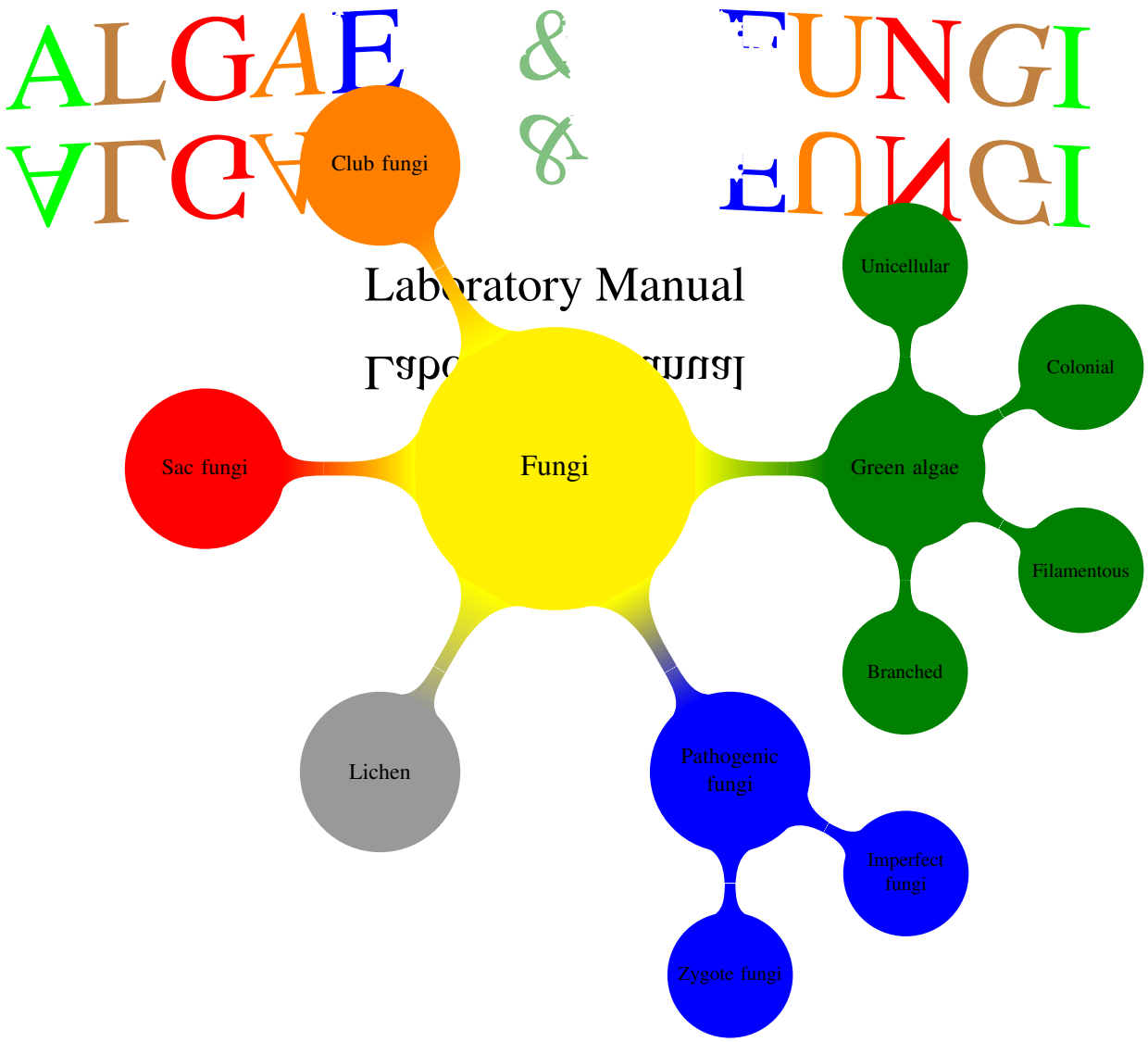

ALGAE AND FUNGI

ALGAE AND FUNGI
Laboratory manual

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SHERUBTSE COLLEGE, ROYAL UNVERSITY OF BHUTAN

KANGLUNG

To my family and.. Students, B.Sc. Life Sciences

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FOREWORD

I am glad to inform you that the Lab Manual on *Algae and Fungi* for B.Sc. Environment and Life Sciences students prepared by **Bimal K. Chetri**, Lecturer in Botany, Department of Environment and Life Sciences will partially fulfil the need of using the practical manual on conducting practicals on freshwater algae, macro-fungi and lichens of Kanglung. I am sure this lab manual will be greatly useful to students for not only in carrying out practicals but also as a reference for more of such work.
I congratulate the author in bringing out this lab manual and wish him success in his future endeavor.

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November 16, 2018

PREFACE

I started working on this laboratory manual of ALGAE AND FUNGI with my students from Sherubtse College, Royal University of Bhutan as a part of their course work. This manual is not exhaustive in itself, however there are references (online and books) cited within the content. I wish this lab manual would be a immense help to students in their academic learning both for their theory and practical components. Algae and fungi are important organisms ranging from microscopic to macro sized which are ubiquitous in their distribution. Study on these organisms from Bhutan is scarce specially on freshwater algae, though there are references available for macro-fungi or in words may be called mushroom. This manual aims to guide undergraduate students with the interest to study these magnificent group of organisms using dichotomous key based on morphological characters and grouping.

Algae are photosynthetic organisms varying in size from unicellular to complex multicellular forms, found in most habitats, ranging from freshwater to marine, hot springs to snow and ice. Their range of shapes and sizes under microscope have always fascinated biologists and researchers. In Bhutan study on freshwater algae would be certainly a new beginning to enthusiastic students who would like to explore microscopic biodiversity. Although their microscopic sizes make difficult for study, their ecosystem services in the freshwater are as important as any other larger flora and fauna in their ecosystem. Algae are considered primary producer at the base of food chain therefore plays fundamental role in providing food to aquatic organisms living in freshwater.

On the other hand fungi are non-chlorophyllous organisms which provides very important economic and ecological services. Many higher plants fail to survive sometimes in absence of their symbiotic association with mycorrhizal fungi that inhabit root systems of higher plants and in return help absorb essential nutrients and water. Other benefits are drugs and antibiotics including edible mushrooms which are essential part of Bhutanese diet. Given the agro-climatic condition of Bhutan, mushroom and molds thrive very well in Bhutan. Mushroom cultivation amongst Bhutanese farmers are picking up as a viable business. Also we often hear or read from news farmers' death in remote villages due to mushroom poisoning.

Therefore this manual would be also a basic guide to help students identify some of the poisonous mushrooms in the wild. Included in this manual are sections on the general features of the freshwater algae, fungi and lichens methods of sample collection and enumeration, using a dichotomous key to the identification of the more frequently occurring genera.

I wish to acknowledge the senior students who have made direct contribution by furnishing this manual with original colour photographs and referencing the articles to help the reader. I hope you may find this Laboratory manual useful and will enjoy working further on these beautiful organisms . This Laboratory Manual on **Algae and Fungi** is to foster an environment of consistent scientific learning and to enable students for self-styled learning by doing. This ***pdf version lab manual is made strictly for academic use and it will be updated regularly***. The template used for writing and editing this book is from Wiley Latex Template.

BIMAL K CHETRI

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August, 2018

ACKNOWLEDGMENTS

My immense gratitude goes to my own students who have put tremendous efforts to in compiling the resources and reviewing the articles which has eventually inspired me in writing this lab Manual. I also acknowledge and express ingratiate to Ms. Jigme Wangmo, Botany Lab Assistant on students' behalf for assisting them in their lab work. Also after my short research program at University of Agricultural Sciences, Dharwad gave idea and confident to work further on my earlier work to complete this book. I also thank Karma Wangchuk (Head, Department of Environment and Life Sciences) for his encouragement and valuable feedback.
To all these hardworking and sincere students I owe a deep sense of gratitude especially now that first edition of this lab manual has been completed.

Bimal K. Chetri

GLOSSARY

Axile: In the centre of the cell.

Asexual spores: Without sex spores or other than sexual spores.

Abaxial: On the side away from an axis or branch.

Acuminate: Having a gradually diminishing point.

Acute: Tapering to a sharp point.

Adaxial: On the side toward an axis or branch.

Adjoined: Lying in contact with.

Akinetes: A resting spore of cyanobacteria.

alga(e): Primitive, often aquatic, plants that carry on photosynthesis but lack the flowers, roots, stems, and leaves of higher plants.

Algal: With reference to an alga.

Anastomose: To unite by cross connections.

Apex (pl. apices): The distal end of a branch or filament, etc.

Apical cell: A specialized cell initiating growth at the apex of a thallus or its branches.

Annulus: Ring of tissue on a mushroom stem left by a torn partial veil

Articulated: Jointed.

Articulation: A joint or a more or less jointed thallus.

Asexual: Reproduction which does not involve a union of gametes.

Assimilatory filament: A superficial filament the cells of which contain chloroplasts.

Attenuate: Narrow and gradually tapering.

Axil: The angle between an axis and an organ borne on it.

Axial filament: A longitudinal series of cells running through the center of an axis or branch.

Axis (pl. axes): The longitudinal portion on which the parts of a plant are arranged.

Acid bark: Bark with a low pH that may be natural or be caused by increasing atmospheric acidity.

aplanospore: Non-flagellated spore (asexual reproduction).

autospore: Internal spore formed by cell division within a mother cell forming a replica of the mother cell, not flagellated (asexual reproduction).

Ascocarp: Fruitbody of an ascomycete fungus

Apothecium/cia: Cup-shaped fruitbody of certain ascomycetes fungi

Appressed: Flattened down onto a surface

Aseptate: Lacking septa, often pertaining to the hyphae seen in zygomycetes (also see coenocytic).

Basidiocarp: A basidium producing organ/fruit of Basidiomycetes.

Basidiospore: Sexual spores in Basidiomycetes after meiosis, on a basidium.

Basidium: The cell or organ, diagnostic for Basidiomycetes, from which after karyogamy and meiosis, basidiospores (generally 4) are produced externally each on an extension (sterigma) of its wall a conidiophores or phialide.

Bifurcate: Divided into two branches; forked.

Binary fission: Formation of two equal daughter cells.

Blade: The more of less broad, flattened, foliose part of an erect alga; lamina.

Bladelet: A small blade.

Branchlet: A small branch.

Benthic: Pertaining to the sea floor.

Benthic-pelagic coupling: The interaction between the benthos or bottom, with the water column, or pelagic ecosystem. It refers to how the dynamics of one ecosystem influences the dynamics of the other.

Benthos: The floor of a sea or ocean; also includes the bottom-dwelling organisms that live there.

Bioassay: A method for quantitatively determining the concentration of a substance by its effect on the survival, development, growth, behavior, or measurable physiological response of a suitable animal, plant, or microorganism under controlled conditions.

Bivalve: Animals having a soft body enclosed in a calcareous two-part shell, e.g., Clams, scallops and oysters.

Biofuel: A gaseous, liquid, or solid substance of biological origin that is used as a fuel.

Basic bark: Bark with a high pH that can be caused by nutrients or by lime.

Cap: Top part of a basidiomycete mushroom that carries the fertile tissue.

Carpophore: Fungal fruitbody comprising stem, cap and gills.

Chlorococcoid alga: A green alga usually with rounded trebouxioid cells in lichens.

Cortex: The skin-like surface on the upper (or lower) surface of a lichen.

Coenocytic: Infrequently septate, multi-nucleate hyphae as in the Zygomycetes.

Cleistothecium (pl. cleistothecia):An enclosed ascocarp containing randomly dispersed asci.

Coenobium: A colony that always has the same number of cells once it is formed.

Concentric: Having a common center.

Conceptacle: A cavity opening to the thallus surface and containing reproductive organs.

Conjugation: Sexual fusion of two ameboid cells (motile is from ameboid movement not flagella). two types.

Chloroplast: A plastid containing chlorophyll only, coloured structure in plant cell which absorbs light used in photosynthesisis usually green or brown and occasionally red or blue-green.

Crustose: Crust-like; in the case of algae, the term is restricted to thin thalli growing flattened against the substratum.

Cortex: The thallus between the epidermis and medulla; if no epidermis is present, the region.

Clamp connection:A hyphal outgro

Chlamydospores: An asexual 1-celled spore originating endogenous and singly within part of a pre- existing cell,/asexual spores formed by the breaking up of fungal hyphae.

Cuticle: A tough, often waterproof, nonliving covering, usually secreted by epidermal cells.

Dikaryon: A cell having two genetically distinct haploid nuclei.

Disc: The spore-bearing part of the fruiting body of a lichen.

Dichotomous: Branching by equal forking.

Dioecous: Unisexual; male and female organs are borne on separate plants.

Diploid: Two sets of homologous chromosomes in the nucleus of the cell.

Dolipore septum: A septum of a dikaryotic basidiomycete hypha which flares out in the middle portion forming a barrel-shaped structure with open ends as shown by electron microscopy.

Endomycorrhiza: Mycorrhiza in which fungal hyphae penetrate cell walls of host plant

Endophytes: Fungus living within a plant without causing visible symptoms of harm.

Epiphytic/epiphyte An organism attached to substrate or another organism.

Fusiform: Spindle-shaped, tapering toward the end.

Foot cell: A basal cell of a conidiophore as seen in *Aspergillus* and *Fusarium*.

Filament: A branched or unbranched row of cells joined end to end.

Filiform: Thread like.

Flagella(e): Whip-like tail(s) attached to the cell used for locomotion.

Filamentous: In the form of a filament.

Frustules: Unique cell walls of silica.

Germ pore: A differentiated, frequently apical area, or hollow, in a spore wall through which a germ tube may come out.

Heterocysts: large, thick-walled cell involved in nitrogen fixation in cyanobacteria.

Holdfast: The part where a bushy lichen is attached to the surface.

Homothallic: Capable of sexual reproduction on a single thallus; monoecious.

Heterothallic: Requires mating between two compatible strains for sexual reproduction to occur; dioecious.

Hormogonium: Short segment of cells that separates from the end of a trichome and initiates a new trichome in some Cyanophyta (asexual reproduction).

Hispid: Having hairs or bristles.

Homoiomerous: Trama composed of only hyphal tissue.

Host: A living organism harbouring a parasite.

Hyaline: Transparent or colorless

Hyphae: The fungal filaments that make up the body of the lichen.

Hymenium: The spore bearing layer (Fertile layer) of a fruit-body.

Hymenophore: A spore bearing structure or that part of it bearing the hymenium.

Isidia: Pin-like structures on the surface of the thallus (vegetative reproduction)

Jam tart fruits: Rounded fruits with a margin that is made up of the thallus and a distinct disc which bears the spores (lecanorine apothecia).

Lobe: The leaf-like part of a leafy (foliose) lichen.

Lentic: Standing water such as wetlands (ponds, sloughs), dugouts, reservoirs and lakes.

Mycelium: A mass of hyphae.

Medulla: The fungal part of the thallus situated below the skin like upper cortex.

Pallisade; Cells arranged vertically.

Papilla: A small rounded process.

Parasite: An organism living on or in the organism obtaining the nutrients from its host, another living organism.

Pathogen: An organism that is capable of causing disease in another organism; generally refers to the viruses, parasitic bacteria and fungi.

Parietal: Arranged along the cell walls.

Papilla: A small wart-like pimple on the surface of the thallus.

Perithecium/cia: A fruiting body resembling a flask-like container with a pore from which spores are released.

Peridium: Outer wall of a fungus, especially a gasteromycetes (e.g. a puffball)

Polypore: A common name for a member of Aphyllophorales that has pores: a bracket fungus.

Pileus: The hymeniumsupporting part of the Basidioma of non-resupinate Agaricomycetes; cap.

Planktonic algae: Single-celled organisms or aggregations of single-celled algae.

planospore: Flagellated spore (asexual reproduction).

Pneumatocyst: . An air-bladder.

Phytoplankton: Microscopic floating plants, mainly algae that live suspended in bodies of water.

Perithecium: Flask-shaped chambers containing asci within pyrenomycetes fungi.

pseudorhiza: a tap-root-like extension at the base of a mushroom stem

Pyrenoid: Protein region inside chloroplast that accumulates carbohydrates or stores starch.

Reticulate: Net-like.

Rhizomorph: Root-like mycelial strand comprising bunched parallel hyphae.

Ring: Membranous remains of the partial veil attached to a stem.

Septum: (pl., septa) a cross wall separating cells of a hyphal thread.

Soredia: Powdery patches of a mixture of fungal hyphae and algal cells which are in structures called soralia (vegetative reproduction).

Somatic: Pertaining to soma; refers to the body phase in plants, the vegetative phase structure or function as distinguished from the reproductive.

Squamule: A small scale which is attached to the bark (or other) surface without a lower cortex or rootlets.

Sinuate: A type of gill attachment specifically gills that are notched at their point of attachment to the stipe.

Stroma: A mass of matrix of vegetative hyphae, with or without tissue of the host or substrate, sometimes sclerotium-like form, in or on which spores or fruit bodies bearing spores are produced.

Sterigma: (pl., sterigmata) Prong at top of basidium on which a spore develops.

Stipe: Stem of algae/fungi.

Striae" Ornamental lines on the cell wall (frustule).

Thallus: The body of the algae, fungi or of lichen formed of hyphae and algae.

Trentepohlioid alga: Filamentous green alga with orange pigments.

Trama: The layer of hyphae in the central part of a lamella of an agaric, spine of Hydnaceae, or the dissepiments between pores in the polypore.

Theca: Cellulose plates that are *armorlike* in appearance found in Pyrrhophyta (dinoflagellates).

Trichome: A thread of cells/branching.

Volva: Remains of the universal veil found at stem base of some fungi

CHAPTER 1

LABORATORY GUIDELINES AND BASICS OF MICROSCOPY

The topics covered are laboratory conduct, tips on writing practical report, basic and selective dichotomous key to identify freshwater algae, fungi and lichens of Kanglung. Images from this lab manual may be freely used for academic purposes, as long as the resources used are cited and acknowledged appropriately.

1.1 General Instructions:

i) Come on time; leave at your own.

ii) The laboratory Assistant and your Tutor will assist you always. Feel free to ask them.

iii) Laboratory provisions should be handled with utmost care and any breakage should be reported to the Tutor or lab technician (lab assistant)

iv) Any breakage/damage, has to be replaced by the student.

v) No eating and chewing during lab hours.

vi) Foot wears to be kept outside while working in the Microbiology laboratory .

vii) Maintain discipline and silence in the laboratory/ institute.

viii) Apparatus and instruments to be handled with great care.

ix) Make sure your working place is clean and dry.

x) Take a microscope, watch glass, petriplate and alpin each. Stains are placed in the common place.

xi) Make sure your microscope is ready for use. Mixture of specimens will be provided to you (use it wisely).

xii) Read the instructions and protocol carefully, collect the materials, organize the work and then commence the experiment.

xiii) Use masks and gloves, whenever required in laboratory.

xiv) Wash your hands with soap before and after work in the laboratory.

xv) Get the remarks/ signature from the Tutor after each practical.

xvi) Your electronic devices must remain switched off throughout the laboratory period, unless permission is granted by the Tutor.

xvii) Electronic devices are allowed for taking notes and writing purposes during lab hours.

xviii) Your practical work is due every week for assessment and should be submitted when you come for the new practical session. Entry will be denied if you don't submit your practical work. You will also loose grade for that particular practical work too.

xix) Leave the lab clean and dry.

1.2 How to write your practical report?

(i) Write aim, date and purpose of the practical.

(ii) Prepare temporary mount firstly with water and later with right stain. Mount the specimen in the center.

(iii) If required use cover slip under higher resolution.

(iv) Result:

(a) Diagram: Left side (Figure 1.1) **i.** Specific to the aim **ii.** Microscopic observations **iii.** Whole figure **iv.** Part (magnified) **v.** Classification in a box.

(b) Write-up:

i. Background **ii.** Identifying points **iii.** Result **iv.** Reference list **v.** All figures and comments should be cited. **vi.** Cite figure/s sources within the text.

1.3 Laboratory provisions:

i. Staining rack **ii.** Dropping bottle **iii.** Slides and cover glass/slips **iv.** Blotting paper and alpin **v.** Microscope
Microscopes are instruments designed to produce magnified visual or photographic images of small objects. The microscope must accomplish three tasks: produce a magnified image of the specimen, separate the details in the image, and render the details visible to the human eye or camera. This group of instruments includes not only multiple-lens designs with objectives and condensers (Figure 1.2), but also very simple single lens devices that are often handheld, such as a magnifying glass (Basic Concepts in Optical Microscopy).

1.4 Light Microscopy

The light microscope, so called because it employs visible light to detect small objects, is probably the most well-known and well-used research tool in biology which can provide spectacular views of nature and can enable students to perform some reasonably sophisticated experiments.

1.5 Optical and mechanical components of the microscope (Figure 1.2)

(i) **Eyepiece or ocular lens:** Eyepiece is the lens, present at the top and is used to see the objects under study. Eyepiece lens contains a magnification of 10X or 15X.

(ii) **Tube:** Tube or the body tube, connects the eyepiece to the objective lenses.

(iii) **Resolving nosepiece:** It is also known as the Turret. Resolving nosepiece has holders for the different objective lenses. It allows the rotation of the lenses while viewing.

(iv) **Objective lenses:** Generally, three or four objective lenses are found on a microscope, with ranges of 10X, 40X, 100X powers. Lenses are colour coded, the shortest lens is of the lowest power, and the longest lens is high power lenses.

(v) **Diaphragm:** Diaphragm helps in controlling the amount of light that is passing through the opening of the stage. It is helpful in the adjustment of the control of light that enters.

(vi) **Coarse adjustment knob:** Used for focus on scanning. Usually the low power lens is used enabling the movement of the tube.

(vii) **Fine adjustment knob:** Used for focus on oil. Moves the body tube for focussing the high power lens.

(viii) **Arm:** It supports the tube of the microscope and connects to the base of the microscope.

(ix) **Stage:** The platform that is flat used for placing the slides under observation.

(x) **Stage clip:** Stage clips hold the slides in proper place.

(xi) **Condensor:** The main function of condenser lens is focussing the light on the specimen under observation. When very high powers of 400X are used, condenser lenses are very important. Presence of condenser lens gives a sharper image as compared to the microscope with no condenser lens.

(xii) **Base:** Provides basal support for the microscope.

(xiii) **Power switch:** The main power switch that turns the illumination on or off.

1.6 Working principle

An object placed on the stage is magnified through the objective lens (Figure 1.3). When the target is focused, a magnified image can be observed through the ocular lens (Source: Working principle).

