CHAPTER-4

MATERIALS AND METHODS

MATARIALS AND METHODS – 4

As an approach to the present study, a well-planned intensive field works covering almost all seasons throughout the year during 2014-2020 were conducted. Detail information's on each and plant species of various habitats of three MPCAs have been recorded. The present dissertation is covering a number of aspects using wide array of methodology that has been discussed below:

4.1. FLORISTICS

Extensive field surveys in different agro-climatic zone of the three MPCAs of West Bengal in various pre-dominant seasons were made during last eightyears. All the vascular plant resources including medicinal plants and their population were recorded in detail. The following methodologies were applied using techniques devised by (Jain & Rao, 1977, Mondal & Chowdhury 2018, Paul et al, 2020) with some modifications wherever it was essential.

4.1.1. Sampling Specimens

Plantsparts were collected in bulk at random from different habitats from almost all the area of MPCAs. During specimens' collection mostly flowering and fruiting stages were targeted for easy identification and good voucher specimens. For this purpose in many cases repeated visit to the same spot were made sometimes even within a week.

4.1.2. Record of Field Data

During collection, plant specimens were tagged properly and necessary field data like colour on different plant parts including flowers, absence or presence of exudate, scent / aroma, habitat structure, association, population structure, etc. were recorded in the *Field Note-Book*. The ethno botanical uses of plants materials were investigated through direct interview using some standard questionnaires to the local ethnic communities and village peoples and also observation on direct uses, were recorded in the *Field Note-Book*.

4.1.3. Processing and Drying of Specimens

At the field camp or at the laboratory, the collected specimens are cleaned and trimmed suitably, displayed properly on blotters (blotting papers and old newsprints) and then dried in wooden Plant Press. Before pressing, most of the specimens were treated with 6

% formaldehyde (HCHO) solution to avoid fragmentation of specimens and to eliminate chances of decomposition through fungal infestation. Soft plants parts are kept in a separate light-weight Plant Press, where the pressure was increased very slowly and much frequent change of blotters during the first few days of the drying operation. For other plants blotters were changed with regular frequency in a heavy wooden Plant-Press until drying. During moist season for proper drying a Hot Air Oven was used with temperature adjusted at 40–45º C. Generally, specimens were completely dried out within one or two week time.

4.1.4. Poisoning of Specimens

After drying all the plant specimens were poisoned with 6% ethanolic solution of Mercuric Chloride ($HgCl_2$) and dried again in blotting papers for a day.

4.1.5. Mounting and Labeling

After poisoning, specimens were mounted on standard Herbarium Sheets. Later on a label was attached, in most cases, near the right hand bottom corner of the sheet, which bears the Field No, date and place of collection, scientific name, family, local name, field-characters and the name of the collector. Mounted and labelled specimens were stored temporarily in a steel cabinet for further use during the present dissertation.

4.1.6. Identification

After the mounting, specimens were taken under critical study and identified initially matched with the pre-identified specimens in NBU-Herbarium and also with character matching with the different Taxonomic literature by various authors (Prain, 1903; Noltie, 1994). Further confirmation of identification the specimen, several virtual herbaria (K, TAI) and online floras were also consulted time to time. Some unidentified specimens were taken to CAL for matching with herbarium and experts in plant taxonomist were also consulted for finalization.

4.1.7. Storing the Herbarium Sheets

Among the entire prepared specimens, one set of voucher specimens were be deposited in the NBU-Herbarium against specific Accession numbers and the duplicates will be deposited at CAL Herbarium for future reference for global scientist and as the evidence of the present work.

4.2. PHYTOSOCIOLOGY

For phytosociological works quadrate sampling is the widely accepted method as suggested by Misra, 1968; Shimwell, 1971; Tripathi & Misra, 1971; Phillip, 1959 and Kadir, 2001; Rai, 2006; Chowdhury, 2009. During this dissertation, most of the samples of tree, shrub & herb species were sampled in different season of the year.

