

Chapter **3**

MATERIALS AND METHODS

MATERIALS AND METHODS

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

3.1. Floristics

The floristic survey of the study area includes all the vascular plants which grow over wetland areas in different seasons of the year. To understand the proper and actual floristic diversity of this part of the country, following methodologies are applied using techniques basically devised by (Jain & Rao, 1977) with modifications wherever it was essential.

3.1.1: *Sampling Specimens*

Plant specimens were collected mostly at random from different wetlands of the district, round the year, covering all the three predominant seasons during 2010 to 2014. In dry season, after receding of water, some plants starts growing in bare wetlands also were collected. Specimens were collected in their reproductive stages, i.e. with flowers and/or fruits, as much as possible. For this purpose in numerous cases repeated visit to the same spot was essential sometimes even within a week.

3.1.2: *Record of Field Data*

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

3.1.3: *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 news-prints) 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 microbial infestation. Soft aquatic plants 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 – 45° C. Generally specimens were completely dried out within one or two week time.

3.1.4: *Poisoning of Specimens*

After drying all the specimens were poisoned with 4% ethanolic solution of Mercuric Chloride (HgCl₂) and dried again in blotting papers.

3.1.5: *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 and determinator. Mounted and labelled specimens were stored temporarily in a steel cabinet for further use during the present dissertation.

3.1.6: *Identification*

After the mounting, specimens were taken under study and identified with the help of different Taxonomic literature by various authors (Prain, 1903; Cook, 1996) and were matched with the pre-identified specimens in NBU-Herbarium, and doubtful specimens were taken to CAL for further matching and or confirmation. In numerous cases, experts in different taxa were also consulted for finalization.

3.1.7: *Storing the Herbarium Sheets*

After the present dissertation is over, the first set of voucher specimens and *Field Note-Books* will be deposited in the NBU-Herbarium and the duplicates will be deposited at CAL Herbarium.

3.2. Growth Forms

Growth forms of different aquatic and semi-aquatic plants are determined by observing how a plant species overcome the adverse environmental conditions. Growth forms were recorded as suggested by (Raunkiaer, 1934); (Chowdhury, 2009) and (Chowdhury & Das, 2013)

3.3. Classification for wetland plants

This area harbors large number of wetland macrophytes and those can be classified (Boutin & Keddy, 1993) into the following habit groups:

- **Free floating hydrophytes:** This type of plants float over the water surface freely. eg. *Pistea stratiotes* Linnaeus, *Eichhornia crassipes* (Martius) Solms., *Azolla pinnata* R. Brown, *Lemna perpusilla* Torrey, *Spirodela polyrrhiza* (Linnaeus) Schleid etc.
- **Rooted with floating leaved hydrophytes:** Plants of this group mainly rooted with bottom soil but leaves with long petioles are floating on water surface. e.g. *Nymphaea nouchali* Burman f., *Nymphaea pubescens* Willdenow, *Nelumbo nucifera* Gaertner, *Nymphodes hydrophylla* (Loureiro) Kuntze etc.
- **Submerged hydrophytes:** Plants of this group mainly lies in submerged condition in the water and rooted at the bottom soil of water bodies. e.g. *Vallisneria spiralis* Linnaeus var. *denseserrulata* Mikino, *Nechamandra alternifolia* (Roxburgh ex Wight) Thwaites, *Hydrilla verticillata* (Linnaeus f.) Royel, *Najas graminea* Delile, *Potamogeton crispus*, *Aponogeton natans* (Linnaeus) Engl. & Krause, *Aponogeton crispum* Thunberg etc.
- **Suspended hydrophytes:** These groups of plants are without root and remain in freely suspended condition inside the water. e.g. *Ceratophyllum demersum* Linnaeus, *Utricularia aurea* Loureiro, *Utricularia gibbosa* Linnaeus ssp. *exoleta* (R. Brown) P. Taylor etc.
- **Emerged hydrophytes:** These groups of plants are rooted and emerged the shoot apex and inflorescence from the water. Eg. *Aponogeton natans* (Linnaeus) Engl. & K. Krause, *Monochoria hastata* (Linnaeus) Solms, *Hydrolea zeylanica* (Linnaeus) Vahl, *Butomopsis latifolia* (D. Don) Kunth etc.

- **Amphibian hydrophytes:** The plants of this group generally grown on marshland and they are able to survive in water and in dry soil for some period. e.g. *Sagittaria guayanensis* Humboldt, Bonpland & Kunth, *Sagittaria sagittifolia* Linnaeus, *Ammannia baccifera* Linnaeus, *Ammannia multiflora* Roxburgh, *Bergia ammannioides* Roxburgh, *Monochoria hastata* (Linnaeus) Solms, *Monochoria vaginalis* (Burman f.) C. Presl ex Kunth etc.
- **Wet marginal hydrophytes:** The plants of this group generally grown on the wet marginal regions of the wetland. e.g. *Ludwigia peruviana* (Linnaeus) H. Hara, *Ranunculus sceleratus* Linnaeus etc.
- **Simulated hydrophytes:** The plants of this group generally grown on dry wetland and it considered to be a simulated hydrophyte. e.g. *Chenopodium album* Linnaeus, *Xanthium strumarium* Linnaeus, *Amaranthus spinosus* Linnaeus, etc.