1.6.1 Numerical Aperture

Numerical Aperture (also termed Object-Side Aperture) is a value (often symbolized by the abbreviation NA) originally defined by Abbe for microscope objectives and condensers. It is given by the simple expression:

$$\text{Numerical Aperture (NA)} = n \times \sin(\mu) \qquad or \qquad n \times \sin(\alpha$$

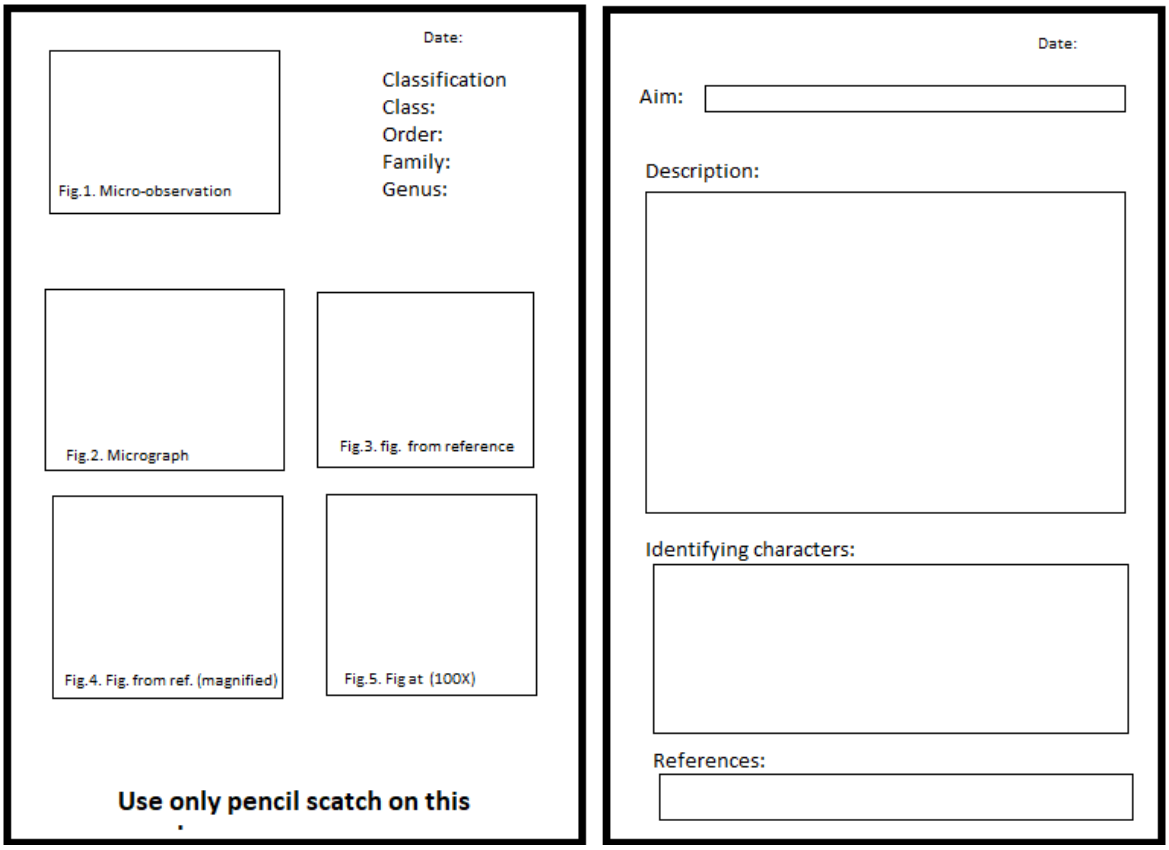


Figure 1.1: Lab manual template

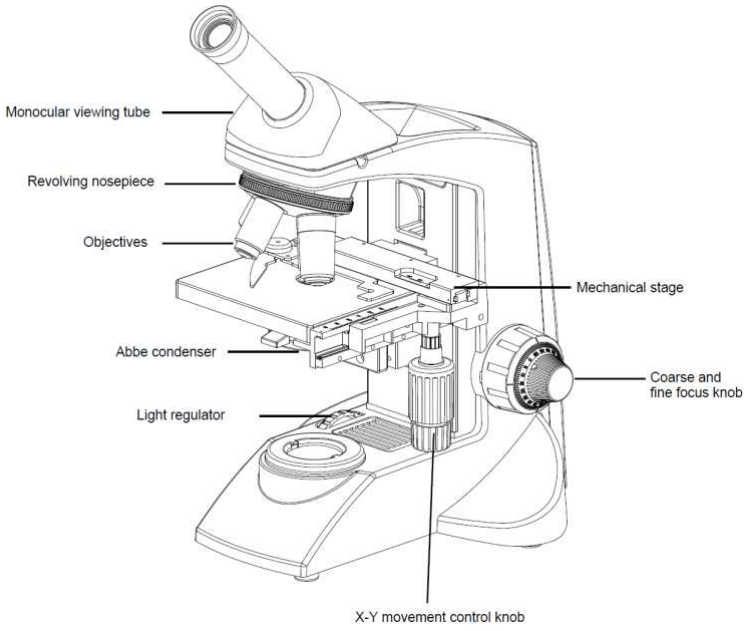


Figure 1.2: Components of microscope

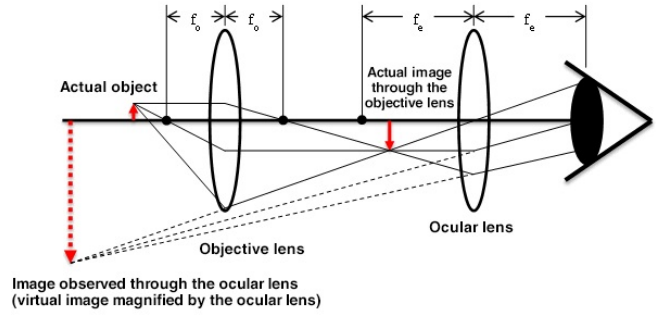


Figure 1.3: Working principle

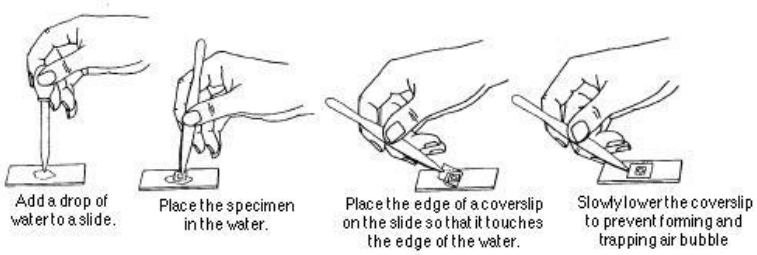
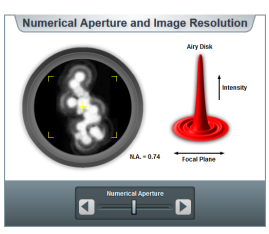


Figure 1.4: Mounting technique

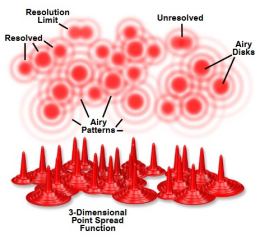
Where, n represents the refractive index of the medium between the objective front lens and the specimen, and μ or α is the one-half angular aperture of the objective. The numerical aperture of a microscope objective is a measure of its ability to gather light and resolve fine specimen detail at a fixed object distance.

1.6.2 Resolution

Human eye fails to recognise object lying closer between 0.01 to 0.25 mm. Resolution can be defined as the ability of a microscope to allow one to distinguish between small objects. The resolution of an optical microscope is defined as the shortest distance between two points on a specimen that can still be distinguished by the observer or camera system as separate entities. An example of this important concept is presented in the Figure 1.5b, where point sources of light from a specimen appear as Airy diffraction patterns at the microscope intermediate image plane (Source: Resolution).



(a) Relationship between NA and r



(b) Resolution

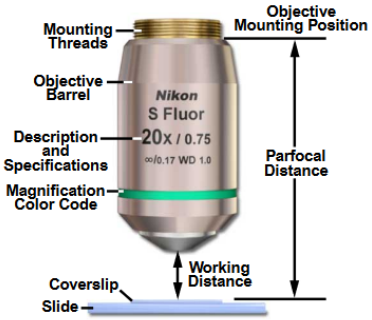
Figure 1.5: Microscopy principle

The bigger a cone of light that can be brought into the lens, the higher its numerical aperture is. Therefore the higher the numerical aperture (Figure 1.5a) of a lens, the better the resolution of a specimen will be which can be obtained with that lens (Source: Numerical Aperture and Image Resolution) .

1.6.3 Working Distance and Parfocal Length

Microscope objectives are generally designed with a short free working distance (Figure 1.7a), which is defined as the distance from the front lens element of the objective to the closest surface of the coverslip when the specimen is in sharp focus. In the case of objectives designed to be used without coverslips, the working distance is determined

by the linear measurement of the objective front lens to the specimen surface (Source: Working distance).



(a) Working principle

$$\text{Field Size} = \frac{\text{Field Number(fn)}}{\text{Objective Magnification(Mo)}}$$

(b) Field of view

Figure 1.7: Microscopy principle

1.7 Using a bright field microscope

Using bright field depends on magnification and resolution you would require.

1.8 Mount the specimen on the stage

The cover slip must be used while working under higher magnification and better resolution. High magnification objective lenses can't focus through a thick glass slide; they must be brought close to the specimen, which is why coverslips are so thin. The stage may be equipped with simple clips (less expensive microscopes), or with some type of slide holder for positioning your specimen.

1.9 Optimize the lighting

Better microscopes have a built-in illuminator, and the best microscopes have controls over light intensity and shape of the light beam. If your microscope requires an external light source, make sure that the light is aimed toward the middle of the condenser. Adjust illumination so that the field is bright without hurting the eyes.

1.10 Focus, locate, and center the specimen

Start with the lowest magnification objective lens, to home in on the specimen and/or the part of the specimen you wish to examine. It is rather easy to find and focus on sections of tissues, especially if they are fixed and stained, as with most prepared slides. A suspension of yeast cells makes a good practice specimen for finding difficult objects. Once you have found the specimen, adjust contrast and intensity of illumination, and move the slide around until you have a good area for viewing.

1.11 Adjust eyepiece separation, focus

With a single ocular, there is nothing to do with the eyepiece except to keep it clean. With a binocular microscope (preferred) you need to adjust the eyepiece separation just like you do a pair of binoculars. Binocular vision is much more sensitive to light and detail than monocular vision, so if you have a binocular microscope, take advantage of it.

1.12 Select an objective lens for viewing

The most frequently used objective lens is the 10x lens, which gives a final magnification of 100x with a 10x ocular lens. For very small protists and for details in prepared slides such as cell organelles or mitotic figures, you will need a higher magnification. Higher magnifications are used exclusively with oil in order to improve resolution (Source: Microscopy).

1.13 When to use bright field microscopy

Bright field microscopy is best suited to viewing stained or naturally pigmented specimens such as stained prepared slides of tissue sections or living photosynthetic organisms. It is useless for living specimens of bacteria, and inferior for non-photosynthetic protists or metazoans, or unstained cell suspensions or tissue sections. Here is a not-so-complete list of specimens that might be observed using bright-field microscopy, and appropriate magnifications (preferred final magnifications are emphasized). Living preparations done using (wet mounts, unstained) - pond water (40x, 100x, 400x), living protists or metazoans (40x, 100x, 400x occasionally), algae and other microscopic plant material (40x, 100x, 400x). Smaller specimens will be difficult to observe without distortion, especially if they have no pigmentation.

1.14 Care of the microscope

(i) EVERYTHING on a good quality microscope is unbelievably expensive, so be careful.

(ii) Hold a microscope firmly by the stand and arm, only. Never grab it by the other parts.

(iii) Since bulbs (if present) are expensive, and have a limited life, turn the illuminator off when you are done.

(iv) Always make sure the stage and lenses are clean before putting away the microscope.

(v) NEVER use a paper towel, any material other than good quality lens tissue or a cotton swab (must be 100% natural cotton) to clean an optical surface. Be gentle!

(vi) Cover the instrument with a dust jacket when not in use.

(vii) Focus smoothly; don't try to speed through the focusing process or force anything. For example if you encounter increased resistance when focusing then

you've probably reached a limit and you are going in the wrong direction.

1.15 Temporary mount

Temporary preparation of materials for light microscopy can be made for quick preliminary investigation. In a wet mount, a drop of water is used to suspend the specimen between the slide and cover slip (Figure 1.4). Place a sample on the slide and then place on edge of the cover slip over the sample and carefully lower the cover slip into place using a alpin or equivalent. This method will help prevent air bubbles from being trapped under the cover slip. The sections should be mounted in a drop of water/stain/saline solution/glycerine. Safranin or Fast green are used for staining filaments of algae, fungi, section of bryophytes, spores of pteridophytes, pollen grains of gymnosperms. Algae can also be stained using Aniline blue. For your work safranin will be used often.

(a) Place the specimen in a watch glass containing stain and leave there until they are stained to the required depth.

(b) Clean and dry glass slide by holding only the sides of the slide and cover slip and locate the center.

(c) Place the stained section at the center of the slide. T Sections can also be placed directly on a drop of stain on a slide.

(d) Cover the stained section with minimum required amount of mounting medium

(e) Air bubbles should be avoided while putting cover slip

(f) Label at the one side

(g) Sealing the cover slip: It is done to prevent mounting medium from drying. It is done by simply painting the edges of cover slip with cover slip with sealing agent in such a way that the space between the slide and the cover slip gets filled with the agent. Canada balsa gum, dammar gum, nail polish, ringing table, etc is used for sealing.

(h) Living specimens do not survive long in the heat from an intense microscope illuminator bulb, usually because the specimen dries up. Glycerine or vaseline may be used to avoid faster drying.

(i) The extra solution (water or mounting medium) can be removed by soaking a piece of blotting paper to the edge of the cover slip.

(j) Always hold slide and cover slip from the side so as to prevent finger marks.

(k) Examine the slide under the microscope.

1.16 Microscopic Drawing

Drawing softwares like (Inkscape) is an open source (free) replacement for Adobe Illustrator and CorelDraw which can be used for diagrams, line drawings, cartoons, etc. GNU Image Manipulation Program (GIMP) is good for photo editing and multipanel figure assembly and labeling while Blender is for 3D graphics. These drawing tools can be used precisely to draw or sketch image of the specimen, but knowing the art of microscope drawing is a skill that is always good to have on hand for Life Science students and Tutors who must be able to produce good quality scientific drawings regardless of your artistic ability. Drawings not only allow you to record an image of the specimen observed, but more importantly, they help you to remember the specimen as well as the important features of the specimen.

1.16.1 Drawing from your temporary mount or permanent slide

Draw what you see directly from the clean slide where specimen is mounted in the center and not from what you think and memorized. The objective of scientific drawing allows Life Science students needed to record their real time observations of a specimen which could be compared with references including textbooks and verify the specimens for correct identifications.

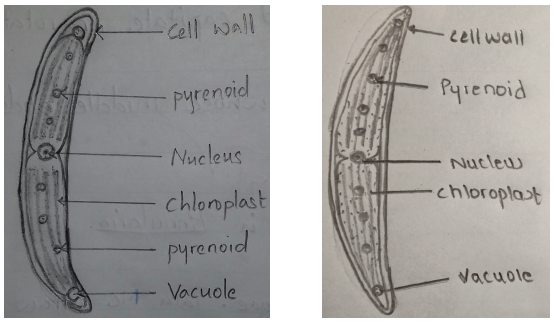
1.16.1.1 Orienting your slide to desired field of view

Use a4 blank paper (unlined) for your drawing. Draw half-inched page margin from all 4 sides. Draw circle per figure of appropriate size accommodating all required number of figures per page. Orient the specimen to it's desire characteristics and focus on the parts to be drawn through the eyepiece of the microscope viewed under bright microscopic field. Look into your microscope and find the largest shape in your field of view (1.7b). Figure out the image and accordingly draw starting with thin light line within the circle.

1.16.1.2 Drawing scientific diagrams

Drawing scientific diagrams requires skill and patience. **You need to draw scientific diagrams and not decorative diagrams.** Do not use soft lines characteristic of sketches (Figure 1.8b). The goal of a scientific diagram is to represent how different parts of a specimen relate to each other. Make the illustration large so that various parts of the specimen are easily distinguish-

able (Figure 1.8a). All drawings must be done in pencil.



(a) Correct drawing **(b)** Wrong drawing

Figure 1.8: Scientific drawing

1.16.1.3 Filling details

When doing scientific drawings, use the following checklist to make sure your drawings have all the required components:

(1) Name of the figure with appropriate short description within the round bracket ().

(2) Labels (always include labels of the important features of the specimen. Each label line must be straight and should not overlap with other label lines; all labels must be to one. Annotations are used to give information about the specimen that cannot be seen on the diagram (e.g. you may want to record that the nucleus was stained blue or that the two flagella on the organism could not be seen and so are not included on the diagram.

(3) Magnification (indicate the magnification at which the specimen was observed e.g. 10x,40x, 100x)

(4) All scientific names underlined (handwritten) or italicized (typed)

CHAPTER 2

COLLECTION OF SPECIMENS

A complete balance between direct contact and independent self-learning will be adopted wherein; the students will be motivated to use library resources extensively and fulfill the expected requirement of this module.
For many species of algae, fungi (macrofungi and pathogenic fungi) and lichens, identification in the field is very often difficult for the students. Collecting the sample specimens, fixing and processing them becomes important for laboratory identification. Permission must be taken while collecting the specimens which require special permit. It is your responsibility to be aware of and adhere to these rules. DO NOT COLLECT MORE THAN ONE SAMPLE unless abundantly available. If the specimen is scare in the area, you are advised to collect only the high quality photographs of the specimens for identification. The best methods for collecting specimens from the field will vary with habitat types and your study objectives. Some quick and easy sampling methods are discussed below, but whatever method you use, make sure you do it safely!

2.1 Expected outcomes

(i) Identify the different algae based on their vegetative structures.

(ii) Identify the freshwater algae of Trashigang Dzongkhag.

(iii) Identify the different pathogenic fungi based on their vegetative and reproductive structures.

(iv) Identify lichens of the region.

2.2 Algae sample collection

Sample collection of freshwater algae varies with habitat types and your study objectives. Simply carry a small plastic bag/vial/jar to transport from the stream/pond/wetland/puddle/drain to the classroom or lab. You needed a small scrape or pinch of the algae to observe under a microscope. You are advised to avoid direct skin contact with algae therefore using tweezers to scrape/pick a sample is recommended. It is always amazing to observe fresh/cool sample same day under microscope to observe them moving and to record their characteristics color for your identification. Do not let your sample to dry up.

2.3 Macrofungi sample collection

Always collect a single good sample. Mushrooms including from wild are wet sticky therefore you are recommended to carry paper bags/newsprint, a pocket knife for safely detaching the sample from the substrate, a marker pen/pen notepad take on-site notes and a basket for transporting fresh specimens to the lab. Carry hand lens to record the visual characters and exudes from the mushroom. This may help you identify the specimens. Your advised to use insect repellent against some ticks bites. Also collect in-situ images of the specimens and record their locations using GPS (Global Positioning System). Be careful not to taste any of the mushroom as it is difficult to differentiate morphologically poisonous from non-poisonous ones. Always keep field record. **Do not rely on your memory.**

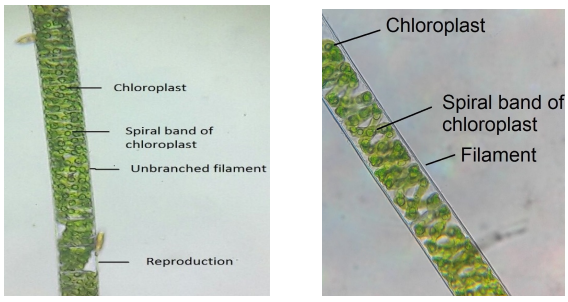
2.4 Lichen collection

Collect a healthy specimen, i.e., without discoloration away from walking trail or from leisure park. Crustose lichens are often difficult to collect. Separating from their substrates often open scars/wound on the bark of the tree. Try taking off only the outer side of the bark. Basic tools required are a knife or chisel and pruner (for detaching from the substrate and cutting small twigs), 7x or 10x magnifier, paper bags notepad, pencils(for on-site observations and recording). GPS for recording exact collecting location for compiling your report. Keep on-site field records including the substrate where lichen was growing on by which you may be able to identify. Lichen distributions and ecology are scarcely documented in Bhutan. Your collections may help future researchers. **Location description and high quality images of the specimen are must for all kind of collections.**

CHAPTER 3

GENERAL ACCOUNT OF ALGAE, FUNGI AND LICHENS

Algae (singular-alga) are photosynthetic organisms that range from microscopic and unicellular (single-celled) to very large and multicellular, predominantly aquatic widely present in freshwater environments, such as lakes and rivers, where they are typically present as micro-organisms-visible only with the aid of a light microscope (Bellinger & Sigee, 2010). This lab manual considers the diversity of freshwater algae which are widely present in drains, stream, ponds, paddy field and rivers of Kanglung.



(a) *Spirogyra* sp. **(b)** *Spirogyra* sp. filament

Figure 3.1: *Spirogyra* filament

3.2 Framing and using dichotomous key

A dichotomous key is a tool that provides sets of statements that act as clues to the users leading to the identification of an organism, such algae, fungi, microbes, flowers and trees

3.1 General account of Algae

Using compound microscopes from Labomed/Olympus with natural light source or with illumination is good enough for the identification of many fresh water algae. You are advised to start your observation with magnification options of 10x and move with 40X, 100X and often greater. A single drop of the sample on the center of the glass slide should be enough to start with your observations and recording. If required more than one mounting could be done. Temporary mounting requires both with and with out staining for noting down all the characteristics of your specimen/s.

species. Keys consist of a series of choices that lead to the correct name of a given item. "Dichotomous" means "splitting into two parts". Therefore, dichotomous keys always give two choices in each step.

(i) In each step a single characteristic is considered at a time.

(ii) Two contrasting statements are put forward to describe the characteristics in such a way as to separate the organisms.

(iii) This continues until all the organisms have been identified.

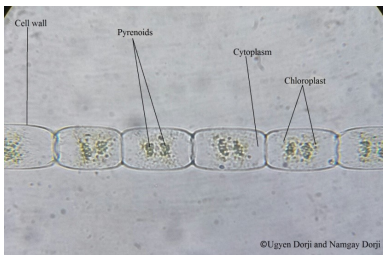
(iv) Eventually your choices should lead you to the genus of the specimen.

(v) From the genus description page you should be able to confirm the genus and species of the specimen.

3.2.1 Morphological grouping

While using dichotomous key, two sets of alternative features are presented at each stage and the user must consider which best fits the specimen being observed. Each alternative leads on to a further (numbered) sequence of choices ultimately reaching the name of the genus to which the specimen is provisionally assigned. This lab manual provides you with limited number of representative genera of a particular group. Therefore for your further reporting on freshwater algae you are recommended to refer (Bellinger & Sigee, 2010; Desikachary, 1959; Hazen, 1899; Prescott & others, 1964; Sharma, 2011; Smith & others, 1933; Vashishta, 1995, and references therein).

(a) *Oedogonium* filament showing oogonium **(b)** *Stigeoclonium* branched filament



(c) *Zygnema* filament showing star-shaped chloroplast

Figure 3.2: Filamentous green algae

3.2.1.1 Key numbers to main genera and species

(I) Green filamentous freshwater algae **(1)** , **(2)** and **(3)** .

(1) Filaments unbranched **go to** (2), sub-item **i.** , **ii.** , **iii.** and **iv.** .

(2) Filaments branched **go to** (3), sub-item **i.** , **ii.** , **iii.** and **iv.** .

i. Green ribbon shaped, spiral chloroplast——*Spirogyra*
Spirogyra has cylindrical cells that are joined end to end to form an unbranched filament. The cell walls are firm and have a thin film of mucilage on the outside, giving them a slimy feel. Chloroplasts have a helical shape (Figure 3.1b) and there can be up to 15 per cell. Numerous pyrenoids are present. The nucleus, often visible in live material, is in the centre of the cell. Cells may be between 10 and 160 μ m in diameter and up to 590 μ m long.

ii. Green ribbon shaped, 2 star shaped cholroplasts per cell———*Zygnema*
Cells of *Zygnema* are cylindrical and have two, characteristic, star-shaped (Figure 3.2c) chloroplasts separated by a clear area. Each chloroplast has a pyrenoid. The filaments usually have a soft mucilage sheath, are unbranched and not very long. They may be attached to a substrate by means of rhizoids. Cells 16-50 μm in diameter and 2 to 3 times long as broad.

iii. Cells cylindrical, sometimes slightly swollen at one end. Some cells along the filament will have ring-like transverse lines at the swollen end (cap cells). —— *Oedogonium*
The chloroplast is parietal with several pyrenoids and netlike. Cells are rectangular (Figure 3.2a) and longer than broad. Cells 10-40 μm wide and 2-5 times as long as broad.

iv. One chloroplast per cell in the form of a flat plate arranged along the long axis of the cell. When viewed from one direction the chloroplast fills most of the cell but when viewed from the other it is a thin line down the middle.——- *Mougeotia*.
The chloroplast of *Mougeotia* is suspended on cytoplasmic strands and can move within the cell depending upon the light. Hence sometimes it may be seen face-on, sometimes edge-on and sometimes twisted. The cells form long unbranched free floating filaments. Cells 3.5-35 μm in diameter and between 5 and 12 times as long as broad.

(3) Filament main axis cells not markedly different to branch cells (except those at the end of branches, which are thinner and hairlike).

i. Branches not usually occurring in distinct whorls——— *Stigeoclonium*
Filaments of *Stigeoclonium* are usually attached by means of basal cells to a variety of surfaces. Branches can be opposite, alternate (Figure**??**) but rarely whorled and terminating in a tapering series of cells sometimes forming a terminal multicellular hair. Filaments surrounded by a thin mucilaginous layer. Chloroplasts parietal with one or more pyrenoids. Cells 5-40 μm wide and many times longer than broad.

3.2.2 General account of Fungi

Fungi (singular fungus) are eukaryotic non-chlorophyllous organisms with absorptive heterotrophic mode of nutrition which include the yeasts, rusts, smuts, mildews, molds, and

mushrooms. There cell wall is made up of chitin. They are neither plants nor animals which have been given the status of separate kingdom of Fungi or Mycota/Mycete. There are also many fungi like organisms, including slime molds and oomycetes (water molds) which have closer relations with aquatic organisms like algae.

3.2.3 Framing and using dichotomous key

Read the descriptive characters in each step and choose the correct option that matches your micrographs and collected specimens. The characters used in the key can be seen with the naked eye or with a 10X hand lens. Micrographs are taken using light microscope at 10x, 40x and 100x resolution. Always use clean glass slides and mount in the center of the slide. Use coverslip while observing under higher resolution. Make use of the following online resources: (Welcome to the MycoKey MMI, Nature first: Fungi Families/Types Identity Parade and MushroonExpert.com)
Macrofungi (Mushrooms) are spore bearing fungi visible to naked eyes mostly club fungi under Basidiomycetes. Alexopoulos, Mims & Blackwell (1996); Alexopoulus, Blackwell & others (2002); Lodge, Ammirati, O'Dell & Mueller (2004); Mata & Pradhan (Mata & Pradhan); McKnight & McKnight (1998); Sethi & Walia (2011); Vashishta (1995, and references therein) could be useful references for identification of macrofungi. High quality colored photographs taken in the field are extremely useful during the time of identification process and for publications.