4.2.1. Sampling

Quadrate samples were taken randomly in all over each MPCA, which includes marshy and exposed wetland areas wherever it is there within each MPCA. For Phytosociological study nested quadrate will be plotted. For herbaceous species 1m × 1m quadrates will be adopted, for shrubs 5m × 5m and for tree 20m × 20m quadrates (Fig. 13) were adopted. Surveys were conducted in three different seasons and are Pre-monsoon, post-monsoon & winter. During sampling all possible plants species including angiosperms, gymnosperms and pteridophytes will be recorded.

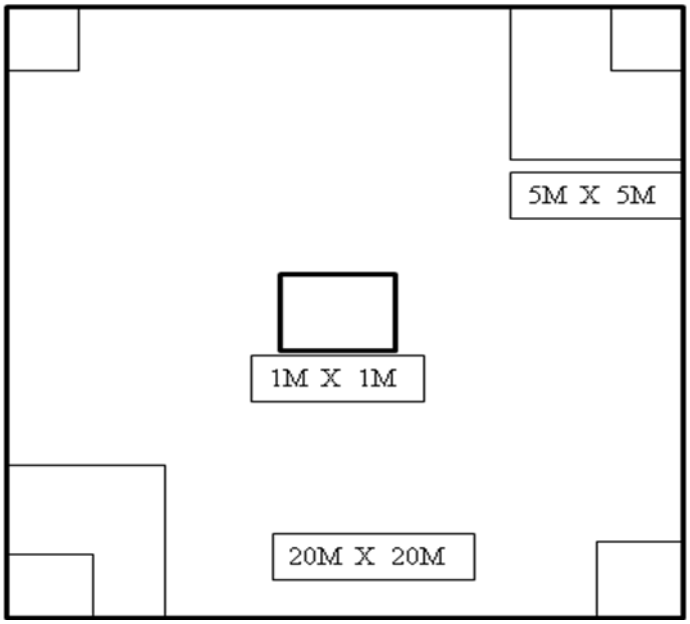

Fig. 13: Sketch of Nested Quadrate Model adopted for phytosociological study

4.2.2. Data processing

Recorded data is transferred to Microsoft Excel worksheet and parameters like Abundance, Density, Frequency and their relativeness along with Important Value Index for each species were determined following the methods as suggested by Phillips (1959), Malhotra (1973), Kadir (2001), Rai (2006) and Chowdhury, (2009).

4.2.3. Biological Diversity Indices

Biological diversity can be quantified in various ways and to determine this, species Richness and Evenness of particular taxa have been measured against unit area. Number of different organisms present in a particular area is a measure as Richness, whereas Evenness can be measure of the relative abundance of the different taxa that makes the richness of an area.

4.2.3.1.1. Simpson's Index (λ)

Simpson's index is a mathematical tool that is used to determine the concentration of dominance of specific species in each MPCA. The value of Simpson's index is varies between 0–1. During measuring the Simpson index both the richness and evenness taken into accounts. The calculating formula for concentration of dominance that is suggested by Simpson (1963) is as follows:

$$\lambda = \Sigma pi^2$$

here, 'pi' denotes the proportional abundance of 'i^{th}' species and pi = ni/N

4.2.3.1.2. Shannon – Weiner Index (H')

Shannon - Weiner index (Shannon–Weiner, 1949) was used to determine species diversity of MPCA:

$$H' = - \Sigma [(ni/N) \ln (ni/N)]$$

Where, 'ni' denotes the number of individuals of each species.

'N' denotes the total number of species studied within the habitat.

4.2.3.2. Species Richness Indices

Species richness can be measurement of number of individual of each species per sample. Species richness can be determined by measuring to species diversity based on the number of species occur per sample plot. For determining the species richness standard indices were adopted:

4.2.3.2.1. Menhinick Indices (D)

Species richness in a community is determined by Menhinick Index (Menhinick, 1964).

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

here, S means total number of species and N denotes total number of individuals that observed.

