

# CHAPTER - V

## MATERIALS AND METHODS

The entire methodology for the present dissertation is primarily based on the survey of plants and documentation of the flora of Rasik Beel complex in the Cooch Behar district of the Indian state of West Bengal. However, in the present survey only the vascular plants have been recorded. Considering from the floristic and vegetation survey the Rasik Beel complex is fully virgin and no literature on such aspects is available on this area and for the Cooch Behar District as well. So, the entire dissertation is based only on the freshly collected materials of vascular plants growing naturally or semi-naturally and cultured with one or more purpose in the study area. Therefore, the entire methodology followed during this dissertation work has been presented below in detail along with appropriate direct or indirect references.

### 5.1. Floristic Survey

The floristic survey of this part of Cooch Behar District includes all the vascular macrophytes those grow over the Rasik Beel wetland and its surrounding areas in different seasons of the year. To understand the proper and actual floristic structure of this part of the Cooch Behar district, mostly the conventional methods as described by Jain and Rao (1977) are followed.

*5.1.1. Mode of Sampling:* This study includes all the vascular macrophytes those grow in the water body of the study area. Plants are collected mostly by random sampling from different wetlands of the study area round the year covering at least three predominant seasons and for a period of 5 years, from 2007 to 2012. During summer, many plants grow in exposed land within wetland also were collected. This method has been followed for all the small and large wetlands, including ponds, rivers and ephemeral water bodies. Specimens were collected in triplicate in most of the cases and in their reproductive stages as far as possible.

*5.1.2. Collection of Specimens:* The voucher specimens were collected with or without flowering and fruiting condition and then processed according to the method as suggested by Jain & Rao (1977). Specimens of all the plants, wild as well as grown, were collected from the entire study area.

*5.1.3. Record of Field Data:* The collected specimens were tagged and necessary field data like flower colour, latex, aroma etc. were recorded in Field Note Book against the tag numbers. Local names, uses, parts used, habitat, distribution pattern, habit, flowering and fruiting, etc. were also recorded in the Field Note Book of the collected plants. The uses and local names of such plants were enquired with the local people and were also recorded in the Field Note Book.

*5.1.4. Drying of Specimens:* The collected specimens were dried in blotting papers and old news prints using a light plant-press in the field and then transferred to heavy wooden press after returning

to the laboratory. But, fleshy soft aquatic (submerged, free floating plant etc.) plants are treated with aqueous 10 % formaldehyde (HCHO) solution to check the fungal growth and to avoid fragmentation. The collected plant specimens were shocked in blotting paper first and then transferred to old newsprint or blotting papers within a short time. Specimens were given changes every 24 hours or even with a shorter interval for the first three days and then in regular intervals till the plants were properly dried.

*5.1.5. Poisoning of Specimens:* Then specimens were poisoned after drying by soaking in the saturated solution of Mercuric Chloride ( $\text{HgCl}_2$ ) in Rectified Spirit (i.e. around 6 % ethanolic solution of  $\text{HgCl}_2$ ) and then again dried under the blotters, sometimes using a Hot Air Oven.

*5.1.6. Mounting and Labeling:* After poisoning, specimens were mounted on herbarium boards using glue and then properly stitched with thread, whenever that was necessary. All the herbarium sheets were labeled properly, preferably on the lower right-hand corner of the sheet. The field number date and place of collection, scientific name, family, local name, phenophase (flowering and/or fruiting) were noted on the label. Also, the note recorded in the Field Note Book were also transferred on the label.

*5.1.7. Identification of Specimens:* Identification of collected specimens was done primarily with the help of different literature including *Flora of Bhutan* (Grierson and Long, 1983, 1987, 1991, 1994, 1999, 2001; Noltie, 1994, 2000), *Flora of India* (Sharma *et al*, 1993; Sharma and Balakrishnan, 1993; Sharma and Sanjappa, 1993; Hajra *et al*, 1995, 1997), *Flora of West Bengal* (Anonymus, 1997), *Flora of Eastern Himalaya* (Hara, 1966, 1971; Ohashi, 1975), *An Enumeration of Flowering Plants of Nepal* (Hara *et al*, 1978, 1979, 1982), *Ferns and Fern-Allies of Arunachal Pradesh* (Singh and Panigrahi, 2005), *The Orchids of Bhutan* (Pearce and Cribb, 2002), and *Diversity and Distribution of Bamboos in Assam* (Barooah and Borthakur, 2003). However, the identity was confirmed by matching specimens with the pre-identified and authenticated specimens available in NBU-Herbarium and at CAL.