3.2.4 General account of Lichen

Theophrastus, the father of botany, introduced the term ***lichen*** which a latin word denoting *superficial growth*. Lichen is a combination of two organisms, an alga and a fungus, living together in symbiotic association. The algal component in the lichen is called phycobiont or photobiont while fungus as mycobiont. Lichen thallus (body) is predominantly of mycobiont which provides shape, structure and colour to the lichen with partial (about 10%) contribution from algae. Among the 20,000 lichen species known in the world 95% belongs to the Ascomycetes group of fungi while Basidiomycetes and Deuteromycetes groups are represented by only 3% and 2% of species respectively (http://wgbis.ces.iisc.ernet.in/biodiversity/sahyadri_enews/newsletter/issue16/identify.htm). Appearance wise lichens are mainly three types:

Crustose type: Crustose lichen is closely attached to the substratum without leaving any free margin and not easy separable from the substrate. The thallus usually lacks lower cortex and rhizines (root like structure). Such lichens are collected along with their substratum for the detailed study.

Foliose type: They are also called as leafy lichens. The thallus in this case is loosely attached to the substratum at least at the margin. Such lichens are collected by scraping them from the substratum.

Fruticose type: Also called shrubby lichens. Thallus is attached to the substratum at one point and remaining major portion is either growing erect or hanging. The lichen usually appears as small shrub or bush and easy to collect with hand.

Lichens can grow in diverse climatic conditions and on diverse substrates. The lichens that are growing on tree trunk and bark are called corticolous lichens, twig inhabiting ones are ramicolous, on wood - legnicolous, on rocks and boulders-saxicolous (epilithic), on moss - muscicolous, on soil - terricolous and on evergreen leaves-epiphyllous. Sufficient moisture, light and altitude, unpolluted air and undisturbed, perennial substratum often favour the growth and abundance of lichens. An annotated checklist is presented of the 287 lichens and lichenicolous fungi known from Bhutan. The vast majority (225) are new records for the country, based on recent collections of 264 species by the second author (Aptroot & Feijen, 2002).

3.2.5 Framing and using dichotomous key

Read the descriptive characters in each step and choose the correct option that matches your collected lichen specimens. The characters used in the key can be seen with the naked eye or with a 10X hand lens. Many lichens contain chemical compounds which act as an important character for identification (chemotaxonomy), therefore spot tests (https://en.wikipedia.org/wiki/Spot_test_(lichen)) using sodium hypochlorite (C), Potassium hydroxide KOH (K) or alternatively Sodium hydroxide (NaOH - caustic soda) as 10% solution in water, including Paraphenylenediamine are used . A small drop to the cortex, or to the medulla after scraping away the cortex. and recording the colour reaction carefully would help identify the lichen specimens. Other online resources available are: A key to common lichens on trees in England and Discovering Lichens in Sri Lanka Source: http://dbiodbs.units.it/carso/chiavi_pub21?sc=351 Source: http://dbiodbs.units.it/carso/chiavi_pub21?sc=351
Lichens are strange and complex life forms. They are symbiotic association between algae (mostly blue-green) and fungi (ascomycetes). Lichens are classified on the basis of fungal component. Mostly lichens are three types based on their morphology. Crustlike, leaflike and shrubby are crustose, foliose and fruticose respectively. Bhutan has rich lichen diversity. All three types of lichens are seen in Kanglung. Online resources like http://dbiodbs.units.it/carso/chiavi_pub21?sc=656 and http://dbiodbs.units.it/carso/chiavi_pub21?sc=351 are useful for lichen identification using dichotomous key. For further references on lichens, you are recommended to refer (Ahmadjian, 1967; Aptroot & Feijen, 2002; Awasthi, 2000a; Malcolm, 1997,?; Rai, Khare & Upreti, 2014; Richardson, 1992; Sinha, 2011; Søchting, 1999, and references therein).

Table 3.1: Fungal identification key

Sl.No.	Key Characteristics	Key
1	Hyphae absent. Colony small, rounded and shiny, commonly white to cream	Go to 2
	Hyphae present, colony cottony, may be coloured	Go to 3
2	Colony with small cells, 0.5 to 2 micro diam, single cells cannot be seen under dissecting microscope	Bacteria
	Colony with cells 3 - 10 micron diam, single cells can just be seen in smear on slide under dissecting microscope	Yeast (*Saccharomyces*)
3	Small cells (spores) on stalks can be seen above or in hyphae using the dissecting or compound microscope. Spores may be in a sac-like or round structure.	4
	Spores are invisible, in colony using the dissecting microscope, and on stained mycelia on slide under the compound microscope	Sterile fungus
4	Hyphae lack cross walls (examine stained tips of young hyphae on a slide under the compound microscope). Spores in sac held above mycelia (sporangium, diagram over page).	5
	Hyphae have cross walls. Spores commonly held away from hyphae, may be in thick-walled sac (pycnidium)	6
5	Spores held in sporangium, or released from sporangium, hyphae with short darkened roots on agar.	*Rhizopus*
	Spores held in ∈sporangiumsporangium, hyphae lack roots into agar	*Mucor*
6	Spores produced in compound pycnidium (diagram over page)	*Phoma*
	Spores formed on free hyphae	7
7	Spores consist of a single cell, not internal walls	8
	Most spores have cross walls, immature spores lack cross walls	14
8	Spores in dry chains when undisturbed	9
	Spores in clumps or clusters, sometimes wet looking	12
9	Chains of spores are unbranched	10
	Chains of spores are branched	11
10	Chains of spores held in a brush - like dry cluster, each chain arises from a bottle-like phialide	*Penicillium*
	Chains of spores emerge from phialides which radiate from a swollen vesicle at the top of a specialized coarse hypha	*Aspergillus*
11	Colonies a deep olive to almost black colour, dry spores are generally rounded, lemon shaped or sometimes irregular	*Cladosporium*
	Colonies fawn, spores uniform in shape and size	*Monilia*
12	Colonies flat, creamy, shiny, when young, turning dark with age	*Aureobasidium*
	Colonies fluffy to flat, usually grey to green	13
13	Green masses of spores, white when immature, common in soil	*Trichoderma*
	Grey masses of spores, colony raised and open	*Botrytis*

Table 3.2: Fungal identification key

Sl.No.	Key Characteristics	Key
14	Spores with both vertical and horizontal walls, dark to black	15
	Spores with walls in one direction only, may be pale or dark	17
15	Spores rounded, with walls radiating from centre of spore, held in clusters on short hyphae, culture often red in the agar	*Epicoccum*
	Spores with longitudinal and lateral walls when mature	16
16	Elongate spores formed in branched chains, youngest at tip	*Alternaria*
	Rounded spores formed singly on the sides of short dark hyphae	*Stemphylium*
17	Spores curved, may be dark or pale	18
	Spores cylindrical to rounded, dark, one to many cross walls	*Helminthosporium*
18	Colonies fluffy, white, with curved spores that have one to many cross walls	*Fusarium*
	Colonies dark, spores short, three celled, with central cell larger than the termini	*Curvularia*

Table 3.3: Lichen identification key

Sl.No.	Key Characteristics	Key
1	Thallus leafy or bushy; attached to the substrate only at the base	Go to 2
	Thallus crusty, powdery or with small overlapping scales	Go to 3
2	Thallus bushy and attached to the substrate/bark only at the base	Fruticose; go to 5
	Thallus leafy and attached to the substrate/bark from the lower surface	Foliose; go to 6
3	Thallus scale or crust-like.	Crustose; go to 4
	Thallus soft and powder, soredia like granules	*Lepraria* spp.
4	Thallus crusty	Go to 7
	Thallus scaly	Go to 8
5	Thallus with erect fruiting structures surrounded by scaly base	*Cladonia* spp.
	Thallus tufted, branched without scaly base	Go to 10
6	Thallus bright yellow to orange	Go to 11
	Thallus neither yellow nor orange	Go to 12
7	Crust bright yellow	*Candelariella*
	Crust white, grey, green or brown	14
8	Scales ascending, grey to green above, white below	*Cladonia* sp.
	Scales closely appressed to bark or wood, bluish green to pale brown	Go to 9
9	Scales pale brown, C+ red	*Hypocenomyce*
	Scales shell-like, bluish green, C-	*Normandina*
10	Thallus with different lower and upper colored surface	Go to 13
	Thallus with same colored surface	Go to 15
11	Lobes narrow and branching, fan-like lemon-yellow, K-	*Candelaria* sp.
	Lobes small or leaf-like, orange yellow to greenish-yellow, K+ red	Go to 16
12	Lobes without soredia and isidia, usually with fruits	*Go to 17*
	Lobes with soredia and isidia, usually without fruits	Go to 18
13	Lobes swollen and hollow, with soredia at the lobes end	*Hypogymnia*
	Lobes flat and solid in section	Go to 19
14	Crust with soredia, fruits often absent	Go to 20
	Crust without soredia, fruits usually present	Go to 27

Sl.No.	Key Characteristics	Key
15	Branches thread-like,breaking when pulling to reveal central white strand	Go to 21
	Branches not thread-like, without a central white strand	Go to 22
16	Lobes with soredia on the margin, fruits absent	*Xanthoria*
	Lobes without soredia on the margin, fruits usually present	Go to 30
17	Lobes with long whiskers on the margin.	*Physcia*
	Lobes without whiskers on the margins	Go to 20
18	Lobes swollen and hollow, soredia on the tips of lobes	Go to 31
	Lobes thin and solid, soredia on the surface of the lobes	Go to 23
19	Lobes greenish above, white beneath, with soredia along the margin	*Evernia* spp.
	Lobes grey above, white to black beneath, with isidia on the surface	*Pseudevernia*
20	Crust with marginal radiating lobes	Go to 28
	Crust without marginal radiating lobes	Go to 29
21	Fruits abundant, with rays on the margin	*Usnea*
	Fruits scarce or absent	Go to 25
22	Branches white to grey, coral-like clustered stem	*Sphaerophorus*
	Branches greenish, flattened irregular in section	Go to 26
23	Lobes with small white dots or lines	Go to 32
	Lobes without small white dots or lines	Go to 33
25	Branches very long and little sausaged like branching at the end	*Usnea articulata*
	Branches many, short and shrubby no sausage-like swelling	Go to 40
26	Branches distinctly channelled in lower half	*Ramalina calicaris*
	Branches not channelled	*Ramalina fastigiata*
27	Fruits enclosed in pimple/volcano like structure	Go to 43
	Fruits disc like or elongated	Go to 44
28	Thallus white, K+ yellow	*Diploicia canescens*
	Thallus grey to brown, K-	*Hyperphyscia adglutinata*
29	Crust, C+ red	*Ochrolechia* sp.
	Crust, C- (C+ orange)	Thallus K+ yellow, *Lepra* sp.
30	Lobes broad, >2 m wide, flat and spreading	*Xanthoria parietina*
	Lobes narrow, <2 m wide, small cushion-like	*Polycauliona*
31	Soredia in knob-like soralia on the lobes end	*Hypogymnia tubulosa*
	Soredia in lip-shaped soralia splits in the lobes end	*Hypogymnia physodes*

Sl.No.	Key Characteristics	Key
32	Lobes with rounded white spots	Go to 34
	Lobes with net-like white lines	Go to 35
33	Lobes tips and fruits appearing white frosted, medulla yellowish	*Physconia enteroxantha*
	Lobes tips and fruits without appearing white frosted, medulla white	Go to 36
34	Lower surface pale brown (medulla and soredia C+ pink)	*Punctelia subrudecta*
	Lower surface black	*Punctelia* sp.
35	Lobes with soredia along with white lines	*Parmelia sulcata* Taylor
	Lobes with dark-tipped pin-like isidia on the surface	*Parmelia saxatilis* (L.) Ach.
36	Lobes olive to chestnut brown, >3 mm wide	Go to 37
	Lobes white to grey , if brown than <3mm wide	Go to 38
37	Lobes with pimple-like conical warts	*Melanohalea exasperata*
	Lobes not with pimple-like conical warts, with isidia or soredia	Go to 39
38	Thallus with isidia on upper surface of lobes	Go to 40
	Thallus with soredia on upper surface of lobes	Go to 41
39	Lobes shiny, coral-like isidia and without soredia	*Melanelixia fuliginosa*
	Lobes matt, with pale yellowish scars	*Melanelixia subaurifera*
40	Main branches angular in section, papillae (small warts) absent	*Usnea hirta*
	Main branch rounded in section, papillae present	Go to 44
41	Lobes attached closely to bark	*Parmelina pastillifera*
	Lobes pale-grey to greenish, loosely attached to bark, turning up-ward at margin, isidia present	Go to 42
42	Lobes broadly cripsed, lettuce like with wrinkle or ridged	*Platismatia glauca*
	Lobes narrow strip-shaped, with numerous isidia covering the lobe surface	*Pseudevernia furfuracea*
43	Thallus grey, fruits raised to grey warts	*Pertusaria pertusa*
	Thallus pale cream too brown, with pimple/volcano like warts	Go to 45
44	Holdfast black at base, branches tough and not stretchy	*Usnea subfloridana*
	Holdfast not black at base, branches inflated and stretchy	*Usnea cornuta*
45	Thallus black pimple like fruits with small hole on top	*Pyrenula spp.*
	Thallus cream with creamy volcano-like fruits	*Thelotrema lepadinum*

CHAPTER 4

FRESHWATER ALGAE

Most of the specimens were collected within Sherubtse campus, from in and around freshwater bodies of Kanglung in Trashigang (*Dzongkhag*) District. The satellite image (Figure 4.3a) shows the area of collections and places within Kanglung in and around of Latitude: 27.2861047 and Longitude: 91.5235929.

Freshwater algae are algae found in non-marine water bodies like in stream, river, pond, drains, paddy field etc. They are usually microscopic, sometimes visible to naked eyes in the form of silky filaments, colonial ball, slimy substances. In Kanglung, different freshwater algae ranging in sizes and structure are available in all types of freshwater bodies. Students are encouraged to collect the samples, recording the actual habitats for identification in the lab. Any beginner's guide to identifying freshwater algae including this lab manual may be used for your record and documentation. Algal classification is given below: (Fritsch, 1944, 1948) classified algae into 11 classes based on **i)** Pigmentation **ii)** Reserve food **iii)** Flagellar arrangement **iv)** Thallus

Class	Pigments	Flagella	Reserve food
Chlorophyceae (green algae)	Chlorophyll-a,b Carotene Xanthophyll	Two identical flagella per cell	Starch
Xanthophyceae	Chlorophyll-a, b Carotene Xanthophyll	Heterokont type, one whiplash type and other tinsel	Fats and Leucosin
Chrysophyceae (diatoms, golden algae)	Chlorophyll-a, b Carotenoids	One,two or more unequal flagella	Oils and Leucosin
Bacillariophyceae	Chlorophyll-a, c Carotenes	Very rare	Leucosin and fats
Cryptophyceae	Chlorophyll-a, c Carotenes and xanthophylls	Heterokont type-one tinsel and other whiplash	Starch
Dinophyceae (Dinoflagellates)	Chlorophyll-a, c Carotenoids Xanthophyll	Two unequal lateral flagella in different plane.	Starch and oil
Chloromonodineae	Chlorophyll-a, b Carotenes Xanthophyll	Isokont type	Oil
Euglenophyceae (Euglenoids)	Chlorophyll-a, b	One,two or three anterior flagella.	Fats and paramylon
Phaeophyceae (brown algae	Chlorophyll-a Xanthophyll	Two dissimilar lateral flagella	Laminarin, fats
Rhodophyceae (Red algae)	Chlorophyll-a Phycocyanin Phycoerythrin	Non-motile	Starch
Myxophyceae	Chlorophyll-a, carotene, phycocyanin, phycoerythrin	Non-motile	Cyanophyce an starch

(a) Algal classification by F.E. Fritsch

4.1 Unicellular green single and colonial (motile/non-motile)

Unicellular green single or colonial algae belong to chlorophyceae. Their size ranges from unicellular microscopic single celled motile (*Chlamydomonas*) to colonial non-motile forms (*Haematococcus, Hydrodictyon*). Bellinger & Sigee, 2010; Desikachary, 1959; Prescottet al. , 1964 refer-

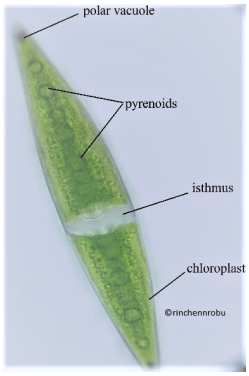
ences could be used for your further study and identification of more of such algae forms.

4.1.1 *Closterium*

4.1.1.1 Habitat

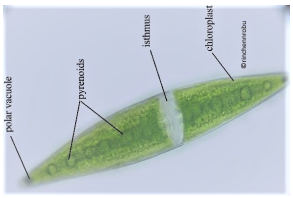
Closterium species are found in drains, ponds, slow streams and stagnant waters, mostly found mixed with slime and some other free-floating fresh water algae in the locality of Kanglung. The places are the surrounding area of upper boys hostel, road side drains, mess area, BHU-area and the paddy fields.

(b) *Closterium* sp. showing habitat.



(c) *Closterium* sp. single cell

Figure 4.1: *Closterium* sp.



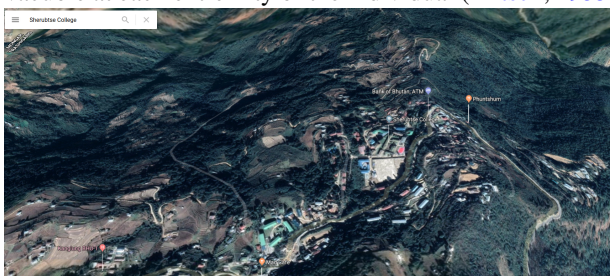
(a) *Closterium* sp.

(b) *Closterium* sp. sickle-shaped.

Figure 4.2: *Closterium* sp. (a. and b.) single celled thallus structure showing semicells joined at the middle (isthmus region).

4.1.1.2 Description

Closterium (Gr. Kloster = spindle) consists of an elongated or cylindrical cell being broadest in the middle and tapering towards the ends, with the cells having straight, spindle, fusiform, crescentiform or lunate shapes (Sharma, 2011). The cells have two halves or semicells joined at the middle (isthmus region). Cells contain two beautiful chloroplasts, located one in each semicell is elongate or rod-shaped having many longitudinal ridges, containing many pyrenoids arranged in a row, with a single nucleus located in the isthmus region with a clear polar vacuole at both tips of each cell (Smithet al. , 1933). In *Closterium*, the cell exhibits a distinct constriction into two perfectly symmetrical halves and the narrower part connecting the two semicells is known as the isthmus region (Fritsch, 1935b). In *Closterium* the elongate chloroplast in each semicell bears more or less numerous longitudinal ridges which are sometimes undulate or anastomose with one another (Fritsch, 1935b). There is also a row of pyrenoids in other cases as single one. In *Closterium* there is a well-marked terminal vacuole at each extremity of the individual (Fritsch, 1935b).



(a) Sherubtse College

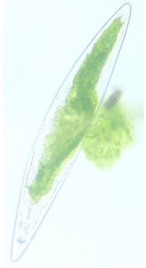
Figure 4.3: Collection site, Kanglung

4.1.1.3 Observation

Desmids are essentially free floatiing and frequently occur in great abundance in small ponds, in the quiet margins of rockey lakes, in *Sphagnum*-blogs, and in other localities where the water is not alkaline. They are altogether restricted to freshwaters (Fritsch, 1935b). The collections rich in species and in number of individuals are usually made only when the waters have a pH of 5 to 6 Smithet al. (1933). The peculiar characteristic of *Closterium* is that there is no asexual reproduction by formation of spores thus, *Closterium* reproduces sexually and vegetatively (Vashishta, 1995).

4.1.1.4 Result

The cell wall of *Closterium* may be colourless or in older specimens it will have various shades of yellow or reddish-brown (Cushman, 1908). Sexual reproduction: is through mode of conjugation. The desmids come together and form a gelatinous sheath parallel to each other. The cell wall at the isthmus splits allowing the protoplasmic content to escape which are amoeboid in nature. The protoplasmic content moves slowly between their parent cells, resulting in the gametic union (outside the cells) (Vashishta, 1995). The resultant zygote develops into zygospore. Some species form conjugating tubes between *Closterium* cells which enter a resting period and changes its colour from green to red (Sharma, 2011).



(a) *Closterium* sp.

(b) *Closterium* sp.

Figure 4.4: *Closterium* sp. (a. and b.) in the process of reproduction.

4.1.1.5 Conclusion

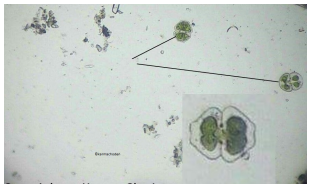
Contribution on this project work may benefit interested students and hope that this work will be continued by the upcoming batches of young enthusiastic, energetic and curious students. The most important thing was to take few best photographs of *Closterium*, which shows a clear structure of the chloroplasts, pyrenoids, the polar vacuoles and the isthmus region.

4.1.2 *Cosmarium*

4.1.2.1 Habitat

Cosmarium is a common free floating freshwater placoderm desmid found in ponds, lakes, ditches, and other reservoirs, though some species (e.g. *C. salium*) are found in brackish water (Sharma, 2011). This specimen was collected from the ponds near physical block and also from the stagnant water which is located just below the MPH Hostel.

(a) *Closterium* sp.

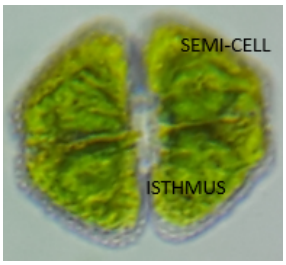


(b) *Closterium* sp.

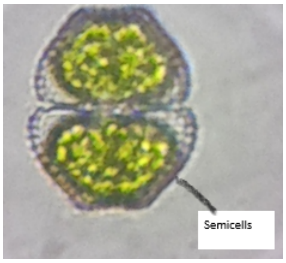
Figure 4.5: *Cosmarium* sp. (a. and b.) showing habitat at Kanglung, Sherubtse.

4.1.2.2 Description

Class: Chlorophyceae
Order: Congugales
Family: Desmidiaceae
Genus: *Cosmarium*
As per Fritsch (1935a).
Cosmarium is the largest desmid genus and is very widespread Sharma (2011). Plant body unicellular and divided into two semi-cells which is joined by isthmus. The walls of the semi-cells (Figure 4.5a) are frequently ornately sculptured, and this, together with the clear brilliant green of chloroplasts make them very attractive. Pyrenoids are present in the chloroplast. Each cell contains a single nucleus located in the isthmus region (Sharma, 2011). *Cosmarium* is a non-motile, freshwater member of division Chlorophyceae (green algae). Asexual reproduction takes place by simple cell-division. Elongation of isthmus region and mitotic division of nucleus into two semi-cells each containing a nucleus.
Sexual reproduction: takes place by conjugation. Interaction of two conjugating cells gives out a thin conjugation tube (Figure 4.7b). Formation of spherical zygospore which after period of dormancy divides reductionally forming four nuclei where two nuclei degenerates. The protoplast divides into two daughter cells, each receiving a daughter nucleus (Fritsch, 1935a; HIRANO, 1965; HIRANO, 1966).



(a) *Closterium* sp.



(b) *Closterium* sp.

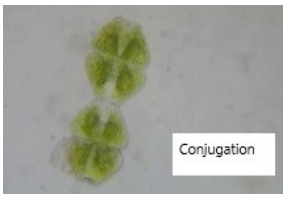
Figure 4.6: *Cosmarium* sp. (a. and b.) showing combined chloroplast and clear isthmus and prominent chloroplast.

4.1.2.3 Observation

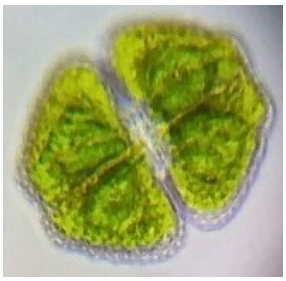
Desmids are distinctive group within the green algae (chlorophyceae). Desmids are essentially free -floating and frequently occur in great abundance in small ponds, in the quiet margins of rocky lakes, in *Sphagnum*-blogs, and in other localities where the water is not alkaline. They are altogether restricted to freshwater (Fritsch, 1935a). It usually appears green in colour and mixed with other free-floating fresh water algae.

4.1.2.4 Results

Micrographs are obtained under 10x, 40x and 100x using Labomed light microscope (Figure 4.7a and 4.7b).



(a) *Closterium* sp.



(b) *Closterium* sp.

Figure 4.7: *Cosmarium* sp. (a. and b.) showing conjugation and prominent chloroplast.

4.1.2.5 Conclusion

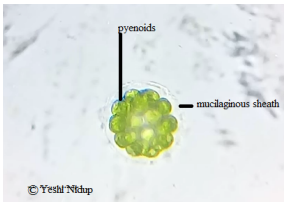
This information regarding *Cosmarium* sp. which is collected from the Kanglung is going to be a short piece of reference to students who are likely to work on *Cosmarium* sp. Specimen being confirmed as a *Cosmarium*, we came to know about its habitat, habits, mode of reproduction and its micrographic study where we can see two semi-cells connected by isthmus and presence of pyrenoids and at least one chloroplast in each semi-cell.

4.1.3 *Pandorina*

4.1.3.1 Habitat

Pandorina specimen was directly collected from dirty water near BHU, Kanglung on 15th of September (Figure**??**). It forms a thick greenish growth in dirty, polluted in water. Basically it inhabits a very wide variety of habitat including ponds, streams, pools and lakes.

(a) *Pandorina* sp. collection site



(b) *Pandorina* sp.

Figure 4.8: *Pandorina* sp. (a. and b.) collection site and cells with mucilaginous sheath.

