CHAPTER: 3

3. MATERIALS AND METHODS

The present dissertation is having a number of aspects using wide array of methodology as has been discussed below:

3.1. Floristics

The floristic survey of this part of Gangetic plains includes all the macrophytes that grown over wetland areas throughout the year. To understand the proper and actual floristic structure of this part of the country, following methodologies are applied. The methods are mostly conventional and as devised by Jain and Rao (1977).

3.1.1. Mode of Sampling: This study includes all the macrophytes which grow in different water bodies of the study area. Plants are collected by random sampling from different wetlands of the district round the year covering three predominant seasons and for a period of 4 years, from 2003 to 2008. In summer, species grown in exposed wetlands are collected and are similar in all small and large wetlands, ponds, rivers and ephemeral water bodies. Specimens were collected in triplicate in most of the cases and during their reproductive stages as far as possible.

3.1.2. Record of Field Data: Specimens were tagged and necessary field data like flower colour, latex colour, scent or odour etc. were recorded in field notebook. Local people are interviewed to know the local names and uses of such plants and were also recorded.

3.1.3. Dry of Specimens: The collected specimens are dried in wooden plant press. However, fleshy and soft aquatic (submerged, free floating plant etc.) plants are treated with concentrated or 10% formaldehyde (HCHO) solution to check the fungal growth and shocked initially in blotting paper and later on transferred to old newsprint or blotting papers within a short time. Next few changes were also quick for first few days. On the other hand some woody and erect herbs, shrubs and tree specimens were

dried up directly in newsprint or in blotting papers in heavy wooden press. Generally specimens were completely dryed within one week. In some cases, a Hot Air Oven was also used for proper and/or quick drying.

3.1.4. Poisoning of Specimens: After drying all the specimens were poisoned with 4% alcoholic solution of Mercuric Chloride ($HgCl_2$) and again dried in blotting papers.

3.1.5. Mounting and Labeling: After poisoning, specimens were mounted on standard herbarium sheets. Later on a label was attached near the right corner on the sheet, which bears the field No, date and place of collection, scientific name, family, local name etc.

3.1.6. Identification: Specimens are studied and identified with the help of different literature and matched with the pre-identified specimens in the Taxonomy & Environmental Biology Laboratory of North Bengal University and in NBU- Herbarium. Doubtful specimens are taken to CAL and BSIS and identified mostly by matching.

3.1.7. Storing the Herbarium sheets: The first set of voucher specimens and field notebooks will be ultimately deposited in the NBU-Herbarium and the duplicates will be deposited at CAL and BSIS.

3.2. Phyto sociology

For phytosociological works quadrate sampling technique is the widely accepted method as suggested by Misra (1968), Shimwell (1971), Tripathi & Misra (1971), Phillip (1959), Das & Lahiri (1997) and Kadir (2001), Rai (2006). During this dissertation 16 larger selected wetlands were sampled in different season of the year.

3.2.1. Sampling: Quadrate samples ere taken randomly in wetland surface, which includes deep-water area, marshy and exposed wetland areas. As the wetland floras are mainly herbaceous, so 1m x 1m quadrates are adopted. 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 Pre-monsoon, monsoon and post-monsoon. In deep water quadrates, free floating, submerged, immerged 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.

3.2.2. Preservation of Specimens: Specimens were collected, preserved and identified as it was discussed under 3.1.

3.2.3. Data processing: Recorded data is transferred to MS Excel worksheet and different parameters like Frequency, Density, Abundance, Relative Frequency, Relative Density, Relative Abundance and Important Value Index of each and every species were determined. The following formulas were used for data analysis as suggested by Misra (1968) and Phillips (1959), Shimwell (1971), Tripathi & Misra (1971), Malhotra (1973), Das & Lahiri (1997), Kadir (2001) and Rai (2006) were applied.

$$\% \text{ Frequency (F)} = \frac{\text{Number of quadrates in which the species occurred}}{\text{Total number of quadrates examined}} \times 100$$

$$\textbf{Density (D)} = \frac{\text{Total number of individuals of a species in all the quadrates}}{\text{Number of quadrates examined}}$$

$$\textbf{Abundance (A)} = \frac{\text{Total number of plants of a species in all the quadrates}}{\text{Number of quadrates in which the species occurred}}$$

$$\textbf{Relative Frequency (RF)} = \frac{\text{Number of occurrence of a Species}}{\text{Number of occurrences of all species}} \times 100$$

$$\textbf{Relative Density (RD)} = \frac{\text{Total number of individuals of a species in all quadrates}}{\text{Total number of individuals of all species in all quadrates}} \times 100$$

$$\textbf{Relative Abundance (RA) Or Relative Dominance (RDm)} = \frac{\text{Abundance of a species per quadrates}}{\text{Total abundance value of total species on quadrates}} \times 100$$

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

3.2.4. 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 an 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.

