CHAPTER - 2

MATERIALS AND METHODS
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2.1. Selectionof the Vegetation for Survey and Collection

An extensive review of literature was done for the study area prior to the field trip and excursion for documentation and Phyto- sociological study. Different areas of the Darjiling hills comprising of three hill subdivision of Darjiling up to Sukuna having altitudinal range from 300m amsl to 3636m amsl were visited during different seasons for the collection of specimens and all relevant field data. In the Darjeeling subdivision the entire locality from Darjeeling – Lebong – Ging-Badamtam (2100-1900-1700); Lebong – Pattabong (1900-1400m), Rungbul – Dhotrey Moonda Kotee (1950-1600 m) Sonada (2000m), Ghoom - Jorebunglow-Jalapahar - (2300–2350m), Jorebunglow - Rangarung - 3rd Mile- Rungli - Rungloit – Takdah- Bara mangwa (2200-1100m), 3rd mile - Mungpoo (2100-1300), Ghoom - Senchal - Tiger Hill (2300-2400- 500m), Sukhia Pokhri - Maney Bhangyang - Megma (2200-2000-2400m). Megma - Tonglu (2400-3000m), Tonglu – Garibans - Sandakphu (3000-2800-3636m), Sandakphu – Sabargam - Phalut (3636-3300-3700), Sandakphu - Samanden –Kalikhola – Ramam – Srikhola - Rimbick (3636-2400-2400-1900-2200m), Rimbick - Lodhoma (2200-1300m), in the Kurseong subdivision, Dilaram - Kurseong (1700-1650m), Kurseong - St. Mary's - Upper Mamring - Lower Mamring (1650-2000-1700–1300m), Mirik - Manjua (1900-1000m); in the Kalimpong sub-division Lopchu - Peshok - Rayang - Kalimpong (1500-900-300-1350m), Kalimpong – Pedong (1350-1400 m), Kalimpong - Rashet (1350-1500), Kalimpong – Labha - Kolbong (1350-1450-2300m); Kafer – Lolaygaon - Munsong (1200-1350-1900m), Latpunchor (1200m and above), Labha - Panmkhasari - Khampong (1450-2400-2000m), Rhenok - Rashet (1400-

2500m); Rechela (1400-3160 m),were visited for obtaining data with respect to seasonal, vertical as well as horizontal distribution of the Pteridophytic flora.

2.1.1. Collection and Preservation of Materials

During the field trips the different species of Fern and fern allies along with other plant specimens relevant to the present dissertation were traced on visual observation and representative specimens were collected. The specimens included the entire plant with intact rhizome and the plant considered included those in Sporulating either in the form of spike, sorus or strobilus. In cases where the reproductive structures were not observed, the mature vegetative plant along with their intact rhizome was collected. Special care was taken to collect all relevant data for a given specimen in a field note book and after proper tagging the specimens was pressed in portable herbarium press between the blotting papers. After the completion of field trip the specimen were brought to the laboratory, where the rhizome were cleaned, trimmed, poisoned with 4% Mercuric Chloride solution in rectified alcohol and was transferred to the wooden plant presses. After proper drying the specimens were poisoned by soaking them in 4% Mercuric Chloride solution in rectified sprit, dried again under pressure using blotting papers. The method followed for the preparation of herbarium from the fresh sample was according to Jain and Rao, 1977.

Dried and poisoned specimen were then pasted on standard herbarium sheets, labeled properly and temporarily stored in the Herbarium cabinet in the department of botany, St Joseph College.

2.1.2. Field note Book

The collected specimens were recorded in a Field Note Book noting all that would not be readily visible in pressed dried specimens. The details included the

Gps data, locations, altitude, date of collections, habit, habitat, type and colours of Hairs, Scales, Spines, Mucoration on stipe, rachis, pinnules and rhizome The field notes were transferred to herbarium labels for ready reference. Moreover, data on local utilization were collected with the parts and purpose for which they are used.

2.1.3. Identification

The specimens were primarily examined and identified in the botany department of St Joseph College using the available literature and matching with the herbarium in the Llyod's botanical garden. Identification was confirmed by matching all specimens at the Central National Herbarium, Howrah (Calcutta).

2.1.4. Enumeration and Description

The enumeration of the fern and fern allies of Darjiling hills in the present study has been done following the classification of Smith et al 2006 with slight modification on the family Thelypterideaceae (Fraser-Jenkins,2009). The fern and fern allies have been classified into 4 classes that include 11 orders and 37 families. The correct name followed by basionym and other available synonyms has been provided for each of the species collected and studied. Proper author citation, protolouge references and record in floristic and taxonomic work as correct name has been provided. A key to the acronym and abbreviation used to denote various journals and books has been provided at the beginning of the enumeration. Local names whenever available have been provided along with language.