*5.1.8. Storing of Voucher Specimens:* Completed herbarium sheets were stored temporarily in a steel cabinet in the Taxonomy & Environmental Biology Laboratory of the Department of Botany, North Bengal University. After finishing of the project work, the main set of voucher specimens will be deposited in the NBU-Herbarium and the duplicates will be deposited in CAL.

## **5.2. Methods of Enumeration**

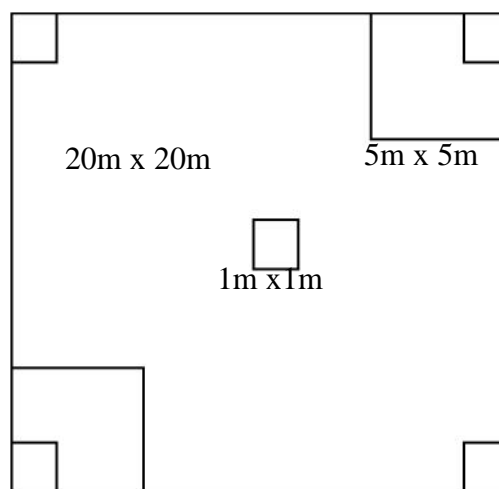
The arrangement of different families in the enumeration of Rasik Beel flora is mainly based on APG III (Chase & Reveal, 2009), followed by the alphabetical presentation of genera and species. For the infraspecific categories the autonym is presented first and the remaining taxa alphabetically. To determine the correct name chiefly the 1.1 version of The Plant List [[www.theplantlist.org](http://www.theplantlist.org)] has been followed. The protologues for family and generic names were mostly taken from Cronquist (1981, Indian reprint in 2002, 1988) and IPNI [[www.ipni.org](http://www.ipni.org)]. The protologues for species and infraspecific categories were taken from different recently published literature, IPNI and The Plant List (version 1.1). A correct name is followed by the basionym and synonym(s), if any and all such names are also

provided with protologue references. Reference to the records of different species in different floras covering this and the adjacent regions were also included. After the references, local or vernacular names, if available, in one or more local languages have been provided. Plants are then described in botanical terminologies following standard references. The phenology in the form of flowering and fruiting periods, exsiccata, general and local distribution and in some cases a 'Note' has been provided to enumerate each and every taxon of species and infraspecific categories.

Several quickly functional and useable numbered dichotomous identification keys to genera and for species and infraspecific categories have been created using mostly the easily observable characters and incorporated within the enumeration for the better utilization of the flora.

### 5.3. Phytosociology

*5.3.1. Sampling:* For the phytosociological understanding of the Rasik Beel vegetation, basic data was recorded through the application of quadrat sampling technique as suggested by Misra (1968), Shimwell (1971), Tripathi & Misra (1971), Phillip (1959), Das & Lahiri (1997), Kadir (2001), Rai (2006), Ghosh (2006) and Chowdhury (2009). During this survey 150 aquatic/ floating but fixed randomly distributed quadrates were taken from wetland surface and 25 random quadrates are taken from surrounding forest areas. As the wetland floras are mainly herbaceous, so 1m x 1m quadrates are adopted for such vegetation. Four pieces plastic of pipes, each 1 m in length, were used to form the frame that can float without any difficulty. Surveys were conducted in three different seasons and are designated as pre-monsoon [March - April], monsoon [May to July] and post-monsoon [September to November]. In deep water quadrates, free floating, submerged, immersed etc. plants are also recorded. During sampling all possible macrophytic plants including angiosperms, pteridophytes and bryophytes were recorded. In wetland algae is very common element, so it is present in almost all the quadrates but were not considered for the present work. In forests areas, Nested Quadrat technique has been used with 20m x 20m quadrates for trees and 5m x 5m quadrates for shrubs and 1m x 1m quadrates for herbaceous plants. The smaller quadrates were nested within the large [i.e. 20m x 20m] quadrates as shown in the figure 5.1. The List-Count data was recorded from each quadrat in three different seasons.