4.2.3.2.2. Margalef Indices (R1)

Margalef Index (Margalef, 1968) is also used to determine Species richness.

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

Where, s = number of species.

n = number or of individuals of a species.

4.2.3.2.3. Similarity Index (SI)

The similarity index determines the interspecific association between the species of plant communities.Sorensen's species similarity index (SI) between the transects and the two sites was calculated

$$SI = \left(\frac{2C}{(a+b)} \right) \times 100$$

Where, C is the number of species in sites a and b; a and b arethe number of species in sites a and b

4.3. ETHNOBOTANY

4.3.1. Economic uses

The indigenous people of the tropical world from the preindustrial period have an intimate relationship with the natural resources of their environment. Wild and

cultivated plants and wild and domesticated animals both provided all the food and others they needed for living. The utility of these three plant families were known to humankind since the ancient time.

MPCAs occupy a very important position among all economic plants, as they are one of the major sources of man's food. Tender leaves, young inflorescence, pith of the stem, fruits and seeds, endosperm of many plants are edible and provide all the nutritive materials for healthy living. Species of wild plants have also local and commercial uses as source of food, sugar, wine, oil, fibers and various other items of uses such as building material, furniture in the form of wood and leaves. Soft young leaves are also useful for making various household items. Due to high nutritive and medicinal values of the edible portion of coconut plant. Few plants they are commercially cultivated. Local, medicinal, commercial and ethnic uses of indigenous plants however are more to be known through extensive survey, wide interaction and document research. Various authors (Jain, 1981, 1987, 1991; Rai *et al.*,1998; Rai & Bhujel, 1999; Rai, 2002; Sarkar, 2011) have been recorded food and medicinal uses of some indigenous plants from this part of India. Now efforts were made to record some more information on different uses of indigenous plants, some are of great interest not earlier known.

Information about the common uses of various semi wild, cultivated and domesticated plants were collected along with their economic values from the existing published works of various authors (Basu 1991, 2012; Basu and Chakraverty 1994; Chowdhury 2009; Basu and Mondal 2013, 2015; Mondal and Chowdhury 2016, 2017, 2018, 2020) or by direct observation on uses of wild plants-based products.

4.3.2. Ethno-botanical Study

The complete methodology for the ethnic uses of plants was primarily based on the interaction with the various ethnic communities of Sub-Himalayan Terai-Duars, plains of Bengal and western plateau. Peoples of various ethnic groups were directly using different parts of plants in their livelihood. During study, entire data in connection to traditional uses of medicinal plants and their mode of utilizations were scientifically documented and properly photographed as evidence. For this part of work conventional methods were followed as suggested by Jain 1981, 1987, 1991; Rai 2002; Sarkar 2011; Chowdhury 2015; Kirtikar and Basu 1935; Chopra *et al.* 1956, 1969; Jain 1991; Shah and Das 2002; Chowdhury 2009; Sarkar 2011; Basu and Mondal 2015; Mondal *et al.*

2017. A set of questionnaire prepared based on the model (Jain 1991; Chowdhury 2009 and Sarkar 2011) for the present study. The extensive fieldworkswere carried out in different villages of Terai and Duars region of North Bengal. Enquiries were made on their daily life, food habit, fodder collection, occupation, health practices, medicines, trade, beliefs, rituals, ceremonies, traditions and customs using a pre-designed questionnaire.

4.4. SOIL ANALYSIS

Physical and chemical parameters of soil of each MPCAhave already analyzed to understand the present status of existing soil of each MPCA. Expert soil analyst and technician have analyzed different physical and chemical parameters of soil samples in Salugara soil testing laboratory of Forest Dept. Govt. of West Bengal following standard methodology.

4.5. RET ASSESSMENT

To assess the threatened status of different wild species of MPCAs were evaluated by matching with the red data book published by Botanical Survey of India (1987). For latest information about the status of selected species, IUCN website was also consulted. During the field study, visual observation and calculated abundance value also help us to determine the local level species availability.