4.1.3.2 Description

Class: Chlorophyceae
Order: Volvocales
Family: Sphaerellaceae
Genus: *Pandorina*

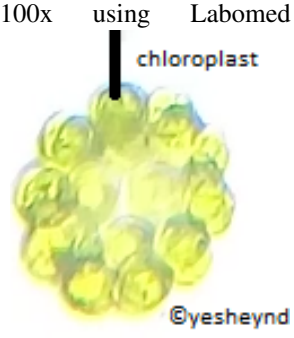
As per (Fritsch, 1935a).
Pandorina is a very common freshwater alga which is green in color. It is motile, multicellular algae. Colonies of *Pandorina* are spherical in shape with 8-32 densely-packed cells (Bellinger & Sigee, 2015). The number of cells held together at their bases to form colony is surrounded by mucilage (Figure 4.8b). The shape of the cells is ovoid or slightly narrowed, it has two flagella with two contractile vacuoles at their base. Chloroplast so present has at least one pyrenoid. *Pandorina* undergoes sexual and asexual reproduction (Sharma, 2011).

4.1.3.3 Observation

Cells embedded in mucilage (Figure 4.8b) that obviously extends beyond the cells at the colony edge (Bellinger & Sigee, 2015). Chloroplast containing at least one pyrenoid, colony center is not hollow. The cells are grouped at the center. Flagella invisible. They are autotrophs due to the presence of chloroplast.
The specimen identified is *Pandorina*. It undergoes asexual reproduction by forming auto-colonies and it also undergoes isogamous sexual reproduction. Cells are uninucleate and contains parietal chloroplast along with pyrenoids (Sharma, 2011).

4.1.3.4 Results

Micrographs are obtained under 10x, 40x and 100x using Labomed light microscope (**??**).



(a) *Pandorina* sp. collection site

(b) *Pandorina* sp.

Figure 4.9: *Pandorina* sp. (a. and b.) cells in colony.

4.1.3.5 Conclusion

This study be the base work on collecting original and authentic information on *Pandorina* found in Kanglung as a reference to the future batch of students. *Pandorina* is considered a Bioindicator. it is used as an indicator of organically polluted waters. It also helps in improving soil fertility.

4.1.4 *Haematococcus*

4.1.4.1 Habitat

The most typical habitats of *Haematococcus* are small temporary water bodies, such as rain water pools, in cliffs and rocky shores of lakes and seas. *Haematococcus* are found in ponds, stagnant water, slow streams and is found mixed with some other fresh water algae. This specimen is collected from the stagnant water which is located near DH-5 hostel.

4.1.4.2 Description

Class: Chlorophyceae
Order: Volvocales
Family: Sphaerellaceae
Genus: *Haematococcus*

As per (Fritsch, 1935b).

Haematococcus is unicellular flagella with chloroplast in the centre of cell and suspended there by strand of cytoplasm. Cells are solitary, ovoid to ellipsoidal, and chloroplast more or less cup-shaped, sometimes reticulate. Their resting cysts are often responsible for the blood-red colour seen in the bottom of dried out rock pools and bird baths. This colour is caused by astaxanthin which is believed to protect the resting cysts from the detrimental effect of UV-radiation, when exposed to direct sunlight. There is a thick mucilaginous wall and there are often protoplasmic extensions into the wide cell wall (Bellinger & Sigee, 2015). Rostafinski concluded that *Haematococcus* is an asexual plant (Hazen, 1899).

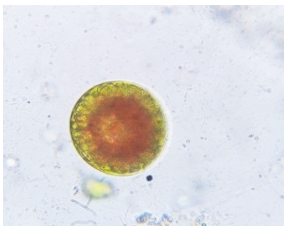
4.1.4.3 Observation

The green pigments in the chloroplast are often masked with red haematochrome so that the cells appear red. The cells are often noticed in an encysted state giving them red color Bellinger & Sigee (2015).

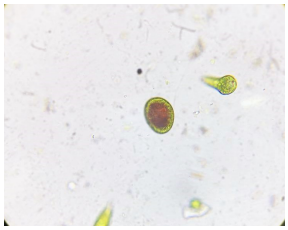
Figure 4.10: *Haematococcus* sp. collection site.

4.1.4.4 Results

Micrographs are obtained under 10x, 40x and 100x using Labomed light microscope (Figure 4.12a and 4.12b).

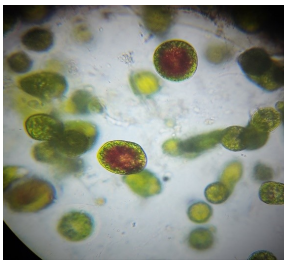


(a) *Haematococcus* sp. collection site

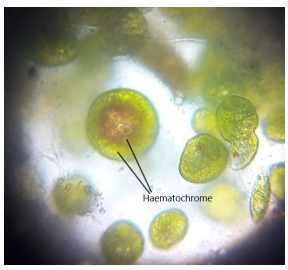


(b) *Haematococcus* sp.

Figure 4.11: *Haematococcus* sp. (a. and b.) cells seen with diatoms.



(a) *Haematococcus* sp. collection site



(b) *Haematococcus* sp.

Figure 4.12: *Haematococcus*: Non-motile cells, ranging from immature (green) to fully mature cysts (a. and b.) filled with red pigment (Haematochrome).

4.1.4.5 Conclusion

This project has been done identify fresh water algae from Kanglung and provide basic information to future researchers to help them identify the species to generic level. The algal specimen is collected from the pond water located nearby DH-5 hostel, which appeared to be mixed with some other algae is identified as *Haematococcus*, where both green and red colored spherical non-motile palmelloid / ellipsoidal cells are seen.

4.1.5 *Euglena*

4.1.5.1 Habitat

Basically, distribution of *Euglena* is cosmopolitan (Verma, Verma). The specimen was collected from the drain where water is still and stagnant, containing rich decaying organic matters having pH value 6-7 behind the boys hostel (DH-I).

4.1.5.2 Description

Class: Euglenineae
Family: Euglenaceae
Genus: *Euglena*
As per Fritsch (1935a).
Euglena are unicellular possessing two or rarely three flagella and motile which also have chloroplast in various shaped, rod-like, disc-like, ribbon-like or stellate. Food is reserved in the form of granules of paramylon Sharma (2011). *Euglena*, despite the presence of chloroplast, they also have a saprophytic tendency. When the *Euglena* is cultured in the solution medium in dark, it loses pigment which is regained by exposing to light again but when it is prolonged, the loss becomes permanent and it becomes colourless leading to saprophytic nutrition Fritsch (1935a). Reproduction by longitudinal division plays the greatest role in the free swimming and encysted forms. Transverse division occurs in the active and encysted forms. Temporary encystment without reproduction occurs in *E. gracilis* Tannreuther (1923).

4.1.5.3 Observation

In ordinary state, *Euglena* is motile having one or two flagella which are of diverse length and inserted apically [1]. *Euglena* displays flagellar movement where it revolves in circle or gyrates or it rotates on its axis. Sometimes it performs a peristaltic activity which is com-

monly known as euglenoid movement (Kotpal et al., 2001).

(a) *Euglena* sp. collection site

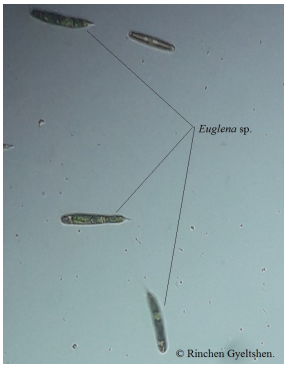
(b) *Euglena* sp.

Figure 4.13: Habitat of *Euglena* and Showing paramylon bodies of *Euglena* sp. (10x40x)

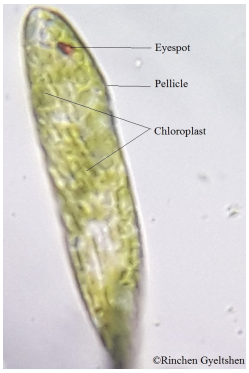
Chief home of *Euglena* is freshwater, where they give a characteristic coloration to the water, for example green in case of *E.varidis*, red in case of *E.sanguinea*, brown in case of species of *Trachelomonas*. A number of species of *Euglena* inhabits in damp mud (Fritsch, 1935a).

4.1.5.4 Results

Single cell of *Euglena* sp. Showing eyespot, pellicle, flagella, chloroplast and paramylon bodies. Micrographs are obtained under 10x, 40x and 100x using Labomed light microscope (Figure 4.13b and 4.14a).



(a) *Euglena* sp.



(b) *Euglena* sp. showing chloroplast and pellicle

(c) *Euglena* sp.

Figure 4.14: Showing whole vegetative part of *Euglena* sp. (10x10x) and eyespot.

4.1.6 Conclusion

This project provides brief information on *Euglena* sp., which is collected from Kanglung area. This information can be used as reference by students who would work on algae and *Euglena* sp. in Kanglung area specifically. The specimen was collected from the drain water behind boys' hostel (DH-I). Studying the characteristics of this specimen and confirming it to be *Euglena* sp., I came to know about their habitat, thallus structure, reproduction, nutrition and its ecological significance.

4.2 *Chlorogonium*

4.2.1 Habitat and occurrence

Chlorogonium (**??**) is widespread freshwater alga in Kanglung. Habitats include soil, temporary pools, and eutrophic lakes.

(a) *Euglena* sp. collection site

(b) *Euglena* sp.

Figure 4.15: *Chlorogonium*, showing clear anterior flagella and distinct pyrenoids

4.2.2 General characteristics

i Unicellular motile and unicellular thallus.

ii Cells are elongating fusiform or spindle in shape tapering at both ends with two flagella arising from the narrow apical end.

iii Typically spindle-shaped (fusiform) or strongly elongated along flagellar axis.

iv Elongate and often almost needle shaped form of cells (Fritsch, 1935a).

v There is anterior eye spot.

vi Cells 2-15 micron m broad and up to 80 micron m long.

vii Can occur in large numbers in small bodies of water especially those rich in humic materials.

viii Several vacuoles are scattered throughout the cell (Sharma, 2011).

ix *Chlorogonium* follows an oogamous type of reproduction (Sharma, 2011).

x Each cell with more than two contractile vacuoles scattered throughout the cytoplasm.

4.2.3 Key characteristics

i Sexual reproduction isogamous to oogamous. Zygotes spherical with thick, flattened wall.

ii *Chlorogonium*, with a parietal diffuse chloroplast contains a number of scattered pyrenoids and with numerous scattered contractile vacuoles (Fritsch, 1935a).

iii *Chlorogonium* is a colourless unicellular flagellate, a genus formerly included in Protomastiginea, a class of colourless Flagellates (Fritsch, 1935a).

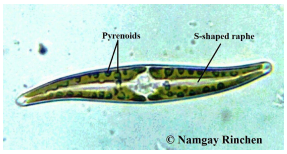
4.2.4 Conclusion

Chlorogonium is a distinctive assemblage of freshwater species characterized by several unusual features including retention of motility, multiple (more than two) contractile vacuoles, and transverse cell division.

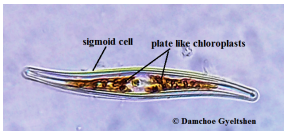
4.3 *Gyrosigma*

4.3.1 Habitat and occurrence

Gyrosigma (4.16a) is a widespread freshwater alga in Kanglung. Its habitat includes small pond, ditches, stagnant waters and drain waters. It rarely occurs in the fast flowing streams.



(a) *Gyrosigma* sp.



(b) *Gyrosigma* sp. showing plate like chloroplast

Figure 4.16: *Gyrosigma* showing clear S-shaped raphe, rounded poles and scattered pyrenoids.

4.3.2 General characteristics

i Pennate diatoms (Bellinger & Sigee, 2015).

ii Cells sigmoid in outline, broad in the central region and narrow towards the apices (Bellinger & Sigee, 2015).

iii Two plates like chloroplasts(Figure 4.16b) on either side of the longitudinal axis or are present lying either side of the gridle (Bellinger & Sigee, 2015; Fritsch, 1935b).

iv The poles are rounded (Bellinger & Sigee, 2015).

v The striate on the valve surface are both parallel to the raphe and also transverse (Bellinger & Sigee, 2015).

vi Reproduce vegetatively by cell-division and sexually by auxospores (Fritsch, 1935b).

vii Radially symmetrical (Fritsch, 1935b).

4.3.3 Key characteristics

i Raphe and axial area are uncommonly curved in Gyrosigma Fritsch (1935b).

ii The raphe is S-shaped Bellinger & Sigee (2015).

iii They exhibit characteristic gliding movement, caused **by streaming cytoplasm by circulation within the raphe, and by the extrusion of mucilage ?**.

4.3.4 Conclusion

Freshwater fossil diatoms are present in the waters indicating high alkalinity (Sharma, 2011). It belongs to pennate type of diatom under Naviculaceae family.

4.4 Phacus

4.4.1 Habitat and occurrence

Phacus are found in freshwater habitats around the Kanglung. Habitats include ponds, pools and still waters. *Phacus* is a member of the class Euglenophyceae, family Euglenaceae and the order Euglenales .

4.4.2 General characteristics

Class euglenophyceae comprises of motile cells and also a small number of palmelloid and non-flagellated species (Sharma, 2011). Flagellated euglenoids posses two or rarely three flagella (Sharma, 2011). The reserved food materials are polysaccharides and paramylon (Bellinger & Sigee, 2015). The chloroplasts in euglenoids are rod-like, disc-like, ribbon-like or stelute and red eyespots is present in most euglenoids (Sharma, 2011). c

4.4.3 Key characteristics

Phacus is solitary and free swimming alga. *Phacus* shows a peculiar twisted shape Sharma (2011). They are morphologically flattened, rigid, leaf-shaped containing many small discoid chloroplasts without pyrenoids and an eyespot (Bellinger & Sigee, 2015). Disc-shaped paramylon bodies (one to many) are present (Bellinger & Sigee, 2015).

4.4.4 Conclusion

Sexual reproduction is not substantially known. It undergoes isogamous type of reproduction Sharma (2011). Euglenoids reproduce solely by cell division (Sharma, 2011).

4.5 *Pleurotaenium*

4.5.1 Habitat

Sample of *Pleurotaenium* was collected on 5th September from the fish pond located in the college botanical garden. They are often found in soft water, more acidic waters and among the mosses.

4.5.2 Description

Class: Chlorophyceae
Order: Conjugales
Family: Desmidiaceae
Genus: *Pleurotaenium*

As per Fritsch (1935b).

Pleurotaenium is a very common freshwater, unbranched single celled green alga. It is commonly found in ponds and pools where there are less disturbances in the water along with some species of *Closterium*. The distinct identifying character of *Pleurotaenium* is that, cells are elongated straight cylinder, about 10 times longer than broad. In *Pleurotaenium* the median constriction is not deep. The swollen area is present in either side of the cells and a ring like thickening seen where two semicells join. Cell wide with numerous long band-shaped chloroplast with pyrenoids Fritsch (1935a).

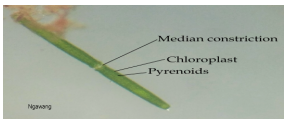
4.5.3 Observation

Pleurotaenium is naturally found in slow streams, ponds and pools in Sherubtse Campus, Kanglung Trashigang.

4.5.4 Conclusion

This project is the basic work on collecting original and authentic information on *Pleurotaenium* found in Kanglung. In future focus could be given on reproduction and if possible identify to species level.

(a) *Pleurotaenium* sp.



(b) *Pleurotaenium* sp. showing plate like chloroplast

(c) *Pleurotaenium* sp. showing plate like chloroplast

Figure 4.17: *Pleurotaenium* showing median constriction, pyrenoids and chloroplast.

4.5.5 Conclusion

Their unique features amongst desmids make us easy to identify them. Similar study on other desmids in Kanglung or from other region of Bhutan could be useful for academic learning. Future project could be done on habitats of *Pleurotaenium* and its effect when its natural habitat is altered due to various reasons.

4.6 Unbranched filamentous green algae

Filamentous unbranched green algae also belong to chlorophyceae. They are slippery scum/silk-like visible to naked eyes (*Spirogyra*) seen floating in the ponds and streams. In Kanglung unbranched filamentous green algae are commonly found floating in pools, ponds and ditches. Alga like *Oedogonium* are attached to substrata by their root-like structure called hold-fast. They basically grow well within pH range of 4-10. Their identification is usually easier than unicellular and colonial forms of algae.

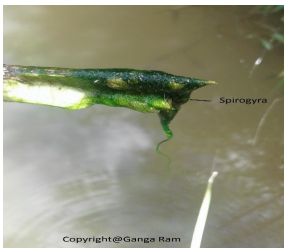
4.6.1 *Spirogyra*

4.6.1.1 Habitat

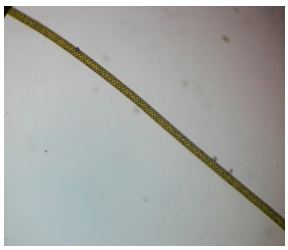
Spirogyra species are found in drains, ponds, slow streams and ditches, mostly found mixed with some other free floating fresh water algae in the locality of Kanglung. The places include roadside drains, Science Park pond, paddy fields and slow streams.

4.6.1.2 Description

Class: Chlorophyceae
Order: Conjugales
Family: Zygnemaceae
Genus: *Spirogyra*
As per (Fritsch, 1935b)
According to Fritsch (1935b), *Spirogyra* is a long and slimy unbranched, filamentous fresh water alga with a very fine filament. It forms a green floating mass of slippery threads which makes it call pond-scum or water silk (Fritsch, 1935b). Randhawa (1959) reported that *Spirogyra* genus includes about 289 species and the study of various *Spirogyra* species is essentially important as it plays a great role in the natural food cycle. It is commonly found in fresh water such as pools, ponds, lakes and ditches (Sharma, 2011). According to the research done by the School of Life Sciences, Chungbuk National University, *Spirogyra* basically grows well in pH range of 4-10 and the temperature range of 10-30. Below pH 4, it showed abnormal growth. While in Kanglung, *Spirogyra* is abundantly found in paddy field, drains, stream side and in the damp moist small temporary ponds where the pH roughly ranges from 6-7.5.



(a) *Spirogyra* sp.

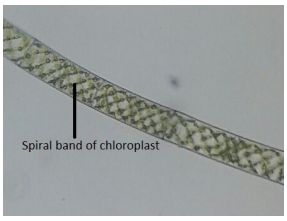


(b) *Spirogyra* sp. showing plate like chloroplast

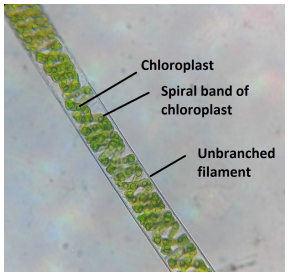
Figure 4.18: *Spirogyra* showing habitat (a.) and unbranched filament (b.).

4.6.1.3 Observation

The cells of *Spirogyra* spp. are joined end to end forming an unbranched and elongated cylindrical filament (Figure 4.19a). Its chloroplast forms a distinct spiral band (Figure 4.19b) within its cell and has numerous pyrenoids which are the main characteristic of *Spirogyra*. Further each cell consists of cell wall and protoplast (Fritsch, 1935a).



(a) *Spirogyra* sp.



(b) *Spirogyra* sp. showing plate like chloroplast

Figure 4.19: *Spirogyra* showing clear spiral chloroplast and pyrenoids.

4.6.1.4 Results

The most favoured reproduction in *Spirogyra* is sexual which occurs basically through conjugation (Smithet al. , 1933). Conjugation is the process of fusion of two gametes of which one acts as active male gamete and passes though the conjugation tube into the female gamete (Fritsch, 1935a). All the cells in filament can form gametes which are morphologically similar. Lateral conjugation occurs only when a male cells lies at the side of female cell and the conjugation tube develops across the wall for fusion of gametes, whereas scalariform conjugation occurs in the monoecious filaments with the formation of conjugation tube.

4.6.1.5 Conclusion

Spirogyra is an unbranched filamentous green alga. The distinctive characteristic for its identification is the spiral band chloroplast containing pyrenoids Fritsch (1935a). Such study *Spirogyra* sp. is informative. So, its going to be a short piece of reference for the upcoming researchers who are likely to work on *Spirogyra* sp.

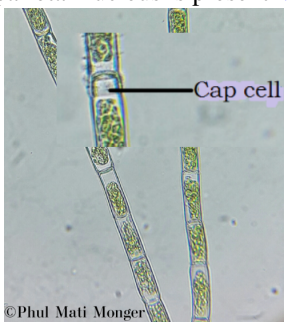
4.6.2 *Oedogonium*

4.6.2.1 Habitat

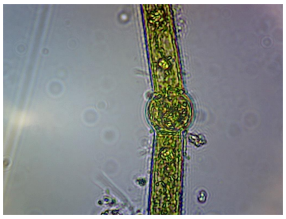
It is an exclusively fresh water alga generally found attached to various substrate such as wood, stones, stems, leaves and many aquatic plants showing epiphytic nature. It occurs commonly in fresh water surroundings of all kinds including pools, ponds, lakes, rivers, and shallow water (Sharma, 2011). The specimen for this project was collected from the pond near College gate, Kanglung and from the stagnant water from the flower pot.

4.6.2.2 Description

Class: Chlorophyceae
Order: Oedogoniales
Family: Oedogoniaceae
Genus: *Oedogonium* sp. As per Fritsch (1935a). *Oedogonium* sp. is a submerged aquatic alga which occurs attached to the solid objects like stones or wood in quite fresh water. The mature filaments are free floating but the younger ones are attached. It is less common in running water (Vashishta, 1995). The plant body is a thallus which is a long, unbranched thread call filament. The cells are elongated and cylindrical (Figure 4.20a). The chloroplast is parietal and extends from one end of the cell to the other end that form a hollow cylindrical network with narrow or broad or broad sub-parallel meshes. Numerous pyrenoid lie at the intersection of the reticulum (Figure 4.20b). Only a single large parietal nucleus is present Vashishta (1995).



(a) *Oedogonium* sp. showing cap cell



(b) *Oedogonium* sp. showing dwarf male

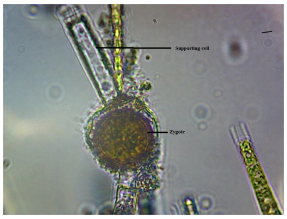
Figure 4.20: *Oedogonium* showing clear reticulate chloroplast and cap cell.

4.6.2.3 Observation

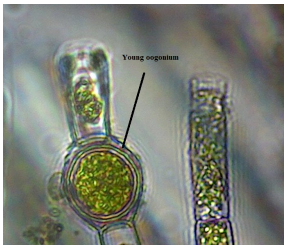
Oedogonium consist of long unbranched threads or filaments. Cells are longer than broad and contains net like parietal chloroplast with many chloroplast. Sometimes, a ring like transverse lines can be seen in between the two cells and is termed as cap cells (Figure 4.20a). In the reproductive part of this specimen, a swollen cell (oogonium) can be observed sometimes(Figure 4.21b).

4.6.2.4 Results

Diatoms reproduce vegetatively by cell division and sexually by the production of auxospores formation (**?**). The process of auxospore formation is actually a 'restorative process' because there is reduction in original size of the cells, during the process of cells division Âţ, is restored during this process. Sexual reproduction in diatoms is influenced by various factors including temperature, light condition and nutrition.



(a) *Oedogonium* sp. showing supporting cell



(b) *Oedogonium* sp. showing young oogonium

Figure 4.21: *Oedogonium* showing supporting cell and oogonium.

4.6.2.5 Conclusion

Oedogonium is found in abundant in fresh water. It looks like a mass of thread-like over the stagnant water giving it a green colour. Its distinct features are: non-branched filamentous, the chloroplast is parietal, presence of cap cell and sometimes presence of oogonium. hough this project has good information about *Oedogonium* sp., there are lots more which has not been mentioned or has been missed out. So, this project can be used a small guide to those who are genuinely looking up for detail study on fresh water algae from Kanglung.

4.6.3 *Zygnema*

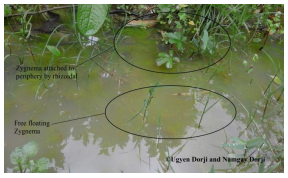
4.6.3.1 Habitat

Zygnema specimen was collected on 17 August 2017 from the puddle near the fountain beside the Kanglung basketball court. *Zygnema* inhabits a very wide variety of habitat including ponds, puddles, streams and sewers having pH 5-6. It forms a thick green mass that is generally seen around the periphery.

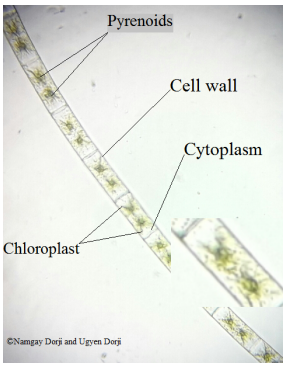
4.6.3.2 Description

Class: Chlorophyceae
Order: Conjugales
Family: Zygnemaceae
Genus: *Zygnema*
As per Fritsch (1935b)

Zygnema is very common fresh water, un-branched, filamentous alga. It is commonly found in stagnant waters but can also be found in running water attached to the periphery by some attaching cells (Sharma, 2011). Generally it also occurs in ponds, lakes, pools and other fresh water reservoirs along with some species of *Spirogyra*. The distinct identification of *Zygnema* is the presence of two star-shaped chloroplast. Each chloroplast has a pyrenoid. The filaments usually have soft mucilage sheath, are unbranched and are not very long (Sharma, 2011).