Detailed description of each species has been provided with all the measurements carried out using metric system only. In case of the measurements if not specifically mentioned, the length of the structure is placed first followed by the breadth punctuated by `x' mark e.g. (6x2 cm denotes that the length = 6cm and the

breadth 2 cm.)

To cite the voucher specimen, the place of collection followed by altitude of the place, local name, local uses, date of collection, the collector's name, name of determinant and field numbers have been provided with the specimens from different places of collections being provided separately. The local distribution of the species given is the data accumulated form the field study. The Global distribution has been reviewed from the past literature and the herbaria visited.

2.2. Phytosociological Studies

The Phytosociological studies were carried out to determine the status of availability, horizontal and vertical distribution and to know the natural associates of the different species of Fern and Fern allies.

2.2.1. Location of the Study area

The study area included all those locations described above in 2.1. The study area was divided into four forest type with respect to climatic zone (Champion and Seth ,1968) which are (1) Tropical Forest (0 -800m (ii) Sub –Tropical Forest (800-1600m) (iii) Temperate forest (1600-3000m) (iv) Sub-alpine forest (3000m and above).

2.2.2. Sampling

A random sampling with nested quadrat was utilized to study the strata of vegetation. Trees and tree saplings above 5 feet in height were classified as trees during the count. The tree and shrub layers were analyzed by quardat method using

the sizes 20 x 20 m and 5 x 5 m respectively and the herbs were analyzed by 1 x 1 m Quadrat. Basically a nested quadrat was placed having a size of 20 x 20m(for trees) within which 5 x 5 m quadrat was placed for analyzing the shrubs within which 1m x 1m quadrat was placed for the study of herbaceous plant. A total of 20 sampling plots were studied at each forest type with the 5 x 5 m quadrate being inside the 20 x 20 m quadrat (Yadav *et al*, 1991, Nath *et al* 1991). Specimens of the different species were collected tagged and herbarium prepared for identification. The diagrammatic sampling design for Phyto-sociological analysis has been provided in figure 2.1.
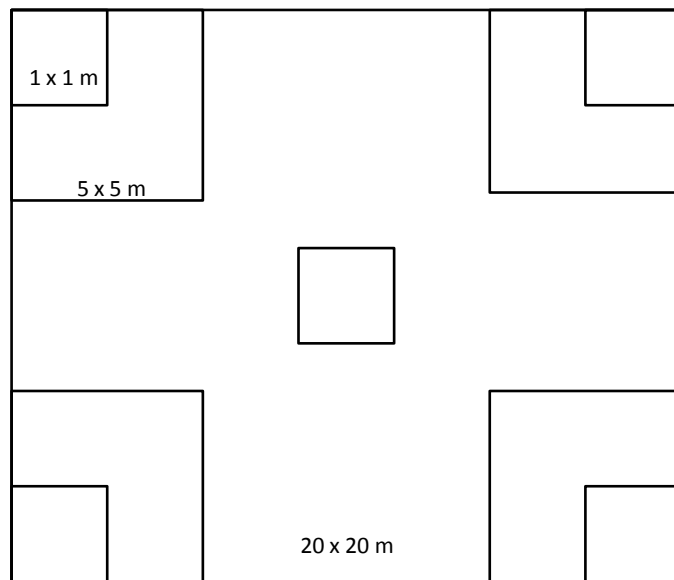

Figure 2.1: Sampling Design for Phytosociological analysis.

2.2.3. Phyto-sociological analysis

The data for each life form were recorded in the study area which were divided into five altitudinal zone and were quantitatively analyzed for Density (D),

Relative density (RD), frequency (F), relative frequency (RF),Abundance (A), relative abundance(RA) etc. The importance value index (IVI) for herbs and shrubs has been calculated through the abundance data according to Samanta and Das(1998). Finally for trees the importance value index (IVI) was calculated according to Philips (1959) and Misra(1969).

2.2.3.1 Density

Density is a numerical strength of a species in relation to a unit area.This parameter gives an idea about the dominance and rarity of a species and is also an indicator of the standing biomass and productivity of the region (Ambashat et al,1995). The percentage of a species with respect to different species in the unit area is called as Relative density. They are calculated as:

Density (D)= Total number of individual of a Species

Total number of quadrat studied

Relative Density (RD) = Density of a Species   x 100

Sum of the Densities of all the Species

2.2.3.2

Frequency is the degree of dispersion in terms of percentage occurrence. In a sampling only the names of the species encountered in the different quadrat are listed. Frequency basically gives us an idea how frequent a species is encountered in the area. The frequency percentage of a species with respect to different species in the unit area is called as Relative Frequency.