To study the Life-Form, the classification as suggested by (Raunkiaer, 1934) is followed in general. 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 which perennate the unfavorable season through seeds or spores and complete their life cycle within a short period.

3.4. Phenology and Mode of Pollination

Study of phenology and pollination technique of various wetland macro-floras were recorded through repeated observation on the different stages of the growth and development in their *in-situ* condition during the period of August 2010 to June 2014. During this study different phenophases of naturally growing plants have been recorded, which includes germination / sprouting, leaf development, tiller formation, stem elongation, inflorescence emergence, flowering, fruit development, ripening of fruits and seeds and senescence have been noted for all aquatic and semi-aquatic wetland plants following different authors (Caprio, 1966); (Wang, 1967); (Leith, 1970); (Croat, 1975); (Bhoj & Ramkrishnan, 1981); (Sivaraj & Krishnamurthy, 1989); (Das & Chanda, 1987); (Sundriyal, 1990); (Chowdhury, 2009). Pollination types and agents were also observed and recorded on *in-situ* condition. For the observation of phenology and pollination, *Mahananda Barrage*, *Gajoldoba*, and the artificial ponds and tanks and other marshy areas located within the campus North Bengal University campus were mainly visited and taken under study.

3.5. Ethnobotanical Investigation

The complete methodology for the ethnic uses of wetland vascular plants is primarily based on the interaction with the *Ethnic* and/or *traditional people*, those who were working in different wetlands at that time and nearby tribal villages were also surveyed to enrich the list, pursuing them to share their traditional knowledge and analyzing the documented data scientifically.

For the ethnobotanical study of wetland plants the conventional methods of survey and record of data as adopted by (Jain, 1981, 1987, 1991), (Rai *et al.*, 1998); (Rai & Bhujel, 1999), (Rai, 2002), (Sarkar, 2011) and (Chowdhury, 2015) were followed. A questionnaire prepared based on the model by (Jain, 1991), and (Sarkar, 2011) for the present study. The extensive fieldwork undertaken was spreading over for three years from 2010 to 2014 and was carried out in different villages of Terai and Duars in West 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.

3.5.1. Establishing Contact and Developing Confidence with Ethnic People

The random demand of information from the people of Ethnic communities generally shows their unwillingness to share their traditional knowledge. The *Ethnic* people in general, are all apparently seemed to be very friendly but maintained a deep secrecy about their traditional knowledge, especially concerning to herbal medicines. So, preliminary development of contact and confidence with Ethnic people is very much essential. For this, basically some contact persons were traced and basic persuasion proceeded through them.

3.5.2. Observing the Daily Life

Direct observation on the daily life in *Ethnic* society including food and traditional liquor preparations, process of making of instruments for different activities etc. helped to record the related plants and to understand the use much easily. The daily or weekly markets (*haats*) were also visited frequently to study the marketability of wild and cultivated plants, vegetables and other plants or plant-parts collected and/or produced in this area. Various plant materials were observed and in some cases introduced into the *NBU Garden of Medicinal Plants* for *ex-situ* conservation. During daily life observation several plants were recorded as edible plants, food plants, medicinal plants, fodder plants, plant related folklores and myths, etc.

3.5.3. Plants and their Economics

The village dwellers are directly depending on many wildy grown economically important plant species. Such economically imperative wetland species of the study areas are also recorded during survey.

To execute this part of the work several literature on various uses of different wetland plants by various ethnic groups, as well as by local poor people were followed and discussed. Apart from that, data procured from literature were matched and authenticated with the same species from the present investigation through door to door interactions with local poor and ethnic people and tried to understand the commercial values of those plant materials especially in the local markets. This part of the work was designed and executed by following workers like (Kirtikar & Basu 1935); (Chopra *et al.* 1956, 1969); (Asolkar *et al.*, 1992); (Hajra & Chakraborty, 1981); (Das & Chanda, 1990); (Jain, 1991), (Bhujel *et al.* 1984 a,b,c); (Shah & Das, 2002); (Chowdhury, 2009); and (Sarkar 2011).

3.6: Wetland Destruction

Being civilized we are very rapidly destroying our life land *i.e.* wetland throughout the world due to several reasons. The visible physical threats were observed and documented from different wetlands of study areas. The reasons of wetland degradation are categorized in to two broad segments: **Anthropogenic stresses**-Through the visual observation of human activities leading to the loss of wetland were recorded and photographed. Duration of such activities was tried to measure through direct interaction with field workers during survey.; and

Natural stresses: Through the observation natural stresses of wetland also have been categorized and recorded.