(a) *Zygnema* sp. showing habitat



(b) *Zygnema* sp.

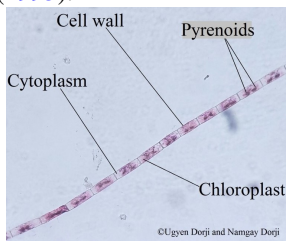
Figure 4.22: *Zygnema* habitat (a) and star-chloroplast

4.6.3.3 Observation

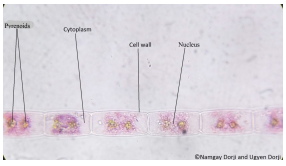
Most of the species of *Zygnema* are free floating but some species are also found attached to a substratum with the help of rhizoidal outgrowths (Sharma, 2011). This specimen was collected from the puddle near the college fountain where the pH of the water is about 5-6. It was found clustered in a group forming a green floating mass. They appear to be essentially autotrophic (Sharma, 2011).

4.6.3.4 Results

Zygnema is an unbranched filamentous alga. Filament consists of many cylindrical cells of almost similar structure. They are 1-9 times longer than they are wide Vashishta (1995).



(a) *Zygnema* sp.



(b) *Zygnema* sp.

Figure 4.23: *Zygnema* showing star shaped chloroplast and pyrenoids

4.6.3.5 Conclusion

It is a simple filamentous green alga with two distinct **star-shaped** nuclei. The filaments develop unicellular or multicellular outgrowths which aids in attachment to the substratum. The cell is usually longer in breadth than it is in width. It is a free floating alga usually found in water that has a pH of 5-6. his project will be the basic work on collecting original and authentic information on *Zygnema* found in

Kanglung. Future researches should be done on habitats of *Zygnema* and its effects when its natural habitat is changed. Moreover, the focus should be given on reproduction and if possible classify even down to species level.

4.6.4 Branched filamentous green

Unlike-unbranched filamentous green algae, filamentous branched algal forms are medium to profusely branched. In this type thallus is differentiated into a prostrate system of branched filaments growing on the substrate and erect system away from the substrate, e.g. *Batrachospermum*, *Stigeoclonium*. Branches can be alternate, opposite or whorled.

4.6.5 *Stigeoclonium*

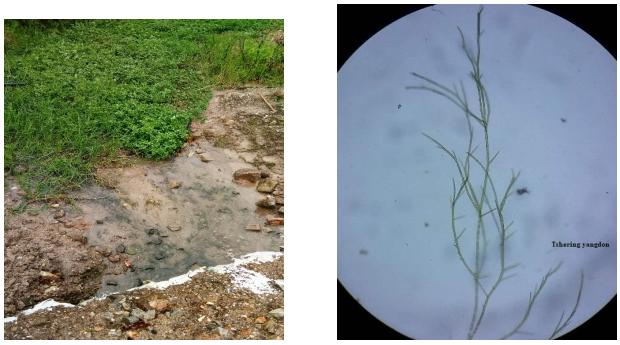
4.6.5.1 Habitat

Stigeoclonium is a freshwater alga growing usually in slow running or stagnant water Fritsch (1948). The algae grows attached to wood work, stones and submerged aquatics and commonly form green hairy mats. This specimen is collected from the slow running water near the college Zangdopelri where new residential hostels are being built.

4.6.5.2 Description

Class: Chlorophyceae
Order: Chaetophorales
Family: Chaetophoraceae
Genus: *Stigeoclonium*
As per Fritsch (1935b)

Plant body of *Stigeoclonium* is heterotrichous and the filamentous thallus is differentiated into a prostrate portion and an erect portion (Sharma, 2011). The more developed the prostrate system, the less developed is the projecting system and vice-versa. The cells are uninucleate and each with a single chloroplast. The terminal elongated cells of the branches taper into hairs and are usually devoid of chloroplast (Figure 4.24b). Branches does not usually occur in distinct whorl. Filaments are usually attached by means of basal cells to variety of surfaces. Branches can be opposite, alternate but rarely whorled and terminating in a tapering series of cells sometimes forming a terminal multicellular hair (Sharma, 2011).

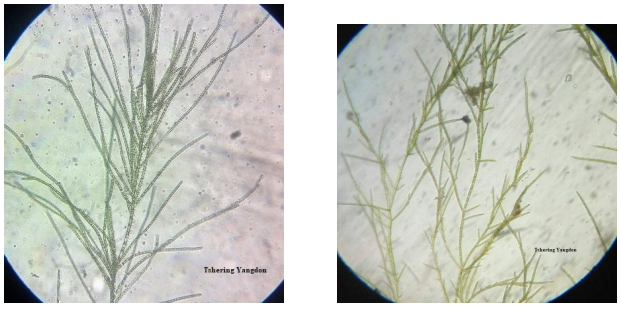


(a) */Stegeoclonium* sp. **(b)** *Stegeoclonium* sp.

Figure 4.24: *Stigeoclonium* showing habitat (a.) and branched filament (b.)

4.6.5.3 Observation

This is found in the slow running water which has a pH of 6.14 (slightly acidic) (**?**). *Stigeoclonium* is known to reproduce by vegetative, asexual and sexual methods. However, the most favored mode of reproduction is asexual reproduction . Filaments of *Stigeoclonium* are usually attached by means of basal cells to a variety of surfaces such as rocks or stones but may break away and be found free floating (Sharma, 2011). Branches can be opposite, alternate but rarely whorled and terminating in a tapering series of cells sometimes forming a terminal multicellular hair (Figure 4.25b). It is common and is often used as an indicator of enriched or organically-polluted situations (also reported as tolerant to heavy metal pollution) (Bellinger & Sigee, 2015). *Zygnema* is an unbranched filamentous alga. Filament consists of many cylindrical cells of almost similar structure. They are 1-9 times longer than they are wide (Vashishta, 1995).



(a) *Stigeoclonium* sp. **(b)** *Stigeoclonium* sp.

Figure 4.25: *Stigeoclonium* showing branched filaments.

4.6.5.4 Conclusion

The specimen which was collected has all the specific characteristics as that of *Stigeoclonium* and available at Sherubtse campus, Kanglung. I hope that this project on *Stigeoclonium* would be a helpful information to students who would like to work more on fresh water algae in Bhutan.

4.7 Blue-green algae (Cyanobacteria)

Blue-green algae are found in almost as diverse a variety of habitats as other true bacteria. They are common in temporary pools or ditches, particularly if the water is polluted. In the common genera *Nostoc* and *Anabaena*, which form chains of cells, fragmentation often occurs at special, larger, colorless, nitrogen-fixing cells called heterocysts, which are produced at intervals in the chains. Members of these two genera also may produce thick-walled cells called akinetes, which can resist freezing and other adverse conditions. When favorable conditions return, this survival feature enables the cells to germinate and become new chains or filaments. *Oscillatoria* filaments rotates on axes and move in a gliding fashion, apparently by the twisting of minute fibrils inside the cell walls while secreting mucilage that reduces friction.

4.7.1 *Nostoc*

4.7.1.1 Habitat

Nostoc is a diverse genus of cyanobacteria which are common in both aquatic and terrestrial areas (https://microbewiki.kenyon.edu/index.php/Nostoc). Terrestrial species are found abundantly in rice fields where the soil is moist, mixed with many small plants like lichens, mosses, etc, on moist rocks, bottom of lakes and springs (https://en.m.wikipedia.org/wiki/Nostoc). In Kanglung, *Nostoc* sp. are found in paddy fields and stagnant water.

4.7.1.2 Description

Class: Myxophyceae
Order: Nostocales
Family: Nostocaceae
Genus: *Nostoc*
As per Fritsch (1935a).
Nostoc is a filamentous blue green algae. It is usually formed of ball-like gelatinous colonies composed of filaments called trichomes (https://microbewiki.kenyon.edu/index.php/Nostoc). Along the filament some large, spherical or cylindrical, colourless empty cells called heterocyst are found (Sharma, 2011). It helps in fixing nitrogen during nitrogen starvation of cyanobacteria. Moreover, an essential survival structure called akinetes is present in *Nostoc*. It also has photosynthetic pigment in their cytoplasm; hence they can also perform photosynthesis (https://en.m.wikipedia.org/wiki/Nostoc). Though sexual reproduction is absent. It reproduces asexually or by vegetative methods (Sharma, 2011).

(a) *Nostoc* sp. collection site

(b) *Nostoc* sp.

Figure 4.26: Habitat of *Nostoc* and showing filament

4.7.1.3 Observation

It is filamentous blue green alga (Sharma, 2011). The trichomes of *Nostoc* is similar to Anabaena but they are embedded in the firm, extensive mucilaginous sheath (Fritsch, 1935a). Akinetes may occur in the older parts of the colony and are produced between heterocyst (unlike in *Anabaena*). The cells are spherical to barrel-shaped.

4.7.1.4 Results

(a) *Nostoc* sp. collection site

(b) *Nostoc* sp.

Figure 4.27: *Nostoc* filament showing mucilage sheath

4.7.1.5 Conclusion

After completion of this work, we have come to know some of the characteristic differences between *Nostoc* and *Anabaena*. Not only that, we learned about its habitats, habit and its uses as important biofertilizer. An efficient amount of energy is vital to achieve a clear vision. So we hope that our contribution will add necessary ingredients to success for the upcoming batches.

4.7.2 *Oscillatoria*

4.7.2.1 Habitat

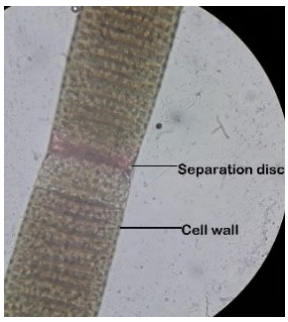
Oscillatoria specimen was directly collected from the dirty stagnant water near Thragom, Kanglung on 17th of September. *Oscillatoria* inhabits a wide variety of habitats. It forms a thick blackish growth in dirty, polluted and stagnant water in Kanglung. Generally it also occurs in ponds, banks of river, canals, streams and sewersr (Sharma, 2011).

4.7.2.2 Description

Class: Myxophyceae
Order: Nostocales
Family: Oscillatoriaceae
Genus: *Oscillatoria*

As per Fritsch (1935a).
Oscillatoria is a very common freshwater, unbranched, filamentous blue-green algae Bellinger & Sigee (2015). The trichomes of *Oscillatoria* are either solitary or matted together to form a well-developed spongy sheet Sharma (2011). The trichomes are generally not surrounded by mucilaginous sheath. The trichomes are capable of gilding movement Vashishta (1995). The end cell may be rounded or have characteristic shape Bellinger & Sigee (2015). It reproduces only vegetatively through methods like formation of hormogonia and fragmentation Fritsch (1935a). *Oscillatoria* is a genus of filamentous cyanobacterium which is named after oscillation in its movement. The tip of the trichome oscillates like a pendulum. https://sussle.org/t/Oscillatoria.

(a) *Oscillatoria* sp. collection site

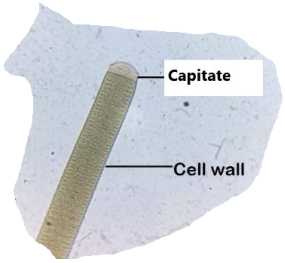


(b) *Nostoc* sp.

Figure 4.28: *Oscillatoria* habitat and a filament

4.7.2.3 Observation

Oscillatoria was found in the polluted stagnant water. Trichomes were free-living or found forming a compact tangled floating mass in the stagnant water. It was barely perceptible.
The species identified is *Oscillatoria*. Individual filaments of *Oscillatoria* move in a pendulum-like oscillating or swinging movement and it reproduces with formation of separation disc.



(a) *Oscillatoria* sp. collection site

(b) *Nostoc* sp.

Figure 4.29: *Oscillatoria* sp. single filament showing capitate and separation disc, 10x.

4.7.2.4 Conclusion

Oscillatoria sp. is not only a source of nitrogen, but also used as organic matter and growth promoting substances for rice cultivation. It can reduce ecological and biochemical imbalance in a rice field. It can also be used as alternative to chemical fertilizers. Therefore, *Oscillatoria* sp. truly a unique and interesting blue-green algae. This project will be the base work on collecting original and authentic information on *Oscillatoria* found in Kanglung for reference to students. Future researchers might want to research on *Oscillatoria* sp. regarding the natural production of butylated hydroxytoluene (antioxidant, food additive and industrial chemical). If possible, explore even down to species level.

4.8 Pennate diatoms

Diatoms are divided into 2 types viz., Centric and Pennate diatoms based on shape of the frustule. Pennate diatoms are elongate in both âĂIJvalve viewâĂİ and âĂIJgirdle viewâĂİ and are bilaterally symmetric (e.g. *Navicula*). Presence of a **raphe** (slit in the **frustules**) on both **valves** related to **gliding motility**. This may be an ancestral trait found only in the benthic pennate diatoms. In addition many benthic forms are heavily silicified (e.g. *Pinnularia*) to increase their buoyancy. Source: (Bacillariophyceae).

4.8.1 *Navicula*

4.8.1.1 Habitat

It is freshwater alga which is commonly found in ponds and rivers usually free floating with other algae. It is found in the marshy and stagnant water which is contaminated and is collected from behind the DH5 hostel in Sherubtse College.

4.8.1.2 Description

Class: Bacillariophyceae
Order: Navicules
Family: Naviculaeceae
Genus: *Navicula*
(-Bory de Saint-Vincent, 1822)
Navicula falls under class Bacilliariophyceae which is commonly known for its ornamentation. Cells of Navicula are solitary, motile and are in boat shaped (https://www.eoas.ubc.ca/research/phytoplankton/diatoms/pennate/navicula/navicula_spp.html). At the central nodule, which is a solid internal thickening of the wall, the two over lying (external and internal) fissures of the raphe approaching from either pole bare connected by a loop like, somewhat sinuous canal Fritsch (1935a). Raphe is present in both the side of valve which bears three enlargement or nodules, one central nodules and two polar nodules. Raphe is responsible for the gliding movement in *Navicula*. It has two chloroplast at each side of raphe each with single rod-shaped pyrenoids (can be view only through girdle view) (http://craticula.ncl.

ac.uk/EADiatomKey/html/Navicula.htmel).
The cell wall along with plasma membrane encloses cell protoplast which is further differentiated into a single nucleus and cytoplasm. The cytoplasm encloses nucleus which is centrally located and two large vacuoles. They undergo asexual reproduction in favorable condition and sexual reproduction which is very rare.

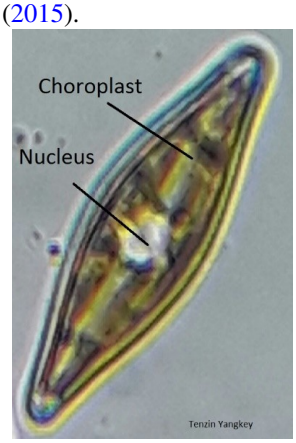
(a) *Oscillatoria* sp. collection site

(b) *Nostoc* sp.

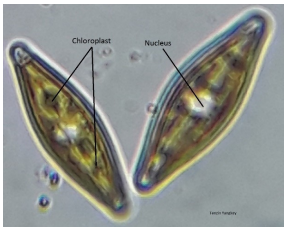
Figure 4.30: *Oscillatoria* sp. single filament showing capitate and separation disc, 10x.

4.8.1.3 Observation

Navicula is a motile diatom which lives singly. It has the cell wall enclosing raphe which is located near the cell wall in the cytoplasm, two chloroplast located at the each sides of the raphe along with the pyrenoids and nucleus centrally located with two large vacuoles. It is boat shaped with yellow brownish color.
True *Navicula* species have lanceloate valve with narrow axial area flanked by fine striae which are slightly radiate at the center but parallel towards the cell apices Bellinger & Sigee (2015).



(a) *Navicula* sp. collection site



(b) *Navicula* sp.

Figure 4.31: *Navicula* sp. showing chloroplast and central nucleus.

4.8.1.4 Conclusion

The specimen collected from Sherubtse college campus near hostel DH5 has been identified as *Navicula*. It is found together with other freshwater algae which is boat shaped. This project gives you information especially on *Navicula* specimen and can be used as reference to know its habitat. It would be useful for working on diatoms of Kanglung and Bhutan.

4.8.2 *Pinnularia*

4.8.2.1 Habitat

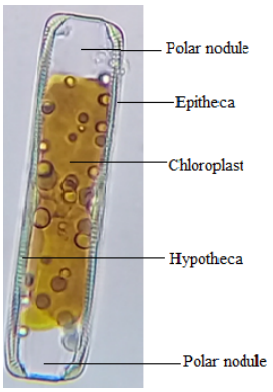
Pinnularia sp. is found in shallow water having slightly acidic nature in Kanglung. The suitable habitat for *Pinnularia* is found above Bhutan Telecom Office, spring water below the fish pond located near the college main gate, beside college football ground in moist soil.

4.8.2.2 Description

Class: Bacillariophyceae
Order: Naviculates
Family: Pinnulariaceae
Genus: *Pinnularia*
According to (Fritsch, 1935a).

According to (**?**), members of the genus Pinnularia are found mostly in acidic, freshwater pools, or they may be epilithic on acidic rocks, epiphytic on Bryophyte and in moist soils, where they may form the food of soil Protozoa and rotifers. It occurs where the pH is low, in the region of 3.5 to 4.5. *Pinnularia* is brown in color, striated rib-like in appearance and cells are linear, lanceolate or even elliptical. There is a central raphe whose middle ends bend in the same direction and there is usually two plate-like chloroplast, one either side of the midline Bellinger & Sigee (2015). Since they belong to the class Bacillariophyceae, they are ornamental in nature.

(a) *Navicula* sp. collection site



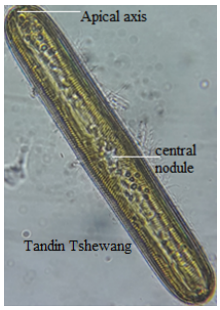
(b) *Navicula* sp.

Figure 4.32: *Pinnularia* sp. habitat and girdle view of *Pinnularia* showing polar nodule.

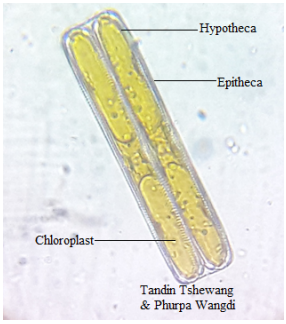
4.8.2.3 Observation

It is brown in colour, striated, rib like in appearance, the poles are usually rounded, capitate or rostrate (https://en.m.wikipedia.org/wiki/Pinnularia). There are usually two plate-like chloroplast, one either side of the mid-

line. The cells of *Pinnularia* are linear or elliptical.



(a) *Pinnularia* sp. collection site



(b) *Pinnularia* sp.

Figure 4.33: *Pinnularia* sp. showing simple cell division

4.8.2.4 Conclusion

Pinnularia are found mostly in acidic and freshwater pools in Kanglung region asily visible as the place where they reside is brownish yellow in color. Though many species of algae which is the ancestors to the higher plants are found in different places of Bhutan, it is left out without being studied.

4.8.3 *Surirella*

4.8.3.1 Habitat

Diatoms exist in two major forms, (i) benthic,non-planktonic, bottom loving and attached to some substratum (ii) planktonic which can float from one place to other Morris (1988). In Kanglung area, species could be found in the water bodies with optimum temperature of 22° C to 25° C and pH value of 6.7 to 8 specifically during late summer season.

4.8.3.2 Description

Class: Bacillariophyceae
Order: Pennales
Sub-order: Biraphidineae
Family: Surirellaceae
Genus: *Surirella*
As per Fritsch (1935a).

Member of Bacillariophyceae, commonly called diatoms, are found commonly in all kinds of fresh waters, sea waters as well as in air or on the soil and other terrestrial conditions. Diatoms are represented by approximately 200genera and 6000 species (Bold & Wynne, 1978).
The diatom cells remain covered by a siliceous wall, called frustules. The wall consist of two overlapping and perforated halves, called epitheca and hypotheca. Epitheca remains fitted over the hypotheca as a lid over the box, two overlapping halves called valves (**?**). The valves remain joined with the connecting bands or cingula. Two connecting bands together form a girdle. In Pennales, the walls are bilaterally symmetrical. Diatoms reproduce vegetatively by cell division and sexually by the production of auxospores which is actually a *restorative process* (**?**).

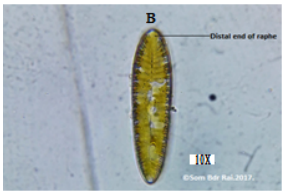
(a) *Surirella* sp. collection site

(b) *Surirella* sp.

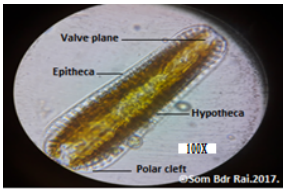
Figure 4.34: *Surirella* sp.habitat and showing theca

4.8.3.3 Observation

Diatoms are without doubt among the most beautiful of microscopic objects because of the wonderful sculpturing of their cell walls (Fritsch, 1935a). Diatoms are cosmopolitan and ubiquitous in distribution, occurring commonly in nearly all types of fresh waters, sea waters, or within the soil and other terrestrial conditions. They also occur as epiphytes on other algae and higher plants. A large proportion of the bottom flora of lakes, ponds and other similar aquatic surroundings is being constituted by diatoms. Diatoms forms major part of planktonic vegetation.
Diatoms reproduce vegetatively by cell division and sexually by the production of auxospores formation (**?**). The process of auxospore formation is actually a *restorative process* because there is reduction in original size of the cells, during the process of cells division (μ), is restored during this process. Sexual reproduction in diatoms is influenced by various factors including temperature, light condition and nutrition.



(a) *Surirella* sp. valve view



(b) *Surirella* sp. girdle view

Figure 4.35: *Surirella* sp.valve view and girdle view.

4.8.3.4 Conclusion

The important thing in this project was to show clear micrographs of *Surirella* species with some of the distinct characters like view of raphe, central nodule, girdle and polar nodule region. This short study has helped me to know the place and the quality of water require for diatom species to grow. This study on *Surirella* species may be useful reference to interested students. Constructive feedback from the readers, for the improvement for possible future edition of this study, shall be gratefully acknowledged.

CHAPTER 5

MACROFUNGI

They are fungi that grow in grassland ecosystems degrade plant cellulose to access carbon for growth. Unlike most species of *Amanita*, which are mycorrhizal, *Amanita thiersii* is a saprobic decomposers of dead grassroots, and is not found in woods or in association with trees. *Lactarius piperatus* are terrestrial mushrooms and it is usually found scattered or in groups. In Kanglung region it is found to be grown in the soil containing dead remains of tree leaves. It is mostly found growing scattered in the oak tree forest. It was collected on Friday 0ct-27-2017 from the oak forest located near the Shenangkhar village, Kanglung gewog around 12:30pm on fine sunny day. Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Ut purus elit, vestibulum ut, placerat ac, adipiscing vitae, felis. Curabitur dictum gravida mauris. Nam arcu libero, nonummy eget, consectetuer id, vulputate a, magna. Donec vehicula augue eu neque. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Mauris ut leo. Cras viverra metus rhoncus sem. Nulla et lectus vestibulum urna fringilla ultrices. Phasellus eu tellus sit amet tortor gravida placerat. Integer sapien est, iaculis in, pretium quis, viverra ac, nunc. Praesent eget sem vel leo ultrices bibendum. Aenean faucibus. Morbi dolor nulla, malesuada eu, pulvinar at, mollis ac, nulla. Curabitur auctor semper nulla. Donec varius orci eget risus. Duis nibh mi, congue eu, accumsan eleifend, sagittis quis, diam. Duis eget orci sit amet orci dignissim rutrum.

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5.1 Objectives

i To collect macrofungi from Kanglung.
ii To identify **wild edible macrofungi**.
iii To describe its habitat and characteristics.
iv To collect high quality images.

5.1.1 *Lactarius*

5.1.1.1 Habitat and Edibility

This is an edible mushroom and probably it can be of three types (*Gyeltshen, personal communication, September 9, 2017*) and generally considered edible especially after cooking. *L. pallidus* has been described by some mycologist as inedible.

5.1.1.2 Description

Class: Basidiomycetes
Order: Agaricales
Family: Russulaceae
Genus: *Lactarius* sp.
As per (Alexopoulos et al., 1996; Alexopoulus et al., 2002). All parts of the mushroom, particularly the gills, are gener-

ally brittle. The stalks are usually short and thick, and caps are often coloured, sometimes brilliantly. The sporophores of lactarius exude a watery or milky juice if cut fresh. The cap often exceeds 15 cm in diameter. With age its edges turn up and the mushroom resembles the shape of funnel (Alexopoulos et al., 1996) The color of spores in *Russula* can vary substantially (**?**). The spores leave a spore print that is pale ochre with a slight salmon tinge (**?**). The pale colour, incurved margin and smooth cap are its most distinguishing features (**?**). The cap often exceeds 15 cm in diameter (Vashishta, 1995). Cap of well develop *Lactarius piperatus* is 5-15 cm in diameter which is convex in shape when it is small. Later becomes flat with a shallow central depression, smooth or slightly wrinkled. Sometimes cap will be yellowish tan with even margin . Gills are very narrowly crowded and are attached to the stem. Stem is cylindrical in shape with white in colour becoming pale cream, more or less equal or tapering somewhat to the base. Stem is smooth and solid in nature. Length and size of stem will vary depending on its growing stage. Normally it is 2-8 cm long and up to 2cm wide (Mata & Pradhan, Mata & Pradhan).

