Chapter 4
Materials & Methods

Chapter 4

MATERIALS AND METHODS

4.1. Floristic study

The floristic studies were concentrated within the boundary of the Darjiling district. The altitude of the study site ranged from 130 m amsl to 3660 m amsl covering different ecological and forest vegetational zones from Tropical through Temperate to Sub-Alpine. The forests were located on gentle to steep hill slopes with different degrees of aspect and slope and their areas varied widely. Rigorous field surveys were done during the research period starting from foothill to the higher zones in three different seasons (pre-monsoon, post-monsoon and winter) to understand the diversity, richness, community structure and plant sociology. However, the sub-alpine zones were visited only during the pre and post-monsoon seasons. The voucher specimen of the plant sample recorded were collected and noted in the field note book with proper tagging. The collected specimens were preserved in airtight polythene bags with little amount of water sprinkled. After the field collection, the specimens were brought to the laboratory and only the healthy ones were taken in triplicate and were treated with a formalin solution to avoid infections and fragility of the plant parts. The plant specimens were then kept under the blotting paper and were pressed between heavy wooden press. The blotting papers were changed every day at least for first three to seven days and afterwards the practice was repeated in alternate days and then weekly until the plant sample completely dried. The dried specimens were then duly poisoned with 8 % solution of $HgCl_2$ in rectified spirit (± 95 % ethanol) to keep it safe against fungus, insects and termites. After the completion of drying and preservation, the specimens were then mounted in standard herbarium sheets and were labelled as per the conventional methodology of Jain & Rao (1977).

The voucher specimens were then preliminary identified in the Taxonomy and Environmental Biology Laboratory of the Department of Botany, University of North Bengal following different available literatures like Hooker (1849, 1851,1872-1897, 1904,1954);Prain (1903); Cowan and Cowan (1929); Hara (1966, 1971); Ohashi (1975); Matthew (1981); Das (1986); Polunin & Stainton, (1987); Grierson & Long (1983-1987, 1991, 1999, 2001); Noltie (1994, 2000); Pearce & Cribb (2002) and many more.

However, the final confirmation on the identification was done after matching the specimen with the herbarium of NBU and finally confirming at CAL.

4.2. Phytosociological study

The phytosociological study was performed to understand the overall spectrum of vegetation of the study area. The vegetation study was analysed following the quantitative method of quadrat analysis. The method of quadrat sampling was first designed and applied by Pound and Clements (1898).

Three tier nested quadrat system was followed for vegetation sampling. Most of the main roads entering the hills and moving upward from the terai were taken as transect. Random quadrats were placed along the altitudinal gradient of the hill ranging from 130 m amsl upto 3660 m amsl and the marked quadrat were studied in three different seasons except for the higher altitude (sub-alpine region) where the quadrat was studied only for two seasons (excluding winter). Three sizes of quadrats were adopted in nested manner with 20 x 20 m plots for trees including saplings, within which two 5 x 5 m sampling plots were laid diagonally for shrubs/climbers and five 1 x 1 m plots for recording ground covers i.e. four plots in four corners and one in the centre of the 20 x 20 m plots based on slope and vegetation (Fig. 4.1). In each quadrat, individuals with girth size of >15 cm cbh (circumference at breast height i.e. 1.37 m above the ground) were counted as trees, individuals within the cbh range of 10 – 15 cm were considered as shrubs and individuals with <10 cm cbh were considered as herbs or seedlings. The sapling count for the tree species within the 20 x 20 m quadrat was also taken into account and their regeneration status was followed. The coordinates and altitude of the plots laid were noted using global positioning system (GPS; Garmin eTrex H).