3.2.4.1. Species Diversity Indices

Diversity indices are mathematical measures those show the proper information about community 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:

3.2.4.1.A. Shannon – Weiner Index (H')

To understand the proper plant diversity of wetland species, Shannon - Weiner index that was suggested by Shannon – Weiner (1949) is followed:

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

Where, 'ni' is the number of individuals of a species.

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

3.2.4.1.B. Simpson's Index (λ)

Simpson's index is another mathematical tool for measuring the diversity. To understand the concentration of dominance of particular species in wetlands or to identify the dominating species Simpson's index are used. Its maximum value ranges between 0–1. Simpson index is a measure of

diversity that takes into accounts both the richness and evenness. The calculating formula for concentration of dominance that is suggested by Simpson (1963) is as follows:

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

Where, 'pi' is the proportional abundance of the 'i^{th}' species.

pi = ni/N

3.2.4.2. Species Richness Indices

Species richness means the measurement of number of species per sample. Species richness is 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 index adopted those are:

3.2.4.2. A. Menhinick Indices (D)

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

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

Where, S = Total number of species observed.

N= Total number of individuals observed.

3.2.4.2.B. Margalef Indices (R1)

Species richness in a community is determined by Margalef Index (Margalef, 1968).

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

Where, s = number of species.

n = number or of individuals of a species.

3.3. Phenology

This part of the dissertation was performed by repeated observation on the stages of the life for resident species in vegetation in different seasons to collect proper and authentic data. This work was started in August, 2003 and extended up to 2008 i.e. for period of over 5 years. During this study different phenophases in the life cycle of plants have been recorded. Different phenophases viz. Seed germination, Seedlings appearance, Vegetative growth, Flowering, Pollination, Fruiting, Seed dispersal

and Death or passing into the dormant phase have been noted for all aquatic and semi aquatic wetland plants.

Regular observation of all the species of aquatic and semi aquatic plants helped to collect the detailed information in natural condition. The nearest wetland namely *Gabgachi-chatral beel complex, Sagar dighi, Nayagram beel, Lakshmipur beel, Belatuli beel, Madhaipur beel* are mainly used for natural observation and a artificial tank of Department of Botany, NBU (2003 -2007) and artificial tank and a pond in Malda College campus (2005-2008) are used as experimental garden.

To study the life form, the classification suggested by Christen Raunkiaer (1934) is followed. Following types of life forms have been recognized:

(i). Phanerophytes: Perennating buds are not well protected. They are located in shoots much above the ground surface upto 30 cm height.

(ii). Chamaephytes: Herbaceous perennials or suffrutescent plants bearing perennating buds just above the ground level to 25 cm high or close to the ground.

(iii). Hemicryptophytes: Perennating buds half hidden at the ground level.

(iv). Cryptophytes: Perennating organs below the ground surface. This part has been studied in much details as most of the aquatic and semi aquatic plants belongs either of the three sub categories like geophytes, helophytes and hydrophytes.

(v). Therophytes: Annuals whish perennate the unfavorable season through seeds or spores and complete their life cycle within a short period.

3.4. Reproductive Potential

This part of work is determined by calculating the average seed output and average seed germination for a species under study. The average seed out put of a plant is determined by taking 10 plants were collected at random and counted separately. Mean value is calculated for average seed output. The collected seeds are then dried out in air and stored in a desiccator. During seed count, number of fruit per plant, seeds per fruit also counted. Seed shape, seed colour and other seed morphology along with seed weight also been noted.

The seeds are then shown for germination in Petridishes, with three replicas, on moist blotting paper or sometime on sterilized sand. Proper moisture is maintained with addition some required amount of water. Rate of seedling appearance is observed and noted in 10 days interval.