**Fig. 5.1.** Nested quadrat for study of Tree, Shrubs and Herbs

5.3.2. *Data processing*: Recorded data were transferred to MS Excel worksheet and different parameters like Frequency (F), Density (D), Abundance (A), Relative Frequency (RF), Relative Density (RD), Relative Abundance (RA) and Important Value Index (IVI) of each and every species were determined. The following formulas were used for the analysis of data as suggested by Misra (1968), Phillips (1959), Shimwell (1971), Tripathi and Misra (1971), Malhotra (1973), Das and Lahiri (1997), Kadir (2001), Rai (2006), Ghosh (2006), Chowdhury (2009) and Sarkar (2014):

**Frequency (F%)** = Number of quadrates in which the species occurred x 100/ Total number of quadrates examined

**Density (D)** = Total number of individuals of a species in all the quadrates/ Number of quadrates examined

**Abundance (A)** = Total number of plants of a species in all the quadrates/ Number of quadrates in which the species occurred

**Relative Frequency (RF)** = (Frequency of a species/ of the frequencies of all species) x 100

**Relative Density (RD)** = Total number of individuals of a species in all quadrates x 100/ Total number of individuals of all species in all quadrates

**Relative Abundance (RA)** = Abundance of a species x 100/ of abundance of all species

**Importance Value Index (IVI) or Species Importance Value Index (SIVI)**: This index is generally used to determine the overall importance of each individual species in a Particular community of ecosystem. The Importance Value Index is calculated by summing up the values of RF, RD and RA.

$$IVI = RF + RD + RA$$

## 5.4. Diversity Indices

### 5.4.1. Biological Diversity Indices

Biological diversity can be quantified in many different ways. For this, two main indices, Richness and Evenness of a particular species have been measured in a unit area. Richness is a measure of the number of different kinds of organisms present in a particular area whereas Evenness is a measure of the relative abundance of the different species making up the richness of an area.

### 5.4.2. Species Diversity Indices

Diversity indices are mathematical measures those show the proper information about composition in a particular community, species wise. Diversity indices provide important information about rarity and commonness of different species in a community. The ability to quantify diversity in this way is an important tool for biologists trying to understand community structure.

The actual scenario of plant species complexity in community structure of a particular wetland in season wise or in yearly, two different standard indices being used as follows:

**5.4.2.1. Shannon-Weiner Index (SDI)**: as suggested by Shannon – Weiner (1949) this Index is used to understand the proper plant diversity of a community and is calculated using the formula:

$$SDI = - \sum [(ni/N) \ln (ni/N)]$$

Where,  $H'$  is the index value

' $n_i$ ' is the number of individuals of a species

' $N$ ' is the total number of species in the habitat studied

**5.4.2.2. Simpson's Index (EH):** Simpson's index is another mathematical tool for understanding the concentration of dominance of particular species in the community or to identify the dominating species. Its value ranges between 0 – 1. Simpson index is a measure of diversity that takes into accounts both the richness and evenness. Simpson (1963) suggested the following formula for its calculation:

$$EH = \sum p_i^2$$

Where, ' $p_i$ ' is the proportional abundance of the ' $i^{\text{th}}$ ' species.

$$p_i = n_i/N$$

### 5.4.3. Species Richness Indices

Species richness means the measurement of number of species per sample. Species richness is the mode of determination of species diversity of an area based on the number of species occur in the habitat per unit area or sample plot. For determining the species richness standard and widely used indices adopted are:

**5.4.3.1. Menhinick Indices (D):** Menhinick (1964) provided the following formula for the calculation of Species Richness in a community -

$$D = S/\sqrt{N}$$

Where,  $S$  = Total number of species observed.

$N$  = Total number of individuals of all species.

**5.4.3.2. Margalef Indices (R1):** Margalef (1968) provided the following formula for to calculate the Species Richness in a community -

$$R1 = (s-1)/\ln(n)$$

Where,  $s$  = number of species.

$n$  = number of individuals of a species.

Apart from these, some other minor methodology might have adopted for the better and/or proper presentation or analysis of the result those are mostly self-explanatory and are generally supported with proper references.