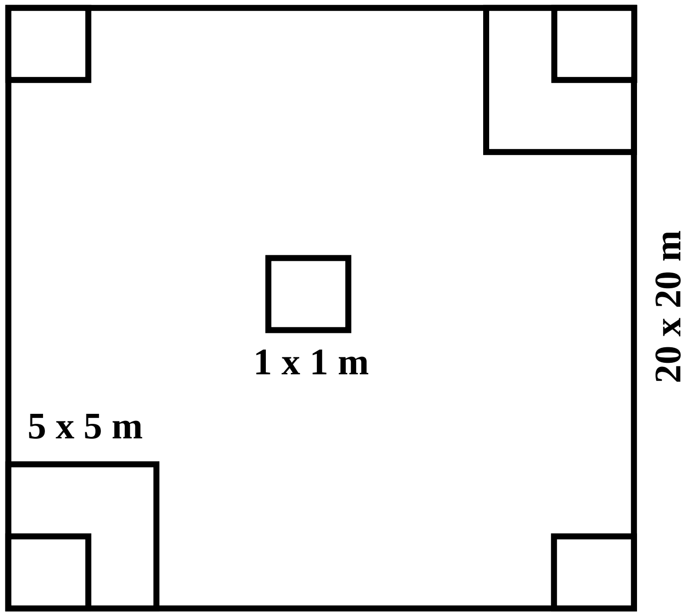

Fig.4.1. Sampling plot design for Phytosociology

4.3. DATA ANALYSIS

The determination of vegetation structure was pooled by plots to compute and estimate frequency, density, abundance, dominance (Curtis & McIntosh, 1950; Phillips, 1959).

4.3.1. Frequency

Frequency is the measure of species distribution in an area expressed in terms of percentage. It is the degree of dispersion of an individual species in a community and it represents the chance of occurrence of species in a given habitat.

$$\text{Frequency (\%)} = \frac{\text{Number of quadrats in which the species occured}}{\text{Total number of quadrats studied}} \text{ X } 100$$

Relative frequency is the degree of species distribution in an area with relation to the frequency of all the species.

$$\text{Relative Frequency (RF)} = \frac{\text{Frequency of the individual species}}{\text{Frequency of all the species}} \text{ X } 100$$

The frequencies of species were further classified into Raunkiaer's five frequency classes *viz.* A, B, C, D, E (Raunkiaer, 1934). The frequency range for these five classes are A (1 – 20 %), B (21 – 40 %), C (41 – 60 %), D (61 – 80 %), and E (81 – 100 %). As per the Raunkiaer's Law of Frequency, poorly dispersed species are likely to be more in number than the species that are better dispersed or distributed *i.e.* A>B>C>=<D<E. The average frequency data for the Raunkiaer's normal frequency diagram for class A was 53 %, B 14 % that of C was 9 %, D was 8 % and E, 16 %.The recorded species were grouped into Raunkiaer's five frequency classes and a comparison between normal and observed frequency was noted.

4.3.2. Density

Density is the numerical strength of a species in a unit area sampled. It indicates the abundance of species in a unit area and gives an idea about the dominance and rarity of a species and also the standing biomass and productivity of the habitat.

$$\text{Density} = \frac{\text{Total number of individual of a species in all quadrats}}{\text{Total number of quadrats studied}}$$

Relative Density is the measure of numerical strength of a species in relation to the total number of individual of a species in a unit area.

$$\text{Relative Density (RD)} = \frac{\text{Density of the individual species}}{\text{Density of all the species}} \text{X } 100$$

4.3.3. Abundance

Abundance is the study of the number of individuals of different species in the community per unit area. It gives an idea about the commonness of a species in a habitat under study.

$$\text{Abundance} = \frac{\text{Total number of individual of a species in all quadrats}}{\text{Total number of quadrats in which the species occured}}$$

Relative Abundance is the ratio of the species to number of quadrat studied for a give vegetation type.

$$\text{Relative Abundance (RA)} = \frac{\text{Abundance of species}}{\text{Abundance of all the species}} \text{ X } 100$$

4.3.4. Dominance

Dominance is the measure of the total basal area of the species per hectare. It indicates the relative size, volume and weight of a tree and provides information on the proportion of its dominance in a forest community. It is ascertained by the formula,

$BA = \frac{(CBH)^2}{4\pi}$ Where, *CBH* is the circumference at breast height and value of π is 3.1416.

Relative Dominance is the basal area covered by a species with respect to the sum of the area covered by rest of the species in the area.

Relative Dominance (RDm) = $\frac{\text{Total basal area of the species}}{\text{Total basal area of all the species}}$ X 100

4.3.5. Importance Value Index (IVI)

The Importance Value Index (IVI) for each species is to determine the overall importance of each species in the community and was ascertained by summing up the values of Relative Frequency (RF), Relative Density (RD) and Relative Dominance (RDm) (Curtis, 1959). It helps to understand the sociological structure of a species in a community.

$$\mathbf{IVI} = \sum[RF + RD + RDm]$$

4.4. DIVERSITY INDICES

The diversity indices are an important tool for measuring the biodiversity. Various indices were estimated to understand the diversity of the species in the forest community.

The diversity indices like the Species diversity was estimated by calculating Shannon & Wiener's Index (1963), the species richness was computed following Menhinick's Index (1964). The concentration of dominance was measured as per Simpson's Index (1949), the species evenness was estimated according to Pielou's Index (1966). The distribution pattern of the species in the community was determined using the formula by Whitford (1949). For estimating the beta diversity between the communities, Sorensen's index of similarity (1948) was used.