Frequency (F%) = Number of Quadrat in which species occurred   x 100

Total number of Quadrat Studied

Relative Frequency (RF) = $\dfrac{\text{Frequency of a Species}}{\text{Sum of Frequencies of all Species}}$ x 100

2.2.3.3 Abundance

Abundance is the total number of individual of a species in a sampling area. It basically gives an idea of the occurrence of a species in the Sampling unit. Relative abundance is the percentage ratio between the Abundance of species with that of sum of abundance of all species in the sampling unit.

Abundance (A) = $\dfrac{\text{Number of Individual of a species occurring}}{\text{Total Number of Quadrat Studied}}$

Relative Abundance (A) = $\dfrac{\text{Abundance of a Species}}{\text{Sum of Abundances of all Species}}$ x 100

2.2.3.4 Basal area

Basal area implies the area covered by the tree which is basically calculated by taking a measurement at breast height i.e. 1.37m of a tree trunk above the ground. Basal area indicates the weight, size, volume and provides information regarding the proportion of its dominance in the sampling area. Basal area is calculated as;

Basal area (BA) = $\dfrac{(CBH)^2}{4\Pi}$

CBH: Circumference at Breast height.

2.2.3.5 Importance value Index (IVI)

The IVI gives an overall picture of a species and its importance in a community. The importance of a species in a community can be obtained by adding the value of relative density, relative dominance and relative frequency.

The IVI is calculated by the following formula;

IVI = RF+RD+ RDm (RA)

2.2.4 Diversity indices

Diversity indices serve as important surrogates for measuring Biodiversity (Sarkar and Margules,2003). A diversity index is a quantitative measure that reflects how many different types (such as species) there are in a dataset, and simultaneously takes into account how evenly the basic entities (such as individuals) are distributed among those types (http://en. wikipedia. org/wiki/ Diversity_index).There are numerous indices formulated for diversity study but the commonly followed diversity indices have been adopted.

2.2.4.1 Species Diversity

It is the effective number of different species that are represented in a collection of individuals. Species richness and species evenness are the two component of Species diversity. Shannon- Wiener index (1963), incorporates both the parameter and is one of the most widely used index for measuring species diversity in an ecosystem (Ilorkar and Khatri ,2003). Lower the dominance higher is the diversity.

Shannon –Wiener index (1963)

$$H' = -\sum [n_i/N)\ln(n_i/N)]$$

Where 'H' is the index value

'ni' is the number of individual of a species

'N' is the total number of species in the Habitat area.

2.2.4.2 Species Richness

The number of different species represented in a sampling unit or the habitat per unit area is called as Species Richness. Species richness is simply a count of species, and it does not take into account the abundances of the species or their relative abundance distributions (Colwell,2009).Menhinick's index (1964) and has been used to understand the Species richness.

Menhinick's Index (1964)

$D = S/\sqrt{N}$

Where 'D' is the index value

'S' is the total number of Species

'N' is the total number of individual of all Species.

2.2.4.3 Concentration of dominance

Simpson index (1949)is used to measure the degree of concentration of dominance of species which basically gives priority to the Dominant species. It maximum value ranges between 0 -1, which basically relates to the number of chance a particular species that can be encountered in a sampling unit.

Simpson's Index (1949)

$\lambda = \sum pi^2$

Where 'pi' is the proportional abundance of the ith species.

Pi= ni/N

'ni' and 'N' are same as Shanon-Wiener index.

2.2.5 Local utilization of Fern and their allies

Surveys were also conducted for the documentation of local use of the fern and fern allies in the Study area. During field trips and excursion the primary data for this purpose were collected through questionnaires and interviews with local people. The knowledge's of elderly people and local medicinal practitioner were thoroughly examined in the way of conversation for utilization of fern Species. At the same time different herbal medicinal man as well as healers comprising Bijuwa, Phedengba etc. Were interviewed for their traditional knowledge and utilization of fern Species . Local market was usually visited at a the time of Hatt (villagers sell their product once in a week) for knowing and listing the fern Species utilized for the domestic consumption . Local nursery and Flower shops were visited to determine the use of ferns as ornamentals. Even local drink and their production was surveyed, examined and documented. Lastly, previous literature was surveyed for darjiling hills to understand the utilization of fern species which proved to be a secondary source.