3.4.1. Data analysis of Reproductive potential

After processing all the necessary data, different phytosociological aspects were calculated using mainly Microsoft excel software. Different parameters were calculated using following formulae:

3.4.1.1. Seed Shape and Size Index

To calculate the seed shape and size the following index suggested by Hill *et al.* (1986) is followed:

$$\textbf{Seed shape Index} = \frac{\text{Length of Seed}}{\text{Breadth of Seed}}$$

Seed size Index = Length of seed x Breadth of Seed

3.4.1.2. Germination Percentage

This was calculated using the following formula:

$$\textbf{Germination \%} = \frac{\text{Total number of seed germinated}}{\text{Total number of seeds sown}} \times 100$$

3.4.1.3. Nonviable Percentage

$$\textbf{Nonviable\%} = \frac{\text{Total number of non-viable seeds}}{\text{Total number seeds sown}} \times 100$$

3.4.1.4. Reproductive Capacity and Seed Output

Reproductive capacity and seed output from a species is determined by standard method as suggested by Salisbury (1942).

$$\textbf{Reproductive Capacity} = \frac{\text{Average seed output of a plant x \% of germination}}{100}$$

Average Seed Output = Average number of fruits per plant x Average number of seeds per fruit

3.5. Economic Botany

Economically important species that grows wildly in these wetlands are also recorded. For proper understanding this work was undertaken in two different ways:

3.5.1. Major Economical uses

This part of work performed to prepare a list of economically important plants growing in these wetlands in different seasons. To perform this work several literature regarding entho botanical use of different tribes living in different parts of India, commercially important plants these are already published were followed. For better presentation this segment is again divided into few small criteria like:

i. Useful Plants: The useful plants those grow in this region are scanned and enlisted. These include plats used as edible, medicinal, fodder etc.

ii. Poisonous Plants: The poisonous plants those are growing in this region also enlisted and include fish poison, cattle poison, human poison etc.

To construct a list of good number of plants, the literature like Kirtikar & Basu (1935), CSIR (1948-1976), Chopra *et al.* (1956, 1969), Asolkar *et al.* (1992), Biswas & Chopra (1940), Hajra & Chakraborty (1981), Das & Chanda (1990), Jain (1991), Bhujel *et al.* (1984, a,b,c), Shah & Das (2002) were consulted.

3.5.2. Ethnobotany

The enthobonanical reports have been prepared by taking interview of different local people those who were working in different wetlands at that time. Asking question to the women, Children and aged people who were collecting plant parts for different use. Nearby tribal villages were also surveyed to enrich the list. This survey was conducted in different blocks of the district. At beginning, different categories of plants like food, fodder, medicine, thatching materials, building materials etc. were recognized. Methods of use of different plants were to be recorded. The local and urban markets were surveyed to know the commercial value of the wild plants or their products. So, the plants were grouped following utilitarian mode of classification.

3.5.2.1. Edible plants: For this study, used part, method of preparation, vernacular names of useful plants were noted.

3.5.2.2. Fodder plants: Vernacular names, useful parts of such plants were recorded.

3.5.2.3. Medicinal plants: Enlisting the useful parts, vernacular names, diseases cure, method of preparation and dose of administration were recorded.

3.5.2.4. Plants for building materials: Vernacular name, part used, purpose of use etc. were recorded.

3.5.2.5. Manure plants: Vernacular names, useful parts, method of application for different plant were noted.

3.5.2.6. Ornamental and utensils: Vernacular names, parts used, purpose of use for different plants were recorded.

3.5.2.7. Broom, religious and fuel plants: Vernacular names, parts used, purpose of use were noted.

3.5.2.8. Miscellaneous: Vernacular names, parts used, purpose of use were noted.

To perform this work standard method that is followed by several earlier authors including Jain (1981, 1987, 1991); Rai *et al.* (1998); Rai & Bhujel (1999), etc. were followed.

3.6. Degradation of Wetland

The wetlands are very rapidly disappearing throughout the world due to several reasons. This segment of work is also trying to determine or search the causes behind the wetland loss within this district.

This work is not included any chemical test but will try to understand the phenomenon through the scanning of physical reasons. The work includes two steps:

i. **Anthropological causes:** Through the observation of human activities leading to the loss of wetland. Several causes have been recorded as photograph and visual observation understanding through interaction during field works. The rate of loss is not determined but only causes have been determined.

ii. **Natural causes:** Same techniques also have been used in case of understanding the natural threats.

Basic information regarding wetland loss has been collected from the entire district considering all, the small and large water bodies. The wetland wise data regarding loss have been collected in different seasons.