(a) Ventral view of *Lactarius* sp.

(b) *Lactarius* sp.

Figure 5.1: *Lactarius* sp. habitat.

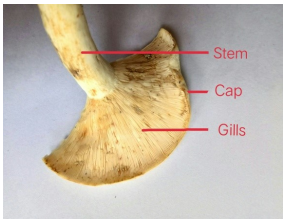
5.1.1.3 Observation

Lactarius piperatus has dull dry white cap and much crowed narrow gills. It has extremely peppery taste and it contains profuse white milk (Mata & Pradhan, Mata & Pradhan). *Lactarius piperatus* is considered edible. Locally it is known as `Nu bamoong`. This mushroom was collected from Kanglung forest, where the vegetation is dominated by growth of pine trees. It is typically half-buried among leaf litter. It can be sometimes found in groups (**?**), and occurs throughout summer and autumn (**?**). The stalks are usually short and thick, and caps are often coloured, sometimes brilliantly. The sporophores of lactarius exude a watery or milky juice if cut fresh.

5.1.1.4 Results

Lactarius spp. (milkcaps) produce a milky latex from their gills when damaged and contain lots of spherical cells called sphaerocysts which makes their texture less pliable and more fragile than other mushrooms (http://www.first-nature.com/fungi/ russulaceae.php.).

(a) Ventral view of *Lactarius* sp.



(b) *Lactarius* sp.

Figure 5.2: Cross-section of *Lactarius* sp.

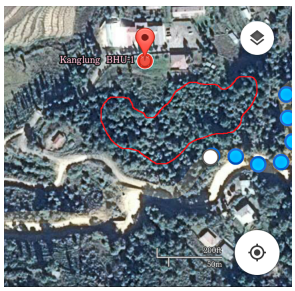
5.1.1.5 Conclusion

Lactarius piperatus growing in soil containing dead remains of tree leaves in the forest above Draktsho in Kanglung region. *Lactarius* belongs to Russulaceae family under Basidiomycetes. Though generally edible especially after cooking, the peppery taste characteristic of this species disappears when the mushroom is cooked. his research might help future researcher/s to get some basic knowledge of *Lactarius* sp. and *Russula* sp . quite similar to former, might create enthusiasm in exploring more information about this particular mushroom.

5.1.2 *Russula*

5.1.2.1 Habitat and distribution

The mushroom was found growing mycorrhizal with hardwoods where there are masses of leaves partially decayed. The mushroom was found growing in group on the forest floor. *Russula* belongs to order Agaricales and family Russulaceae. In kanglung *Russula brevipes* are seen growing on half decayed leaves.



(a) Ventral view of *Russula* sp.

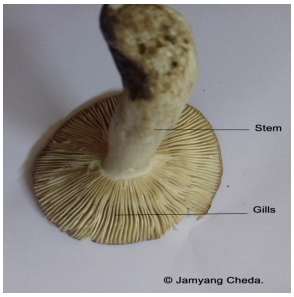
(b) *Russula* sp.

Figure 5.3: *Russula* sp. habitat and collection site

5.1.2.2 General characteristics

All the parts of the mushroom, particularly the gills are generally very brittle. The stalks are usually short and thick and the caps often colored, sometimes brilliantly Alexopoulos et al. (1996). Cap is whitish to dull yellow and funnel-shaped with depression in central `https://en.m.wikipedia.org/wiki/Russula-brevipes`.

(a) Ventral view of *Russula* sp.



(b) *Russula* sp.

Figure 5.4: *Russula* sp.

5.1.2.3 Key characteristics

Cap is convex with a central depression. Gills are attached to the stem and crowded. The young mushroom have whitish cap while some old aged have discoloring brownish Photographs showing the habitat of Russula, growing on masses of decayed leaves. The peculiar character of this mushroom is that they have very short stem and they are therefore called as short-stemmed mushroom (3-5cm long). Odor is faintly fragrant (Source: *Russula brevips*). The flesh is white and changes to discoloring brownish when kept for long time (2-3 days).

5.1.3 *Macrolepiota*

5.1.3.1 Habitat and edibility

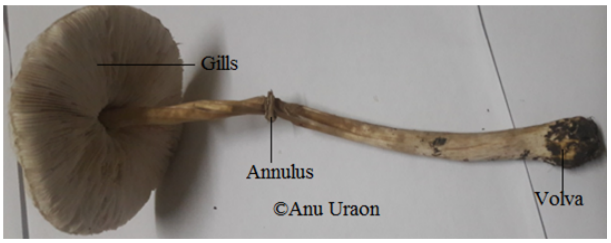
Macrolepiota procera are saprobic. They are mostly found in woodland clearings and in grassy areas next to woodland. They are found alone or in small scattered groups. They are usually found between July to November (http://www.first-nature.com/fungi/macrolepiota-procera.php).

5.1.3.2 Description

Division: Amastigomycota
Sub-division: Basidomycotina
Class: Basidomycetes
Order: Agaricales
Family: Agaricaceae
Genus: *Macrolepiota*
Species: *Macrolepiota procera*
(As per Alexopoulos et al. (1996)).
The cap of *Macrolepiota procera* expends until it is flat with a small central bump called an umbo. The flesh is white and does not change when cut. Initially the cap is spherical and pale brown with a darker brown area near the crown that breaks into scales. At maturity, the cap diameter ranges between 10-25 cm. The gills are white or pale cream and free, terminating some distance from the stipe. The stem consists of large double edged ring called annulus. The stem is smooth and white or cream but it is decorated with small brown scales that gives snakeskin appearance (when matured). Inside the stem, white fibrous flesh is loosely packed (http://www.first-nature.com/fungi/macrolepiota-procera.php).

(a) Ventral view of *Russula* sp.

(b) *Russula* sp.



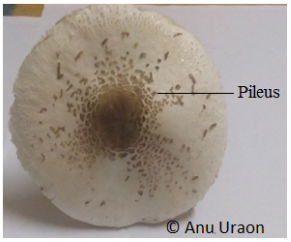
(c) *Russula* sp.

Figure 5.5: *Macrolepiota* sp.

5.1.3.3 Observation

The parts of *Macrolepiota procera* that can be easily notice are pileus, ring like structure called annulus, small central bump called an umbo and the stipe. It is found singly in woodland clearings and grassy areas. It is brightly colored.

(a) Ventral view of *Macrolepiota* sp.



(b) *Macrolepiota* sp.

Figure 5.6: *Macrolepiota* showing gills and bulbous base.

Macrolepiota procera have 19 elements contents and bio concentration potential in fruiting bodies. The cap of this mushroom are unique in taste and can contain a spectrum of important and dangerous mineral compounds (https://www.ncbi.nlm.nih.gov/pubmed/21877977).

5.1.3.4 Conclusion

Macrolepiota ocreata is inedible and poisonous, so it is not recommended to be collected and consumed. This project will help the researchers to gain more idea about inedible mushroom and particularly about the *Macrolepiota* species.Edibilty unknown in Kanglung, Bhutan.

5.1.4 *Auricularia*

5.1.4.1 Habitat and edibility

Auricularia auricula is a saprophyte. It grows on the dead branches of trees, on the main trunk and on decaying logs. It occurs during the monsoon period under humid condition and in wet evergreen forests. *Auricularia* is an edible species (**?**).

5.1.4.2 Description

Class: Basidiomycetes
Order: Auriculariales
Family: Auriculariaceae
Genus: *Auricularia auricula*
As per Alexopoulos et al. (1996); Alexopoulus et al. (2002). The fruit body of *Auricularia auricula* is about 5 to 8 cm; cup shaped; gelatinous, elastic and attached to the substrata by the back surface of the cup. The outer surface is bright reddish to purple color; lower surface is smooth and lighter in color than the upper surface with irregular vein and wrinkles **?**. It has a tough, gelatinous, elastic texture when fresh, but it dries hard and brittle. *Auricularia* grows attached laterally to the wood and has no stem. It also has many medicinal properties Mata & Pradhan (Mata & Pradhan). Locally this mushroom is named as *Bjili Namcho*.

(a) Ventral view of *Auricularia* sp.

(b) *Auricularia* sp.

Figure 5.7: *Auricularia* sp. habitat and bulbous base.

5.1.4.3 Observation

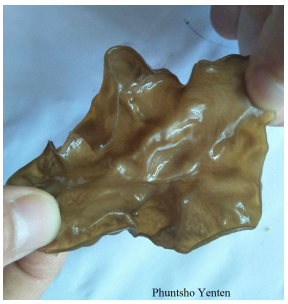
Auricularia auricula is attached to the substrata by the back surface of the cap. The fruiting bodies occur in groups; attached laterally to the substratum **?**.

5.1.4.4 Results

(a) Ventral view of *Auricularia* sp.

(b) *Auricularia* sp.

Figure 5.8: *Auricularia* sp. irregular and wrinkled surface



(a) Ventral view of *Auricularia* sp.

(b) *Auricularia* sp.

Figure 5.9: *Auricularia* sp. wrinkled surface.

5.1.4.5 Conclusion

Auricularia auricula does not have taste, but provides an enjoyable consistency and is able to absorb and concentrate tastes of species and other flavours in the food. his study could be a helpful resource for the interested students to know about *Auricularia auricula* and their habitat, occurrence and edibility. It can be a short reference for future study on macrofungi.

5.1.5 Coprinopsis

5.1.5.1 Habitat and edibility

Coprinopsis ocreata occurs later in the year. They are mostly found solitary and under coast liveoak. It is grown in deciduous and coniferous forest. This mushroom is inedible as it contains highly toxic amatoxins, as well as phallotoxins (www.mykoweb.com>CAF>species>Coprinopsis)

5.1.5.2 Description

Division: Amastigomycota
Sub-division: Basidomycotina
Class: Basidomycetes
Order: Agaricales
Family: Amanitaceae
Genus: *Coprinopsis ocreata*

(As per Alexopoulos et al. (1996))

The cap is usually 5-15 cm wide and hemispherical. It flattens with age. The colour of the cap is white with brownish centre and often it is paler towards the margin. The remains of the partial veil are seen as a floppy annulus (ring) which is usually about 1-1.5 cm below the cap. The crowded white gills are free. The stipe is white and is 8-15 cm long with a swollen sac like base called volva. The stipe bears a thin white membranous ring. The spore print is white. The transparent spores are globular to egg shaped. The ecology is mycorrhizal (http://en.m.wikipedia.org>wiki>Coprinopsis)

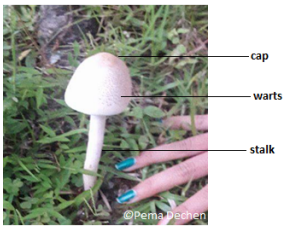


fig1. *Coprinopsis acuminata*

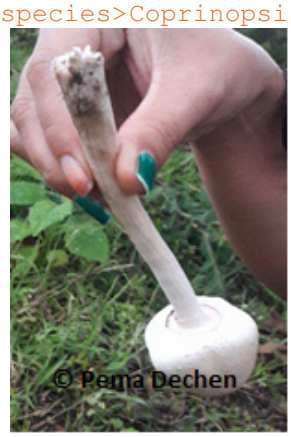
(a) Ventral view of *Auricularia* sp.

(b) *Auricularia* sp.

Figure 5.10: *Coprinopsis* sp. habitat.

5.1.5.3 Observation

The parts of *Coprinopsis ocreata* that can be easily notice are pileus, ring like structure called annulus and the stipe. It is found singly in grass lawn and it is brightly colored. *Coprinopsis ocreata* is highly toxic as it contains highly toxic amatoxins and phallotoxins. Amatoxins consist of at least eight components with a similar structure that of eight amino acid rings. The liver is the principle organ affected, as it is the first organ encountered after absorption by the gastrointestinal tract (www.mykoweb.com>CAF>species>Coprinopsis)



(a) Ventral view of *Auricularia* sp.

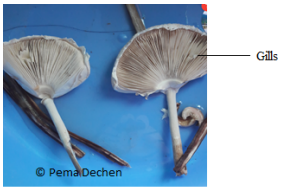


fig3. TS of *Coprinopsis acuminata*

(b) *Auricularia* sp.

Figure 5.11: *Coprinopsis sp.* sp.cross section.

5.1.5.4 Conclusion

Coprinopsis ocreata is inedible and poisonous, so it is not recommended to be collected and consumed. This project will help the researchers to gain more idea about inedible mushroom and particularly about the *Coprinopsis* species.

5.1.6 Strobilomyces

5.1.6.1 Habitat and edibility

Strobilomyces is found as solitary mainly in coniferous and deciduous forests in terrestrial areas. This is an inedible mushroom and if consumed it will lead to certain disorder like vomiting, nausea, headache and to certain extend it is fatal http://blog.crazyaboutmushrooms.com/old-man-of-the-woods-mushroom/.

5.1.7 Description

Phylum: Basidiomycota
Class: Basidiomycetes
Order: Agaricales
Family: Boletaceae
Genus: *Strobilomyces*

As per (Alexopoulos et al., 1996; Alexopoulus et al., 2002).

The fruit body is pileus and stipe and also contains the universal veil (Alexopoulos et al., 1996). It's pileus has the width of 50-160 mm. It is convex, scaly, dry and dull (Alexopoulos et al., 1996) and http://www.fungusfactfriday.com/055-strobilomyces-floccopus/. Stipe is up to 14 cm long and 2 cm thick. The context of the mushroom is fleshy, soft and fragile with rough stem. The spore deposit is brownish to black. The spore globose are ornamented with crests, wings, without germ pore and ranges from 9-15 times 8-12 μ M.

(a) Ventral view of *Strobilomyces* sp.

(b) *Strobilomyces* sp.

Figure 5.12: *Strobilomyces* sp. habitat.

5.1.7.1 Observation

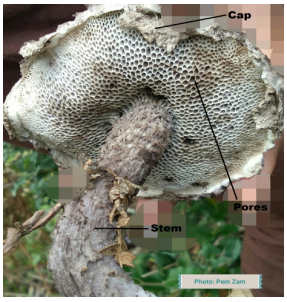
Strobilomyces is initially white in color but turns reddish brown and then slowly darkens into black after cutting and exposing it in the air http://www.mushroomexpert.com/strobilomyces.

html and http://www.fungusfactfriday.com/055-strobilomyces-floccopus/.
It has pores instead of gills and the cap is directly attached to stem(Alexopoulos et al., 1996) Convex when young but flattens during maturation (McKnight & McKnight, 1998).

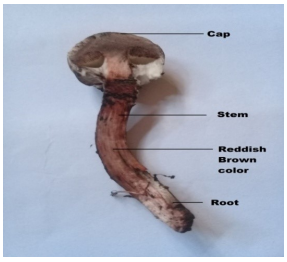
(a) Ventral view of *Strobilomyces* sp.

(b) *Strobilomyces* sp.

Figure 5.13: *Strobilomyces* sp. Dorsal view.



(a) Ventral view of *Strobilomyces* sp.



(b) *Strobilomyces* sp.

Figure 5.14: Underside view of *Strobilomyces* sp.with white pores and cross section.

5.1.7.2 Conclusion

It is an inedible mushroom and can be fatal if consumed. *Strobilomyces* is rarely found in Kanglung between July to mid September month. This specimen was collected from nearby Kanglung BHU area. This project would greatly help the farmers and students to identify *Strobilomyces* sp. as inedible macrofungus and it would also help the people to know about its poisonous nature and the precautions.

5.1.8 *Trichoglossum*

5.1.8.1 Habitat and edibility

Solitary to gregarious. Terrestrial on among moss and on rotting wood or leaf litter (Mata & Pradhan, Mata & Pradhan). It is inedible.

5.1.8.2 Description

Class: Geoglossomycetes
Order: Geoglossales
Family: Orbiliaceae
Genus:*Trichoglossum*
Species: *Trichoglossum hirstum*
As per (Alexopoulos et al., 1996; Alexopoulus et al., 2002).
Vacate and 4-7cm long. Head is oblong, flattened, often groveed, 1-3cm long and up to 2cm wide. Stem 3-8cm long and up to 3mm thick and round, equal and conspicuously pubescent (Mata & Pradhan, Mata & Pradhan; **?**). The asci each have 8 spores and the flesh is thin, tough and brownish. The paraphyses are brown, cylindrical and coiled at the tips. Spores are long and thon, rod-like with 13 to 15 septa. These are commonly called black earth tongue (http://www.leifgoodwin.co.uk/Fungi/Trichoglossum%20hirsutum.html). Setae abundant, acuminate ,dark brown, projecting one quarter to one third of their length above hymenium. Apospore arranged in parallel, nearly cylindrical but often gradually tapering towards both ends (http://fungi.myspecies.info/all-fungi/trichoglossum-hirsutum).

(a) Ventral view of *Trichoglossum* sp.

(b) *Trichoglossum* sp.

Figure 5.15: *Trichoglossum* sp. habitat.

5.1.8.3 Observation

Fruit bodies are black and paraphyses are slender, septate, with slightly swollen apical cells that are usually curved. It looks like a lollipop and these are commonly called Black Earth Tongue (http://fungus.org.uk/nwfg/earth-tongues.htm#4).

(a) Ventral view of *Trichoglossum* sp.

(b) *Trichoglossum* sp.

Figure 5.16: *Trichoglossum* sp. habitat.

(a) Ventral view of *Trichoglossum* sp.

(b) *Trichoglossum* sp.

Figure 5.17: textitTrichoglossum sp. of different sizes and labeled parts.

5.1.8.4 Conclusion

Trichoglossum hirstum have tiny hairs known as setae on spore bearing surface. It is endible and local people here do not eat this fungus. This project may be used as short reference to learn about *Trichoglossum hirstum* found in Kanglung region and help the students also to identify the characteristics and habitat of *Trichoglossum hirstum*.

5.1.9 *Suillus*

5.1.9.1 Habitat and edibility

Terrestrial under pine scattered to gregarious and *Suillus spraguei is edible* Mata & Pradhan (Mata & Pradhan).

5.1.9.2 Description

Class: Agaricomycetes
Order: Agaricales
Family: Boletaceae
Genus: *Suillus spraguei*
As per Alexopoulos et al. (1996); Alexopoulus et al. (2002). Cap: 5-10 cm in diameter, cone-shaped or convex becoming flat, with an incurved margin often hung with veil remnants, red to reddish yellow with coarse scales, surface dry and sometimes viscid in wet young specimens. Hymenophore: Tubes adnate to decurrent and bright yellow. Stem: 5-10cm long and up to 2cm wide, solid, sometimes wider at the bottom yellow at the top above the ring, scaly and patchy below similar to the cap. White veil with pink patches, delicate, fibrous, leaving a dull colored ring on the stem. Flesh: downy yellow changing to dull pink or reddish if bruised. Spore Print: Olive brown Mata & Pradhan (Mata & Pradhan). In Kanglung locality it is popularly known as **DhonBamong**(Gyeltshen, personal communication, September 09, 2017).

(a) Ventral view of *Suillus* sp.

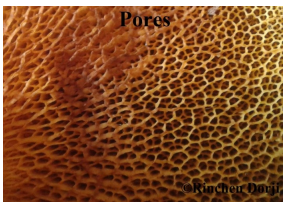
(b) *Suillus* sp.

Figure 5.18: *Suillus* sp. habitat.

5.1.9.3 Observation

Suillus auricule-judae is attached to the substrata by the back surface of the cap. The fruiting bodies occur in groups; attached laterally to the substratum **?**.

(a) Ventral view of *Suillus* sp.



(b) *Suillus* sp.

Figure 5.19: *Suillus* sp. showing pores.

5.1.9.4 Conclusion

Suillus spraguei can often be found in large quantities in pine woods where it is the frequently the dominant species. *Suillus spraguei* starts decaying before it is fully matures. Though it has bright red color which usually fades away on maturity Mata & Pradhan (Mata & Pradhan). This study could be a helpful resource for the interested students to know about edible mushrooms within the Kanglung locality and also to publish poster on edible wild mushroom.

5.1.10 *Hydropus*

5.1.10.1 Habitat and edibility

Hydropus nigrita occurs later in the year. They are mostly found solitary and under coast liveoak. It is grown in deciduous and coniferous forest. This mushroom is inedible as it contains highly toxic amatoxins, as well as phallotoxins (www.mykoweb.com>CAF>species>Hydropus)

5.1.10.2 Description

Division: Amastigomycota
Sub-division: Basidomycotina
Class: Basidomycetes
Order: Agaricales
Family: Amanitaceae
Genus: *Hydropus nigrita*
(As per Alexopoulos et al. (1996))

The cap is usually 5-15 cm wide and hemispherical. It flattens with age. The colour of the cap is white with brownish centre and often it is paler towards the margin. The remains of the partial veil are seen as a floppy annulus (ring) which is usually about 1-1.5 cm below the cap. The crowded white gills are free. The stipe is white and is 8-15 cm long with a swollen sac like base called volva. The stipe bears a thin white membranous ring. The spore print is white. The transparent spores are globular to egg shaped. The ecology is mycorrhizal (http://en.m.wikipedia.org>wiki>Hydropus)

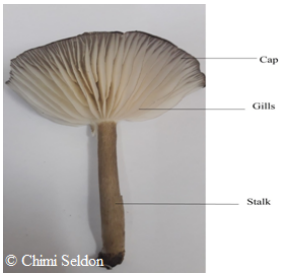
(a) Ventral view of *Hydropus* sp.

(b) *Hydropus* sp.

Figure 5.20: *Hydropus* sp. habitat.

5.1.10.3 Observation

The parts of *Hydropus nigrita* that can be easily notice are pileus, ring like structure called annulus and the stipe. It is found singly in grass lawn and it is brightly colored. *Hydropus nigrita* is highly toxic as it contains highly toxic amatoxins and phallotoxins. Amatoxins consist of at least eight components with a similar structure that of eight amino acid rings. The liver is the principle organ affected, as it is the first organ encountered after absorption by the gastrointestinal tract (www.mykoweb.com>CAF>species>Hydropus)



(a) Ventral view of *Hydropus* sp.

(b) *Hydropus* sp.

Figure 5.21: *Hydropus* sp.cross section.

Hydropus nigrita is inedible and poisonous, so it is not recommended to be collected and consumed. This little contribution of mine maybe helpful to future students to get some basic knowledge of *Hydropus nigrita*. People in the future who like to contribute on this project will be highly encouraged.

5.1.11 *Hygrophorus*

5.1.11.1 Habitat and edibility

Hygrophorus olivaceoalbus creates mycorrhizae with conifers and pine. It usually favors acidic and chalky ground with mosses in higher altitudes as well as conifer forests and occasionally in mixed forests and it has a good food value.

5.1.11.2 Description

Phylum: Basidiomycota
Order: Agaricales
Family: Hygrophoreceae
Genus: *Hygrophorus*

Elias Fries in 1815 (wikipedia, 2017) As per (Alexopoulos et al., 1996; Alexopoulus et al., 2002).

Hygrophorus are fleshy and sometimes tough umbrella like sporophores that bear holobasidia of the chaistobasidial type on the surface of the gills that hang down from the cap. (Aexopoulos, 1907-1906) Cap: 3-12 cm, convex when young, becomes broadly convex or more or less flat; sticky when fresh with a streaked appearance from stretched-out fibers beneath the slime. It is dark brown to gray-brown little lighter towards the margin and the margin is somewhat in rolled when young. Gills: Attached to the stem or running down it, distant or nearly. Stem: 3-10 cm long; up to 1 cm thick; equal or with a somewhat tapered apex. It is sheathed with slime over the lower portion when fresh. The apex is white colored covered below the slime with brown fibers that stretch out as the mushroom grows and often become disposed as vaguely concentric stripes or bands by maturity, often with a fragile and imperfect or somewhat gelatinized ring; basal mycelium white (http://www.mushroomexpert.com/hygrophorus_olivaceoalbus.html).

(a) Ventral view of *Hygrophorus* sp.

(b) *Hygrophorus* sp.

Figure 5.22: *Hygrophorus* sp. habitat.

5.1.11.3 Observation

The fungus typically fruits between late summer and early winter, and occasionally (depending on the geographical location and climate) as early as in June or in December. The fruit bodies are often found singly, but sometimes may also grow in clusters.

(a) Ventral view of *Hygrophorus* sp.

(b) *Hygrophorus* sp.

Figure 5.23: *Hygrophorus* sp. showing size and different view.

5.1.11.4 Conclusion

Hygrophorus is collected from Kanglung locality near lower Pangthang. This mushroom is an edible mushroom and has good food value. This project can be a short reference for future study on macrofungi. This also can be a study material to students working on more macrofungus from kanglung.

5.1.12 *Amanita*

5.1.12.1 Habitat and distribution

Amanita vaginata is a terrestrial mushroom, which is usually found solitary to gregarious. Moreover, in Kanglung region, it is usually found on land surface having decaying leaves (fertile soil, rich in manure). Specimen was collected on 29 October 2017 at 11:30 am.