4.4.1. Shannon-Wiener's Index (1963)

The species diversity is the expression of community structure and indicates the distribution and complexity of the species in the habitat. It was estimated following Shannon-Wiener's Index ($\boldsymbol{H'}$) (Shannon and Wiener, 1963).

$$\boldsymbol{H'} = -\sum[(ni/N)\ln(ni/N)]$$

Where, *ni* is the number of individual of a species and *N* is the sum total of all the individual species

4.4.2. Menhinick's Index (1964)

The species richness is the number of species in a sample or habitat per unit area and is based on the total number of species and the total number of individuals in a sample or habitat. It is calculated by Menhinick's Index (***D***) (Menhinick, 1964).

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

Where, *S* is the number of individual species and *N* is the sum total of all the individual species

4.4.3. Simpson's Index (1949)

The concentration of dominance puts more emphasis on the dominant species in the habitat. It determines the chances that a species could be encountered during the sampling and its value lies between 0 and 1. It was calculated by Simpson's Index (***CD***) (Simpson, 1949).

$\boldsymbol{CD} = \sum(ni/N)^2$ Where, *ni/N* is the same as that of Shannon-Wiener values

4.4.4. Pielou's Index (1966)

The species evenness recognizes how close in number each species is in a particular habitat. It was estimated using Pielou's Index (***J***) (Pielou, 1966)

$$\boldsymbol{J} = H'/\ln S$$

Where, *H'* is the Shannon Index and *S* is the number of individual

4.4.5. Whitford Index (1949)

The abundance to frequency ratio (A/F) was studied to understand the spatial distribution pattern of the species (Whitford, 1949). The ratio of <0.025 indicates regular distribution, 0.025 – 0.050 means random distribution and >0.050 indicates contiguous distribution (Curtis and Cottam, 1956).

WI = Abundance/Frequency

4.4.6. Sorensen's Index (1948)

The indices of Similarity (***S***) and dissimilarity were calculated using the formula by Sorensen (1948). It is the simplest measure of beta diversity whose value ranges from zero when there is no species overlap, to one when the species overlaps between the communities. It helps to compare different habitat types and their suitability for migration and evolution and speciation of the species.

$\boldsymbol{S} = 2C/\mathrm{A} + \mathrm{B}$

Where, A = number of species in community A; B = number of species in community B; C = number of species common to both the communities.

Dissimilarity Index = $1 - \boldsymbol{S}$

4.5. SOIL ANALYSIS

Soil samples were collected during the field survey from different locations and altitudes. The top layer of the soil (0 – 15 cm) and the sub-layer (15 – 40 cm) were collected and analysed to determine soil physico-chemical properties. The estimation of the pH was done by McKeague method (McKeague, 1978), total organic carbon by Walkley and Black method (Walkley & Black, 1934), Nitrogen by Kjeldahl method (Bremner, 1965), Potassium by ammonium acetate extraction method (Pratt, 1965) and Phosphorus by Bray I method (Bray & Kurtz, 1945).

4.6. STATISTICAL ANALYSIS

The data collected were interpreted by statistical methods wherever necessary. A Two-way *ANOVA* test was performed to understand the differences between different parameters. The standard error was tested using the formula.

$\boldsymbol{SE} = \mathrm{S}/\sqrt{\mathrm{N}}$

Where, S is the standard deviation and N is the number of observations.

4.7. GEOGRAPHIC INFORMATION SYSTEM & REMOTE SENSING

The ecological studies of larger areas over a span of time have been enriched by the development of computer based Geographic Information System that is superior over manual methods (Johnston & Naiman, 1990). It has been effectively used to map the forest vegetation cover at micro, macro and meso levels (Rai, 2006). Remote sensing can be useful in analyzing and monitoring diversity patterns and making conservation strategies (Stohlgren *et al.*, 1997). In the present study the Aster Digital Elevation Model (DEM) was used to prepare the elevation, slope and aspect map using ERDAS software (ver. 8.5) and it was classified further in different range/class in ArcGIS software (ver. 9). The Aster DEM was then subset using the study area boundary in ERDAS software. Further this subset image file was classified in different altitudinal band in ArcGIS. The forest type was classified into three classes- Dense forest, Open forest and Non-forest area. The digitalized image was then classified according to elevation, slope and aspect. The Land Sat imagery of the study area was geo-rectified with the help of base image and classified using unsupervised classification techniques. Different maps have been constructed and calculated from the study area.