collections were done between 8 am to 10 am. For microscopic analysis samples were brought to the laboratory and were thoroughly mixed before the examination.

3.3 B.2 Plankton analysis

The microscopic analysis was done following Sourins (1978). Identifications of phytoplankton species were done by following Desikachary (1959), Prescott (1961), Bellinger and Sigee (2010) and Likens (2010).

In Laboratory the microscopic analysis of zooplankton species were done following Hosmani and Bharathi (1980). The zooplankton species observed under research binocular microscope and identified following the works of Tonapi (1980), Needham and Needham (1986), Battish (1992), Kodarkar and Chandrasekhar (1995) and Sharma and Sharma (2008, 2013).

3.3B.3 Quantitative Analysis of Plankton

For the quantitative analysis of plankton, a Sedgwick – Rafter (SR) plankton counting cell was used. A sub-sample of 1 ml was transferred to plankton counting cell for numerical counts. Average of 10 counts was done for the number of planktonic organisms. The number of each species or genus was calculated by the following formula (Welch, 1952).

$$N = \frac{a \times 1000 \times c}{l}$$

Where,

N = Number of plankton per litre.

a = average number of plankton in all counts in a counting cell of 1ml capacity.

c = the volume of original concentrate in ml.

l = the volume of original water filtered expressed in litres.

3.3. C MACROPHYTIC DIVERSITY

For macrophytic observation monthly field surveys were done during the study period. The macrophytes collected were pressed under newspapers in the field itself after proper washing and smaller ones were placed in polythine bag and tied up with thread tightly. The specimens collected were then taken in to laboratory and mounted on herbarium sheets by following the usual laboratory techniques. Some specimens were preserved in FAA solution. The collected plant species were identified following standard key and literature (Kanjilal *et. al*., 1934-1940; Bor, 1940; Baruah, 1992) and consulting the standard book of Fassett (2006) and comparing the herbaria of Department of Botany, Gauhati University, Guwahati. For the up to date nomenclature www.theplantlist.org and Plant Diversity of Assam (Barua and Ahmed, 2014) has been consulted. Phenological characteristics of the plant species were recorded in the field itself by visiting the wetlands at regular intervals (Haggerty and Mazer, 2008; Deka *et al*., 2010; Deka, 2015).

To study the phytosociological characters of the beel, quadrates of 1 m x 1m size were used within the communities. Every month each of 25 quadrates was randomly placed in all the five collection sites of the beel to find out the Importance Value Index (IVI) of species, by following the methods as described by Mishra (1969).

The quantitative characteristics of the macrophytes were calculated using following formula:

$$\text{Relative Frequency (RF)} = \frac{\text{Frequency}}{\text{Total no.of species frequency}}$$

$$\text{Relative Density (RD)} = \frac{\text{Density}}{\text{Total no.of species density}}$$

$$\text{Relative Abundance (RA)} = \frac{\text{Abundance}}{\text{Total no.of species abundance}}$$

Important Value Index (IVI) = RF + RD + RA

3.4 DETERMINATION OF SPECIES DIVERSITY INDICES

Diversity indices studied in the present investigations were Simpson Index, Dominance Index, Shanon –Wienner Diversity Index, Berger-Parker Dominance Index, Margalef Index and Sorensen's Similarity Index. These indices were calculated with the help of the following formulae:

1. Simpson Index = $\left(\frac{\sum n_i(n_i-1)}{N(N-1)}\right)$
2. Dominance Index = 1 - $\left(\frac{\sum n_i(n_i-1)}{N(N-1)}\right)$
3. Shanon – Weinner Diversity Index, $H^{-} = -\sum\left(\frac{n_i}{N} . \log_2\left(\frac{n_i}{N}\right)\right)$

Where,

ni = Importance value for each species

N = Total importance values

4. Berger-Parker Dominance Index = $\frac{N_{max}}{N}$

Where,

N_{max} = Number of individuals in the most abundant species

N = Total number of individuals

5. Margalef Index = $\frac{S-1}{\log N}$

Where,

S = Number of species

N = Number of individuals

6. Sorensen's Similarity Index (SI) = $\frac{2C}{A+B}$

Where,

A = Number of species present in site A

B = Number of species present in site B

C = Number of species common in both the sites

3.5 STATISTICAL ANALYSIS

To find out the degree of relationships between different groups of plankton and physico-chemical parameters of water Karl Pearson's coefficient of correlation (r) was calculated among all these variables. The level of significance in the correlations was tested by performing the t-test. All the relevant statistical analyses are done by using SPSS version 16.

3.6 CANONICAL CORRESPONDENCE ANALYSIS (CCA)

Canonical Correspondence Analysis (CCA) was done by using Palaeontological Statistics (PAST) Software Version 3.06 to determine relationship to determine the relationship between plankton and physico-chemical parameters.