(a) Ventral view of *Amanita* sp.

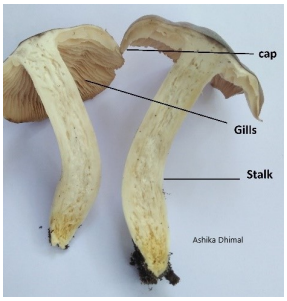
(b) *Amanita* sp.

Figure 5.24: *Amanita* sp. habitat.

5.1.12.2 General characteristics

The cap is grey or brown in color and slightly paler towards the margin. It is smooth and slightly sticky when moist with a diameter of 5-10 cm, ovoid at first and expanding to almost flat with a low umbo and a distinctly grooved margin (Mata & Pradhan, Mata & Pradhan). It has free gills which are arranged closely and are white to creamy in color. The stem is 5-10 cm long, slender, hollow and tapering towards the apex having white tinge with orange brown and very fine white hairs. The stem neither has ring nor basal bulb unlike other *Amanita* species (www.mushroomexpert.com/amanita_vaginata.html).

(a) Ventral view of *Amanita* sp.



(b) *Amanita* sp.

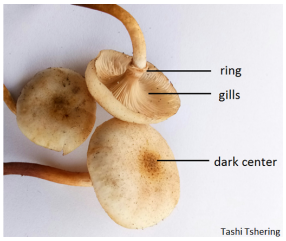
Figure 5.25: *Amanita* belongs to Amanitaceae family of Basidiomycetes

5.1.13 Armillaria

5.1.13.1 Habitat and distribution

This mushroom belongs to family Physalacriaceae and genus *Armillaria*. On 20th October, 2017 it was around 12:45 p.m. when I found this mushroom growing on the root of a cut oak tree in the forest near Shenangkhar, Kanglung. It was a sunny day, but few days before it was raining and the forest looked damp. This mushroom is usually pathogenic and parasitic on broad-leaf trees. It also occurs as saprobe on stumps and dead roots and even on fallen branches. Commonly found in deciduous, coniferous and mixed forest.

(a) Ventral view of *Armillaria* sp.



(b) *Armillaria* sp.

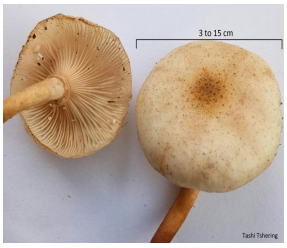
Figure 5.26: *Armillaria* habitat.

5.1.13.2 General characteristics

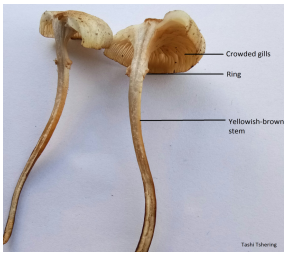
Wood-rotting gilled mushrooms with white spore prints and gills are attached to the stem. Partial veil present in most species, but the veil can manifest in different forms from cob-webby ring zones to full-blown rings. Parasitic and many form visible dark rhizomorphs in the wood (Armillaria).

5.1.13.3 Key characteristics

The basidiocarp of each has a smooth cap 3 to 15 cm in diameter. Caps are convex, but become flattened with age often with a central raised umbo. The cap is honey-yellow to red-brown in colour with a darker area near the centre. Flesh of the cap is white and firm. Its caps are flattened develop wavy, striate margins. On the young caps, fine scales are found more towards the centre. As the caps reach maturity, the scales do not remain evident. The weakly decurrent gills are crowded and flesh coloured. At maturity gills become yellowish and develop rusty spots. The basidia lack basal clamps (**?**). Stems are white while young, but turn yellowish-brown and finely woolly on maturity. The mushrooms are bundled together in rhizomorphs that are black in colour. Due to clustering growth, the stipe possesses tapering base, fairly tough, white ring usually features as a pastel yellow edge (Armillaria) .



(a) Ventral view of *Armillaria* sp.



(b) *Armillaria* sp.

Figure 5.27: *Armillaria* habitat.

(a) Ventral view of *Armillaria* sp.

(b) *Armillaria* sp.

Figure 5.28: *Armillaria* habitat.

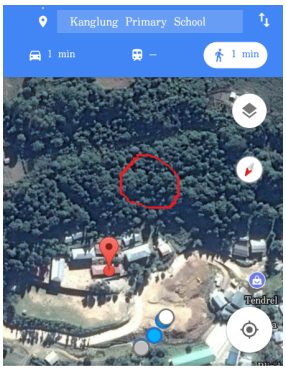
Edibility: Considered conditionally edible and it is often used for its culinary value. Raw fruiting bodies of this species are considered to be toxic. *A. mellea* contains compounds such as polysaccharides (glucans) and sesquiterpenes which exhibit anticancer properties and also help in treatment of diseases like hypertension, atherosclerosis, diabetes and obesity (**?**).

5.1.14 *Mycena*

5.1.14.1 Habitat and distribution

Mycena strobilinoides was seen buried under partially decayed leaves. The weather condition was partly sunny. Specimen was collected on 31st October, 2017 at around 2:05 pm from the forest located above Kanglung Primary School.

(a) Ventral view of *Mycena* sp.



(b) *Mycena* sp.

Figure 5.29: *Mycena* habitat.

5.1.14.2 General characteristics

The cap is small, round, or conic at first with margin incurved and expanding to bell-shaped or convex. The surface is smooth, moist, slippery with vivid reddish orange, fading to strong orange or orange-yellow. The gills are yellow to orange with reddish edge. The stalk is cylindrical, fragile with reddish orange in color and has a white spore print (McKnight & McKnight, 1998).

5.1.14.3 Key characteristics

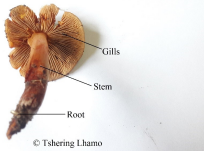
The cap is 2-5 cm wide with conical shaped when it is young and expands to bell-shaped or slightly flattens as it matures. The surface of the cap smooth, moist and slippery in nature. The gills are yellow to orange n color and are adnexed and crowded. The stem ranges from 6 cm to 11 cm long with reddish-orange color and are fragile and hollow inside (McKnight & McKnight, 1998).

(a) Habitat of *Mycena* sp.

(b) *Mycena* sp.

Figure 5.30: *Mycena* habitat.

(a) Dorsal view of *Mycena* sp.



(b) *Mycena* sp.

Figure 5.31: *Mycena strobilinoides*, pictures showing location, their habitat and their parts.

Edibility: *Mycena strobilinoides* can easily be recognized by its slippery nature and its attractive color and according to the villagers these are inedible and also as per http://identification.growing-mushrooms.com/isedible/mycena-strobilinoides-1107/ it was found inedible. This mushroom can be found growing on the moist and loose soil with partially decayed leaves. *Mycena strobilinoides* is an agaric fungus in the family mycenaceae 9 https://en.wikipedia.org/wiki/Mycena_strobilinoides.

5.1.15 *Coltricia*

5.1.15.1 Habitat and distribution

Coltricia perennis commonly known as the **Tiger's Eye** is found growing scattered or mostly in clusters or gregariously in the moist sandy soil. This specimen was found growing along roadside area in the coniferous forest located above BHU, Kanglung. The mushroom was collected on 26th of October, 2017 at around 2:00 pm.

(a) Dorsal view of *Coltricia* sp.

(b) *Coltricia* sp.

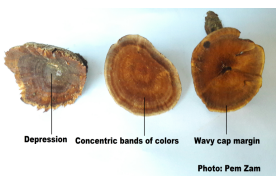
Figure 5.32: *Coltricia* habitat.

5.1.15.2 General characteristics

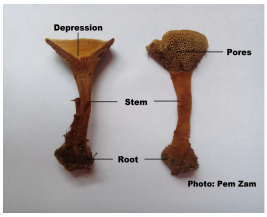
Coltricia belongs division Basidiomycota https://en.wikipedia.org/wiki/Coltricia. The cap is 3.3 to 3.8 cm in diameter. It has a flat to vase-shaped and silky-shiny cap when fresh with cinnamon brown color usually with concentric bands of colors having straight to thin margin, sometimes eroding with age http://www.mushroomexpert.com/coltricia_cinnamomea.html. The cap is also depressed in the centre or somewhat funnel-shaped **?**. The pores are circular to angular, 2-3 per mm; tubes 3 mm deep. Yellowish brown to cinnamon brown in color running down the stem. The stem is tough and dry with rusty brown color http://www.mushroomexpert.com/coltricia_cinnamomea.html ranging from 2.8 to 5 cm in height and 7 to 9 mm in thickness.

5.1.15.3 Key characteristics

In *Coltricia perennis*, the cap is cinnamon brown in color with wrinkled or wavy margins and is hard and rigid when dry. The cap is silky-shiny with concentric bands of colors http://www.mykoweb.com/CAF/species/Coltricia_perennis.html. This mushroom is considered as an inedible mushroom and are hard and tough in nature (**?**).



(a) Dorsal view of *Coltricia* sp.



(b) *Coltricia* sp.

Figure 5.33: *Coltricia* dorsal and ventral view.

Photographs showing the habitat of *Coltricia*, growing on on mossy soil along the road side abobe Kanglung BHU. It was found growing in a sandy soil (heath) especially on

the edges above BHU region. It belongs to the family Hymenochaetaceae and order Hymenochaetales (**?**).

5.1.16 Microporous

5.1.16.1 Habitat and distribution

Microporous xanthopus is a terrestrial mushroom which grows in group. It is found in the humid subtropical climate at the altitude between 1800m -1900m. It is found in Kanglung area near BHU. It is found in humid place on dead wood. It was collected on 26th October, 2017 at 1:15PM. The weather was sunny.

(a) Dorsal view of *Microporus* sp.

(b) *Microporus* sp.

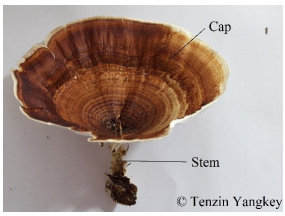
Figure 5.34: *Coltricia* dorsal and ventral view.

5.1.16.2 General characteristics

The fruiting body is a funnel shaped and are thin. They have various shades concentrically zoned at center. The shades are brown and they are wavy. The lower surface are white- to yellow dull color with lots of micropore on it. The cap is supported by the stem which is white to yellow dull in color (http://australianfungi.blogspot.com/2011/05/53-microporus-xanthopus.html). It is generally found on the dead wood, fallen branches and barks and are saprotrophic (https://www.fungimap.org.au/index.php/fduonline-home/115/294/polypores/P-microporus-xanthopus).

5.1.16.3 Key characteristics

The fruiting bodies have thin, funnel-shaped cap and are concentrically zoned in various shades of brown (australianfungi.blogspot.com/2011/05/53-microporus-xanthopus.html) with the diameter of 7.2 to 10.1 cm. The shades are eccentric or lateral with pileus (cap) lustrous, glabrous **?**. The cap holds water in it (australianfungi.blogspot.com/2011/05/53-microporus-xanthopus.html). The cap is supported by yellow foot stem which has the height of 2.1 to 2.6cm. The under surface has numerous tiny pores and thus it has genus name as *Microporus* (https://www.anbg.gov.au/fungi/case-studies/microporus-xanthopus-growth.html). It is inedible **?**.



(a) Dorsal view of *Microporus* sp.

(b) *Microporus* sp.

Figure 5.35: *Coltricia* dorsal and ventral view.

(a) Dorsal view of *Microporus* sp.

(b) *Microporus* sp.

Figure 5.36: *Coltricia* dorsal and ventral view.
Photographs showing the habitat of Microporus, growing on dead wood near to Kanglung BHU. Microporus belongs to class basidiomycetes under polyporales order.

5.1.17 *Craterellus*

5.1.17.1 Habitat and distribution

It grow mostly as mycorrhiza with hard wood and less with conifers. It is grown scattered, gregariously, or (usually) in tightly closed packed clusters, often mostly in mossy areas during summer and autumn http://www.mushroomexpert.com/craterellus_cornucopioides.html. In Kanglung area, it is found growing as closely packed clusters on the mossy soil.

(a) Dorsal view of *Craterellus* sp.

(b) *Craterellus* sp.

Figure 5.37: *Craterellus* habitat.

5.1.17.2 General characteristics

Fruiting body is 3-5 cm wide, 5-9 cm height; without a clearly defined stem and cap. Surface black to dark grey with hollow tubular stem.

5.1.17.3 Key characteristics

Fruiting body is without a clearly defined cap and stem: tubular at first, becoming deeply vase-shaped: upper edge rolled under when young and often partly rolled under in maturity;

thin fleshed http://www.mushroomexpert.com/craterellus_cornucopioides.html.

(a) Dorsal view of *Craterellus* sp.

(b) *Craterellus* sp.

Figure 5.38: *Craterellus* dorsal and ventral view.
Photographs showing the habitat of Craterellus, growing on on mossy soil.**Craterellus cornucopioides** showing upper dark/ grey surface, deeply depressed inner surface and hollow tubular stem.
Upper/inner surface is deeply depressed, scaly surface, not viscid, its colour appears to be black when it is wet and become dark grey when dried, wavy margin and sometimes torn Mata & Pradhan (Mata & Pradhan). *Craterellus* belongs to Cantharellaceae family under Basidiomycetes.

5.1.18 *Fomes*

5.1.18.1 Habitat and distribution

This mushroom is classified under family Polyporaceae under Basidiomycota and genus *Fomes*. It was collected on 22nd October, 2017 around 10:50 a.m. near DH-1 of Sherubtse College on a sunny morning. I found this mushroom growing on a fallen log of Oak tree. They are parasitic on dead/fallen hardwoods and persist all year round. Grows on broken barks and decomposes on the bark itself after they die.

(a) Dorsal view of *Fomes* sp.

(b) *Fomes* sp.

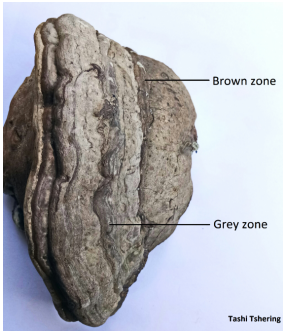
Figure 5.39: *Fomes* habitat.

5.1.18.2 General characteristics

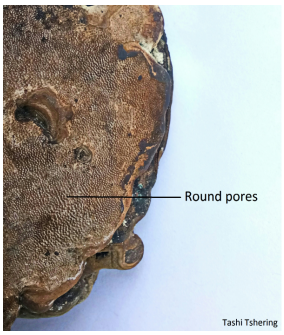
The fruiting body is 8-10 cm in diameter with the thickenss 2-8 cm Mata & Pradhan (Mata & Pradhan). This species produces large polypore fruiting bodies with silver grey to almost black, though they are normally brown. The fruiting body is attached to the substratum without stem.

5.1.18.3 Key characteristics

The upper surface is found grey and often brownish growing zones are seen at the outer edge. The fruiting body is hoof shaped with annual layers of grey and brown zones with distinct attachment lines. The lower pore surface is brownish with round pores 2-3 per mm. The flesh inside the fruiting body is pale brown and hard with numerous tubes. Tubes are pale grey-brown to dark brown in colour (http://www.first-nature.com/fungi/fomes-fomentarius.php).



(a) Dorsal view of *Fomes* sp.



(b) *Fomes* sp.

Figure 5.40: *Fomes* habitat.
Photographs showing the habitat of Fomes, growing on masses of decayed leaves.

5.1.19 *Coprinus*

5.1.19.1 Habitat and distribution

Coprinus comatus also known as the Shaggy ink cap was found growing in the open area located in the coniferous and deciduous forest above the highway going towards Samdrup Jhongkhar and the BHU in Kanglung. The specimen was collected on September 21st, 2017 at 5:03 pm exactly. The mushroom was completely hidden by the plants around it and was growing in an open coniferous forest. The soil was very loose and the location of the plant was in a dark area which was also dense and damp. The mushroom was solitary and very fragile.

(a) Dorsal view of *Coprinus* sp.

(b) *Coprinus* sp.

Figure 5.41: *Coprinus* dorsal and ventral view.

5.1.19.2 General characteristics

Cap of *Coprinus comatus* is approximately 3-15cm which can be oval or rounded during a young age and growing to

a bell shaped with a lifting margin along with large shaggy scales. As it ages, the mushroom becomes black. The mushroom is whitish with the center being brown. Their odor is not distinctive (http://www.mushroomexpert.com/coprinus_comatus.html). The gills are white which can be free as well as closed. Their stem is fibrous and hollow while their flesh being soft and white (http://mushroom-collecting.com/mushroomshaggy.html). The mushroom before it turns black is edible and has a mild taste (https://en.wikipedia.org/wiki/Coprinus_comatus).

5.1.19.3 Key characteristics

The mushroom is distinct due to its cylindrical cap covering half of the stem. It consists of shaggy scales which are brown at the apex. Black and brown spores are found and the flesh is white in colour (https://en.wikipedia.org/wiki/Coprinus_comatus).

(a) Dorsal view of *Coprinus* sp.

(b) *Coprinus* sp.

Figure 5.42: *Coprinus* dorsal and ventral view.

Photographs showing the habitat of *Coprinus comatus*, is found solitary and growing in an open forest on loose soil in a damp and dark environment. *Coprinus* belongs to Agaricaceae family under Basidiomycetes.

5.1.20 *Pycnoporus*

5.1.20.1 Habitat and distribution

Pycnoporus cinnabarinus is a terrestrial and lignicolous fungi, mostly found solitary or caespitose. In Kanglung, it occurs on dead and decaying deciduous hardwoods. Commonly called Cinnaber Bracket.

(a) Habitat of *Pycnoporus* sp.

(b) *Pycnoporus* sp.

Figure 5.43: *Pycnoporus* habitat.

5.1.20.2 General characteristics

The fruiting body is 5-13 cm wide and almost 1cm thick. The upper surface is reddish orange in color which fades to whitish with age along with wrinkles and warts. The lower surface is orange-red, less fading than cap with 2-4 pores per mm and has white spore print Mata & Pradhan (Mata & Pradhan).

5.1.20.3 Key characteristics

Attractive and distinct red in color. This fungus is thick and they have densely-packed pores. Grows mostly on decaying hardwoods.

(a) Habitat of *Pycnoporus* sp.

(b) *Pycnoporus* sp.

Figure 5.44: *Pycnoporus* views.

Pycnoporus commune growing in soil containing dead remains of tree leaves in the forest above Draktsho in Kanglung region; growing on dead logs.

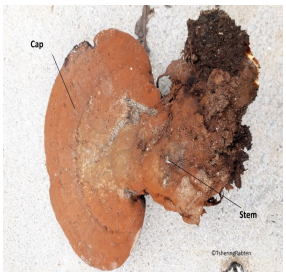
Edibility: It is inedible but in any case because of its rarity, it should not be collected [http://www.first-nature.com/fungi/pycnoporus-cinnabarinus.php].

5.1.21 *Gadoderma*

5.1.21.1 Habitat and distribution

This project will be useful for those students, who are interested to work more on bracket fungi. This fungus belongs to Ganodermataceae family under Basidiomycetes. The local name is shing namcho. The local name is shing namcho.

(a) Habitat of *Ganoderma* sp.



(b) *Ganoderma* sp.

Figure 5.45: *Ganoderma* habitat.

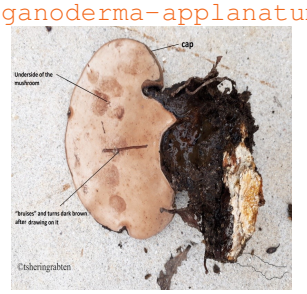
5.1.21.2 General characteristics

This fungus grows as a mycelium within the wood of living and dead trees Alexopoulos et al. (1996). It forms fruiting bodies that are up to 30-60 cm (12-24 in) across, hard as leather, woody-textured, and inedible in raw form. They are

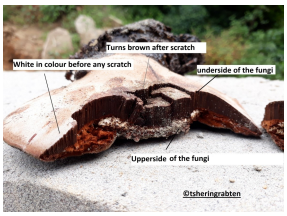
white at first but soon turn dark red-brown. Brown spores are released from the pores on the underside of the fruiting body. The fruiting bodies are perennial, and may persist for multiple years, increasing in size and forming new layers of pores as they grow.

5.1.21.3 Key characteristics

It grows out of fallen logs or wounds in trees and forms a shelf-like knob. The top of the cap is pale brown with a white margin that leads into the white underside. Before touched, the pore surface is a perfect white color. After it is touched, the mushroom **bruises** and turns dark brown very quickly, making it easy to draw on! Artists use this mushroom as a canvas to sketch beautiful illustrations on. http://blogs.evergreen.edu/fungalkingdom/ganoderma-applanatum-the-artists-conk/



(a) Habitat of *Ganoderma* sp.



(b) *Ganoderma* sp.

Figure 5.46: *Ganoderma* side view.

Photographs showing the habitat of *Russula*, growing on masses of decayed leaves. *Ganoderma applanatum* comes in all sorts of shapes and sizes, which can be about 8 inches long.It has no distinct cap, caphymenium is decurrent and lacks a stipe. Spore print is brown **?**.

5.1.22 *Clitocybe*

5.1.22.1 Habitat and distribution

Clitocybe (Lepista) nuda is a terrestrial mushroom, which is usually found growing in fairy rings. In Kanglung, it is usually found growing on substrate like decaying leaves, logs and under hedgerows during autumn and winter. Specimen was collected on 30th October 2017 at 1:30 pm.

(a) Dorsal view of *Lepista* sp.

(b) *Lepista* sp.

Figure 5.47: *Lepista* habitat.

5.1.22.2 General characteristics

It has violet tinge on the cap and retains a slightly enrolled margin, having diameter 6-15cm.Usually, old specimen develops wavy margin Mata & Pradhan (Mata & Pradhan). Gills are arranged closely, adnate to adnexed with purple or pale purple in color and turns pinkish buff or brownish with age. The stem is 5-10 cm long, and 1-2 cm wide which is almost equal and solid. Their flesh is thick, soft and purplish to liliac buff and has pale pinkish-buff spore print. Perhaps surprisingly, Wood Blewits (common name) can be used to dye fabrics or paper a grass green color rather than lilak, purple or blue. To make a green dye the fungi are chopped up and then boiled in water in an iron cooking pot (http://www.first-nature.com/fungi/Clitocybe-nuda.php).

(a) Dorsal view of *Lepista* sp.

(b) *Lepista* sp.

Figure 5.48: *Lepista* cross section.

-

5.1.22.3 Key characteristics

The violet-tinged cap and gills of the young wood blewit, together with its stocky build, distinguish it from other purple or lilac colored fungi Mata & Pradhan (Mata & Pradhan). It is considered edible but not sure and should not be confused with some of the larger purple species of *Cortinarius* fungi. *Cortinarius* fungi all have rusty brown spores, so it is fairly easy to distinguish them from *Clitocybe nuda* by making a spore print (http://www.first-nature.com/fungi/Clitocybe-nuda.php).
Photographs showing the habitat of *Clitocybe/Lepista*, growing on decaying leaves in below Sherubtse center catering mess.

5.1.23 *Oudemansiella*

5.1.23.1 Habitat and distribution

Oudemansiella radicata is found on the ground where it is being covered by decayed leaves and branches of the tree. It was collected from the forest just above the Kanglung Primary School, in the subtropical climate at the altitude of 1800m to 1900m. It was collected on 31st October, 2017 at 1:30 PM. The weather was sunny during

the collection of specimen. They are solitary mushroom.

(a) Habitat of *Oudemansiella* sp.

(b) *Oudemansiella* sp.

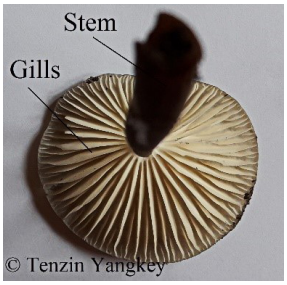
Figure 5.49: *Oudemansiella* habitat.

5.1.23.2 General characteristics

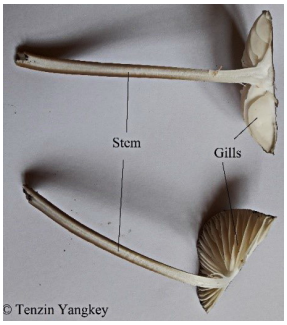
Fruiting body is 3-5 cm wide, *Oudemansiella radicata* is recognized by their tall and slender stature abundantly grown in summer and autumn where the other mushrooms are rare (https://unicornbags.com/cultivation/oudemansiella-radicata/). The fruiting body are broadly convex but flattening toward middle depression. They have light brown or greyish brown or yellowish brown. The stem is cylindrical, slender and tapering towards the apex. Gills are well spaced and short with white in color.

5.1.23.3 Key characteristics

The fruiting body is broadly convex that flattens towards the middle depression. They are in light brown or greyish brown or yellowish brown. It is radially wrinkled on the disc with margin or faintly striate. The flesh of the mushroom is thin and white in color. It has a diameter of 6.1 cm. The gills are slightly sparse with different length (https://unicornbags.com/cultivation/oudemansiella-radicata/). The stems are 12 cm long with 0.6 cm thick and are slender, brittle that extends down into the soil. The spores are white in color.



(a) Habitat of *Oudemansiella* sp.



(b) *Oudemansiella* sp.

Figure 5.50: *Oudemansiella* gills and cross section. Photographs showing the habitat of *Oudemansiella*, growing covered with decayed leaves and branches. The mushroom belongs class of Basidiomycetes which is edible as well as it is considered medicinal. It is also known for its secretion of oudamanism which shows the antifungal activities in pathogenic plant.

5.1.24 *Schizophyllum*

5.1.24.1 Habitat and distribution

Schizophyllum commune is found growing scattered or mostly in clustered as saprobic on dead wood or twigs in the forest above Draktsho. Sometimes it is also found as parasite on living trees http://www.mushroomexpert.com/schizophyllum_commune.html

(a) Habitat of *Schizophyllum* sp.

(b) *Schizophyllum* sp.

Figure 5.51: *Schizophyllum* habitat.

5.1.24.2 General characteristics

Fruiting body is 1-3 cm long and 1-4 cm wide depending on its age. It looks like the claws of cat with short finger like margin. Upper surface is with dirty white gray short hairs and appears like wool Mata & Pradhan (Mata & Pradhan). Under surface is composed of gill-like folds, split down in the middle which appears whitish to grayish, without a stem. Flesh is leathery, tough and pallid http://www.mushroomexpert.com/schizophyllum_commune.html.

5.1.24.3 Key characteristics

It has tiny fruiting bodies without stem and are attached themselves to decaying hard wood sticks and logs like tiny bracket fungi. Underside of *Schizophyllum commune* has gill like folds that are split down in middle rather than pores or simple flat surface unlike other bracket fungi. It is known as ***Aie zimpo*** in local language (Dzalakha).

(a) Habitat of *Schizophyllum* sp.

(b) *Schizophyllum* sp.

Figure 5.52: *Schizophyllum* dorsal and ventral view. *Schizophyllum commune* growing in soil containing dead remains of tree leaves in the forest above Draktsho in Kanglung region; growing on dead logs. **Edibility**: It is con-

sidered as edible and believe to have medicinal properties Mata & Pradhan (Mata & Pradhan). It belongs to family Schizophyllaceae under Basidiomycetes.

5.1.25 *Trametes*

5.1.25.1 Habitat and distribution

This mushroom can be found on most kinds of hardwood trees but most commonly on Beech, forming brackets on standing timber and more often rosettes on the tops of stumps [2]. In oak tree forest besides the Shinangkhar village, under Kanglung gewog Trametes gibbosa are found mostly growing on dead logs of oak. I have collected this mushroom specimen on 29/10/2017 at about 2:30 pm under the warm sunny day.

(a) Habitat of *Trametes* sp.

(b) *Trametes* sp.

Figure 5.53: *Trametes* habitat.

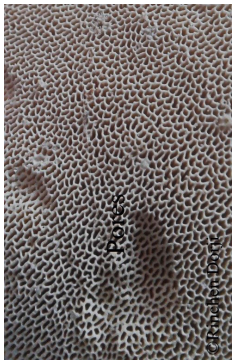
5.1.25.2 General characteristics

Trametes gibbosa, commonly known as lumpy bracket, is a polypore mushroom that causes white rot (https://en.m.wikipedia.org/wiki/Trametes_gibbosa). A fruit body of Trametes gibbosa is fleshy, typically it measures 5 to 20 cm across and roughly semicircular when growing as brackets but more or less circular when growing as a fan on the top of a stump. Brackets of *Trametes gibbosa* vary greatly in thickness but are usually between 1 and 6 cm thick (http://www.first-nature.com/fungi/trametes-gibbosa.php).

5.1.25.3 Key characteristics

Usually the dorsal surface of this mushroom is gray or white but due to aging we can see the green colour too because of the decomposition of the algae or growth of algae. Elongated pores are located on the ventral surface with spore print white to cream colour (https://en.m.wikipedia.org/wiki/Trametes_gibbosa).

(a) Habitat of *Trametes* sp.



(b) *Trametes* sp.

Figure 5.54: *Trametes* habitat and porous gills.

Photographs showing the habitat of *Trametes*, algal growth on the dorsal side of *Trametes gibbosa*. Although not generally reported as seriously poisonous, these bracket fungi are much too tough to be considered edible (http://www.first-nature.com/fungi/trametes-gibbosa.php).

5.1.26 *Russula*

5.1.26.1 Habitat and distribution

Russula emetica also known as the sickener is found growing terrestrial or solitary in the moist and damp soil with partially decayed leaves in the coniferous forest located near BHU, Kanglung. The mushroom was found growing singly, scattered and in groups in an environment with loose and humid soil. The mushroom was collected on 26th of October at around 1:30 pm and it was a sunny day.

(a) Habitat of *Russula* sp.

(b) *Russula* sp.

Figure 5.55: *Russula* habitat.

5.1.26.2 General characteristics

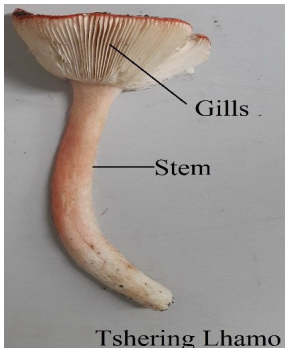
It is a basidiomycete mushroom which has a red, convex to flat cap and sometimes becoming slightly depressed when fully matured and it measures up to 8-8.5 cm in diameter, with a cuticle that can be peeled off almost tio the centre. Gills are white or pale cream in colour and are free or crowded. The stem is cylindrical, white in colour and it turns slightly yellow with its age. It measures up to 4-9 cm long and 0.7-3 cm in diameter. It has a faint fruity odour and white or pale cream spore print (http://www.first-nature.com/fungi/russula-emetica.php). It has a very

acrid or peppery hot taste (http://botit.botany.wisc.edu/toms_fungi/sep2004.html).

5.1.26.3 Key characteristics

Russula emetica, the cap is red or cherry red in colour with finely ridged margins and is convex to flattening and is slightly depressed in the middle, with white stem and closed gills

(a) Habitat of *Russula* sp.



(b) *Russula* sp.

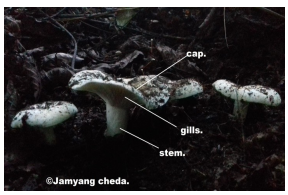
Figure 5.56: *Russula* habitat.
Photographs showing the habitat of *Russula emetica* and their parts. *Russula emetica* belongs to order Agaricales and family Russulaceae Alexopoulos et al. (1996) This is a poisonous mushroom and if consumed it causes gastrointestinal distress like vomiting and abdominal cramps. It is found growing in damp soil with partially decayed leaves of pine trees in Kanglung region.

5.1.27 *Russula*

5.1.27.1 Habitat and distribution

The mushroom was found growing mycorrhizal with hardwoods where there are masses of leaves partially decayed. The mushroom was found growing in group on the forest floor. *Russula* belongs to order Agaricales and family Russulaceae. In kanglung *Russula brevipes* are seen growing on half decayed leaves.

(a) Habitat of *Russula* sp.



(b) *Russula* sp.

Figure 5.57: *Russula* habitat.

5.1.27.2 General characteristics

All the parts of the mushroom, particularly the gills are generally very brittle. The stalks are usually short and thick and the caps often colored, sometimes brilliantly Alexopoulos et al. (1996). Cap is whitish to dull yellow and funnel-shaped with depression in central https://en.m.wikipedia.org/wiki/Russula-brevipes.

5.1.27.3 Key characteristics

Cap is convex with a central depression. Gills are attached to the stem and crowded. The young mushroom have whitish cap while some old aged have discoloring brownish http://www.mushroomexpert.com/russula_brevipes.html.

(a) Habitat of *Russula* sp.

(b) *Russula* sp.

Figure 5.58: *Russula* habitat.
Photographs showing the habitat of *Russula*, growing on masses of decayed leaves. The peculiar character of this mushroom is that they have very short stem and they are therefore called as short-stemmed mushroom (3-5cm long). Odor is faintly fragrant. The flesh is white and changes to discoloring brownish when kept for long time (2-3 days) http://www.mushroomexpert.com/russula_brevipes.html.

5.1.28 *Russula nigricans*

5.1.28.1 Habitat and distribution

The specimen *Russula nigricans* was collected from below the Kanglung BHU on 29th October 2017 at 1:55pm. It was growing in a damp soil, where there was remains of dry leaves and branches. The small fresh and young mushrooms were found growing on the mother mushroom, which are white and soft. The mother fungus was very large and was brownish dark, with black patches. The wide spread gills and the growth of new ones inside the cup shaped cap made it easier to identify. The place where it was found was humid and shady area where sunlight could hardly reach the surface.

(a) Habitat of *Russula* sp.

(b) *Russula* sp.

Figure 5.59: *Russula* cross section.

5.1.28.2 General characteristics

The widely spread adnate gills of *Russula nigricans* are thick and extremely brittle; they are interspersed with many shorter gills (known as lamellae.) Stems are 1 to 4cm in in diameter and 3 to 8 cm tall. The smooth blackening stems are cylindrical or taper in slightly towards the base. The stem flesh is white at first, blackening with age; reddening and then blackening when bruised. There is no stem ring. **Spore**: Ellisoidal or ovoid; 7-8 x 6-7 micron Warts to typically 0.3 micron tall, finely linked in a partial reticulum(mesh- like network). Spores have white spore print (http://www.first-nature.com/fungi/russula-nigricans.php).

5.1.28.3 Key characteristics

The changing colour of the mushroom marks its special identifying characteristic. Though it is considered edible but their tough nature and deteriorate flavor with blackening makes it unfavorable of consumption. The thick and widely spaced gills makes it easy to identify with confidence. (https://en.m.wikipedia.org/wiki/Russula_nigricans). Its cap was 5-20 cm in diameter, the cap was broadly convex when young, later flat with a central depression or shallowly vase-shaped, dry, more or less smooth but with a waxy feel. The color is initially whitish but soon discoloring to brownish ashy grey or brown and later it turns nearly black. It gains both its common name and scientific name from its propensity to turn black from cutting or bruising. The margin is not lined and the skin does not peels of easily. The cap cuticle often cracks in dry weather, the flesh is white, graying with age.

(a) Habitat of *Russula* sp.

(b) *Russula* sp.

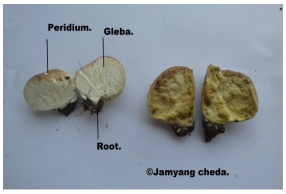
Figure 5.60: *Russula* cross section.
Photographs showing the habitat of *Russula nigricans* and showing Hymenophore; gills and stem. *Russula* belongs to class basidiomycetes under Russulaceae family.

5.1.29 *Calvatia*

5.1.29.1 Habitat and distribution

The mushroom was found growing scattered in a moist soil and also was spotted in the deciduous forest floor above the boy's hostel (minjurling) where there are grass growing. Another matured *Calvatia cyathiformis* was found growing as saprophytes in grassy ground where there are tree stumps and decayed leaves.

(a) Habitat of *Calvatia* sp.



(b) *Calvatia* sp.

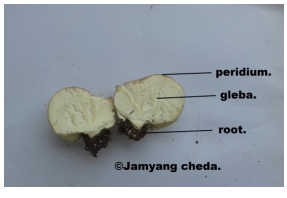
Figure 5.61: *Calvatia* species habitat.

5.1.29.2 General characteristics

They are typically between the size of a soft ball when they are young and gradually become pear shaped as it matured. It is also called as puffball. The peridium is sculptured over the top and becomes brown with age. The gleba usually has a purplish tinge at maturity Alexopoulos et al. (1996). *Calvatia cyathiformis* when matures, it begins to develop a basal portion that is a bit narrower than the ball-like portion, creating a shape of an inverted pear http://www.mushroomexpert.com/calvatia_cyathiformis.html.The members of the lycoperdaceae are distinguished by the peridium of two layers, the outer of which may be further differentiated, and by the presence of a capillitium among the spores Gwynne-Vaughan & Barnes (1930).

5.1.29.3 Key characteristics

They are usually globose, pear-shaped or ovoid and fleshy when young. They are sessile and surrounded by thick peridium. The peridium that encloses the gleba is differentiated into two distinct layers, exoperidium and endoperidium Vashishta (1995).



(a) Habitat of *Calvatia* sp.

(b) *Calvatia* sp.

Figure 5.62: *Calvatia* cross section.
The fleshes are whitish but turns to pale brown when aged. The capillitium is present among the spores and the chambered gleba. Peridium opens at maturity to emit clouds of spores Gwynne-Vaughan & Barnes (1930).

CHAPTER 6

CRUSTOSE, FOLIOSE AND FRUTICOSE LICHEN

The temperate region of the country ranges from 1,800m to 3,500m and exhibits the greatest abundance in variety and density of lichen growth. Corticolous Lichens of genera *Buellia, Collema, Leptogium, Heterodermia, Hypotrachyna, Parmotrema, Pertusaria, Ramalina* and *Usnea* are dominant in this region and prefer the bark of the trees and rocks as their substratum, but could be found in variety of other substrata too. *Quercus* provides a suitable habitat for the growth of light loving saxicolous or terricolous lichen species of *Lithothelium, Pertusaria, Lecanora, Parmalia, Cetraria, Ramalina* and *Heterodermia* (Awasthi, 2000b). The mixed forest of *Quercus* and *Pinus* at lower elevations (up to 2000m) are, moderately dry and preferred by species of lichen genera *Phaeophyscia, Dirinaria* and *Heterodermia*. At higher elevation, the region is dominated by coniferous forests, having dry bark, therefore, only few species of *Parmelia, Lecanora, Lecanora, Physica* on trunks and *Usnea and Ramalina* on twigs at canopy height are seen in this region. Additionally, other trees and shrubs also acts as a favourable substrata for number of lichen species in temperate region of the country. Inside the dense forest, shade loving terricolous lichen species of *Leptogium, Collema, Peltigera, Sticta* and *Lobaria* grow abundantly on vertical slopes along with mosses (Awasthi, 2000a,b). Thus, in the temperate region of the country the dominant lichen species present are Corticolous and saxicolous lichens.

Lichens are mutualistic association of fungus and an alga or cyanobacterium and occur as crusty patches or bushy growths on tress, rocks and bare ground. Lichens are very sensitive to sulphur dioxide pollution in the air. Since industrialization, many lichen species have become extinct. This is mainly due to sulphurdioxide because their efficient absorption systems results in rapid accumulation of sulphur when exposed to high levels of sulphur dioxide pollution. The algal partner seems to be mosly effected by the sulphur dioxide pollution; chlorophyll is destroyed and photosynthesis is inhibited, lichens also absorb sulphur dioxide dissolved in water Richardson (1981). Bioindicators are living organisms that respond in an especially clear way to a change in the environment. Lichens are also be used to measure toxic elemental pollutants and radioactive metals because they bind these substance in their fungal threads where they concentrate them over time (NASH & GRIES, 2002).

6.1 Foliose lichen

These are some of the largest and perhaps most complex lichens. The thallus generally forms flat, leaf-like lobes, with differentiated layers of tissue, the upper and lower cortices, forming the upper and lower surfaces. The lobes are commonly, but not always, appressed to the substrate surface, but can be lifted away. The lower cortex is often differently coloured, frequently brown or black and usually bears rhizines. In *Peltigera* the lower surface is ecorticate. In foliose lichens with multiple branches of the thallus that may stand away from the substrate, the differentiated lower cortex distinguishes them from fruticose lichens, e.g. *Evernia prunastri*, in which the thallus lobes are white beneath, and *Pseudevernia furfuracea*, in which the undersides are black when mature. Foliose lichens take their name from the fact that they are vaguely similar to 'foliage', or leaves. These have and upper and lower cortex like a sandwich of fungal layers with algae mat in middle. They are generally raised

to some extent above the substrate but connected to it by rhizines (hair like growths that anchor the thallus to its substrate). They are easier to remove from their substrate because of rhizines.

6.1.1 Objectives

i To collect single species of foliose lichen from kanglung area.

ii To identify the foliose lichens being collected.

iii To study their habitat.

6.1.2 *Parmelia*

6.1.2.1 Habitat

This lichen was found growing on the bark of a tree near Choekortse lhakhang of Kanglung. Relatively common on deciduous and coniferous trees in open habitats, occasionally on rocks (http://eol.org/pages/16320/details).

6.1.2.2 Description

Phylum: Ascomycota
Class: Lecanoromycetes
Order: Lecanorales
Family: Parmeliaceae
Genus: *Parmelia*
Species: *Parmelia sulcata*
As per (https://en.m.wikipedia.org/wiki/Parmelia_sulcata). Lichens are classified on the basis of the nature of the fungal element and the kind of the fructification Sinha (2011).
Thallus is adnate, foliose, 4-20 cm in diameter, and lobate. The upper surface is gray, smooth to strongly foveolate, shiny and sometime purinose. It is also protective, gelatinized mycelial layer which is pseudoparenchymatous in structure. The lower cortex bears hyphae or root-like structure called rhizines which is attached to the substratum Sethi & Walia (2011). The rhizines function as anchorage and absorptive organs, the thallus is attached by several rhizines. They are dark or dark brown in colour Sinha (2011). It is blue-green lichen, becomes bronze-tinged as it ages. The lobe has conspicuous white pseudocyphellae which forms a fine network .

(a) Habitat of *Parmelia* sp.

(b) *Parmelia* sp.

Figure 6.1: *Parmelia* species habitat.

6.1.2.3 Observation

Parmelia sulcata, or hammered shield lichen, is a silvery foliose species in the Parmeliaceae with dark, nearly black, underside possessing dense rhizines. The photobiont of this symbiont is green algae in the genus Trebouxxia (one of the most common photosynthetic partners in lichen relationships (http://botanyphoto.botanicalgarden.ubc.ca/2015/01/parmelia-sulcata/).

(a) Habitat of *Parmelia* sp.

(b) *Parmelia* sp.

Figure 6.2: *Parmelia* sp. dorsal and ventral side.

6.1.2.4 Conclusion

It is very tolerant of pollution and has a cosmopolitan distribution. Making it one of the most common lichens. This study could be an useful resource for the interested students to know about foliose lichen . *Parmelia sulcata* is generally believed to be fairly pollutant tolerant.

6.1.3 Flavoparmelia

6.1.3.1 Habitat and edibility

Flavoparmelia caperata is usually found attached on the bark of trees. Samples of lichen *Flavoparmelia caperata* were collected from the bark of trees and rocks in Sherubtse college Pemaling residential campus. This lichen material was collected fresh from the nature. The identification was done morphologically using an appropriate literature Awasthi (2000a) This is a common green shield lichen

found attached on the cypress trees and rarely on rocks Awasthi (2000b).

6.1.3.2 Description

Phylum: Ascomycota
Class: Lecanoromycetes
Order: Lecanorales
Family: Parmeliaceae
Genus: *Flavoparmelia* (L.) Hale (1986)
Species: *Flavoparmelia caperata* Awasthi (2000b)
Foliose lichens are leaf-like structures and are attached to substratum loosely Sethi & Walia (2011) Its thallus is large to medium, green in color when the environment is humid and becomes grey in color when dry. They have rounded lobes measuring 3 to 8mm (https://en.wikipedia.org/wiki/Flavoparmelia_caperata). It has a smooth and flat surface, but often have quite wrinkled forms. The leaf-like thallus body is dorsoventrally flattened, growing outwards and attached to its base by simple rhizoids. Rough patches called soredia is present arising from the furuncles Awasthi (2000a). The lower cortex is black color in the center and brown color near the border.

6.1.3.3 Observation

Flavoparmelia caperata or common green shield lichen is found on various trees. This foliose lichen is observed on the cypress trees growing laterally outwards. Two different views are observed; dorsally and ventrally. The rhizines are hardly seen, but it is short.

(a) Habitat of *Flavoparmelia* sp.

(b) *Flavoparmelia* sp.

Figure 6.3: *Flavoparmelia* species belongs to division Ascomycota, order Lecanorales, family Parmeliaceae and genus *Flavoparmelia*.

(a) Habitat of *Flavoparmelia* sp.

(b) *Flavoparmelia* sp.

Figure 6.4: *Flavoparmelia* species belongs to division Ascomycota, order Lecanorales, family Parmeliaceae and genus *Flavoparmelia*.

(a) Habitat of *Flavoparmelia* sp.

(b) *Flavoparmelia* sp.

Figure 6.5: *Flavoparmelia* species belongs to division Ascomycota, order Lecanorales, family Parmeliaceae and genus *Punctelia*.

6.1.3.4 Conclusion

Flavoparmelia caperata is a single species of foliose lichen found in kanglung area which is seen everywhere in Sherubtse campus. *Flavoparmelia caperata* is one of the species that has the ability to check the quality of air. This project gives an information about single species of foliose lichen in Sherubtse locality in terms of its physical characteristics and its habitat.

6.1.4 *Punctelia*

6.1.4.1 Habitat

The samples were collected from the bark of the tree in the small forest near Sherubtse college campus and in the college gene park. It can be found on the bark of the trees and also on the surface of the rocks.

6.1.4.2 Description

Division: Ascomycota
Class: Lecanoromycetes
Order: Lecanorales
Family: Parmeliaceae
Genus: *Punctelia rudecta*

Classification as per (https://species.wikimedia.org/wiki/Punctelia_rudecta) Its thallus is medium sized to 8 cm. Its lobes are often found to be plane, linear or sub irregular spreading 4-7 mm. On the upper surface it is bluish gray to light mineral gray in color. Its margin is tinged with brown (http://lichenportal.org/

portal/taxa/index.php?taxon=54597). Pseudocyphellae is also found, which is a tiny pore that appears on the upper surface of the lichens. Pseudocyphellaria is characterized by the presence of white or yellow Pseudocyphellae on the lower surface of thallus Awasthi (2000a). They also help in the gaseous exchange in the thallus Richardson (1992). An isidia is an outgrowth on the surface of thallus of many lichens Awasthi (2000a) usually with a columnar structure. It is short and smooth, simple to forked or coralloid and its tip is found to be brown, often arising from Pseudocyphellae and cortical cracks not breaking up into soredia-like masses, occasionally becoming extremely dense (http://lichenportal.org/portal/taxa/index.php?taxon=54597).

6.1.4.3 Observation

Punctelia rudecta can be found attached to a rock in the forest near a small stream where there was not enough sunlight. It is found mostly in cool and damp places where the temperature is mostly moderate. It can be also found on the surface of the stones.
Its lower surface is found to be brown in color. The rhizines are simple to forked having the same color as that of its lower surface (http://lichenportal.org/portal/taxa/index.php?taxon=54597).


(a) Habitat of *Punctelia* sp.

(b) *Punctelia* sp.

Figure 6.6: *Punctelia* species belongs to division Ascomycota, order Lecanorales, family Parmeliaceae and genus *Punctelia*.

(a) Habitat of *Punctelia* sp.

(b) *Punctelia* sp.

Figure 6.7: *Punctelia* species belongs to division Ascomycota, order Lecanorales, family Parmeliaceae and genus *Punctelia*.

6.1.4.4 Conclusion

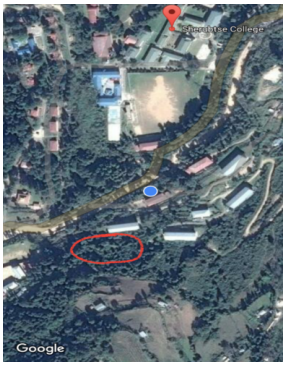
Punctelia rudecta has a sub cosmopolitan distribution and this species is moderately sensitive to air pollution. It is not very sensitive to small pollution differences. Locally it is found abundant in the forests. Lichens are the air pollution indicators and *Punctelia rudecta* is one of the species that has the ability to check the air quality. This project can be a useful reference.

6.1.5 *Usnea*

6.1.5.1 Habitat and distribution

This lichen was found growing on bark of *Ehretia acuminata* tree in which the long thallus body is suspended freely, supported by cluster of rhizoids and it was attached to the bark of the tree. It was found growing in shady area, above the thubtenling hostel in Kanglung region. The lichen was being collected on 24th of October 2017 in afternoon at the time around 12:30pm to 1 pm.

(a) Dorsal view of *Usnea* sp.



(b) *Usnea* sp.

Figure 6.8: *Usnea*, pictures showing location, their habitat and their parts.

6.1.5.2 General characteristics

Thallus is filamentous and radially symmetrical substratum attached by a basal holdfast. It's pale grey-green fruticose lichen with a pendant growth form having a tassels branching (https://en.m.wikipedia.org/wiki/Usnea_filipendula). Thallus is round and hairy with white elastic inner core (Awasti, 2000). It is mutualistic with algal photobiont. The fungi part belongs to the division Ascomycota and the algal part belongs to the division Chlorophyta [https://en.m.wikipedia.org/wiki/Usnea].

6.1.5.3 Key characteristics

They have thin cortex and lateral branches that are constricted at the base. Apothecia is absent in them Awasthi (2000a). They have short branch like appendages called fibrils. (2-3 mm in length) this gives an appearance of a fish bone. The main branches are arranged in transverse and longitudinal sections. Secondary branches may be dis-

tinctly narrowed or not at their point of attachment (Clerc, 1998). Their thallus is not segmented and soralia usually present (Randlane, T., Torra, T., Saag, A. & Saag, L., 2009).

(a) Habitat of *Usnea* sp. **(b)** *Usnea* sp.

Figure 6.9: *Usnea filipendula* species belongs to division Ascomycota, order Lecanorales, family Parmeliaceae and genus *Usnea*.

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