SEMESTER V

17BTU511A PLANT DIVERSITY –I PRACTICAL 3H - 1C

Total hours/week: L:0 T:0 P:3 Marks: Internal: 40 External: 60 Total: 100

Practical

1. Comparative study of thallus and reproductive organs of various algae mentioned in theory.
2. Comparative study of vegetative and reproductive parts of various fungi mentioned in theory.
3. Study and section cutting and lectophenol mount of plant disease materials studied in theory.
4. Study of various types of lichens.
5. Study of external features & anatomy of vegetative and reproductive parts of Marchantia and Funaria.
6. Collection of plant diseases materials and bryophytes available locally.

References

1. Aneja, K.R., & Mehrotra, R.S. (2015). *An Introduction to Mycology* (2nd ed.). New Age International pubishers.
2. Agrios, G.N. (2004). *Plant Pathology* (5th ed.). UK: Academic Press.
3. Kumar, H.D. (1999) *Introductory Phycology*. Aff. East-West Press Pvt Ltd., Delhi.
4. Lee, R.E. (2008). *Phycology* (4th ed.). USA: Cambridge University Press.
5. Sambamurty, (2008). *A Textbook of Bryophytes, Pteridophytes, Gymnosperms and Paleobotany*. IK International Publishers.

SYLLABUS

LIST OF EXPERIMENTS

1. Comparative study of Thallus and reproductive organs of various algae mentioned in theory.
2. Comparative study of vegetative and reproductive parts of various fungi mentioned in theory.
3. Study and section cutting and lactophenol mount of plant disease materials studied in theory.
4. Study of various types of lichens.
5. Study of external features & anatomy of vegetative and reproductive parts of Marchantia and Funaria.
6. Collection of algae, fungi, plant diseases materials and bryophytes available locally.

References

1. Agrios, G.N. (2004). *Plant Pathology* (5th ed.). UK: Academic Press.
2. Aneja, K.R., & Mehrotra, R.S. (2015). *An Introduction to Mycology* (2nd ed.). New Age International pubishers.
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1. **Comparative study of thallus and reproductive organs of various algae mentioned in theory.**

Introduction

Algae is a very large and diverse group of eukaryotic organisms, ranging from unicellular genera such as Chlorella and the diatoms to multicellular forms such as the giant kelp, a large brown alga that may grow up to 50 meters in length. Most are autotrophic and lack many of the distinct cell and tissue types found in land plants such as stomata, xylem and phloem. The largest and most complex marine algae are called seaweeds, while the most complex freshwater forms are the Charophyta, a division of algae that includes Spirogyra and the stoneworts. There is no generally accepted definition of algae. One definition is that algae "have chlorophyll as their primary photosynthetic pigment and lack a sterile covering of cells around their reproductive cells". Other authors exclude all prokaryotes and thus do not consider cyanobacteria (blue-green algae) as algae. A thallus is a flattened growth form, which does not have discrete organs like stems, leaves and roots. However, some of these have a leaf-shoot organization similar to other land plants.

Aim:

To compare the thallus and reproductive organs of various algae mentioned in theory.

1. Vaucheria

Division Xanthophyta

Class Xanthophyeae

Order Heterosiphonales

Family Vaucheriaceae

Genus Vaucheria

The genus Vaucheria has about 40 species, out of which about 9 are reported form India. The most common species are V. sessilis and V. geminata, which occur during winters. The alga is aquatic as well as terristrial. Most of the species grow in damp garden soil, moist wall, in stagnant ponds, ditches and slow moving streams. Some species are marine. The thallus: 1. The plant body is filamentous, branched multinucleated, acellular and coenocytic. 2. The filaments are extensively branched. Branching is lateral but looks dichotomous. 3. The filaments are cylinderical and aseptate. They appear like siphons. 4. The terristrial species are attached to the substratum by means of small tufts of colorless, lobed hapteron (rhizoids). 5. The cell wall is thin made up of two layers. 6. There is a big central vacuole which runs throughout the plant body. The vacuole is surrounded by thin layer of cytoplasm. 7. A large number of small, disc-shaped, yellow green chromatophores are scattered in the cytoplasm. 8. The pyrenoids are completely absent.

Reproductive structure:

Asexual (a) Zoospores:

1. The asexual reproduction occurs by the formation of zoospores. They are formed in aquatic species.
2. Single zoospore is formed inside the terminal zoosporangium.
3. The zoosporangium is cut off from rest of filament by transverse septum.
4. Each zoospore is large, oval shaped, yellow-green in color and bears many flagella.
5. The zoospore is regarded as compound zoospore. It is called synzoospore.
6. It has a big central vacuole surrounded by many chromatophores. It is multinucleated.

Vaucheria- Habit of the thallus and different Reproductive stages

(b) Aplanospores: 1. The terrestrial species develop thin walled, non-motile, rounded or elongated spores called aplanospores.

2. They serve as means of asexual reproduction.

(c) Gongrosira stage:

1. Some aquatic or terrestrial species, under adverse conditions, develop a row of short, thick walled, gelatinous segments called akinetes.
2. This stage is called Gongrisira stage because the plant body looks like another alga Gongrisira.

Sexual:

1. The plants are mostly monoecious but a few species are dieocious.

2. The sexual reproduction is oogamous. The male sex organs are anthredia and female are oogonuia.

3. Each antheridium is borne on a short stalk. It is cylindrical, curved and hook like.

4. The antheredium produces numerous, biflagellate male gametes (antherozoids) which librate through a small apical pore.

5. The oogonia are oval in shape, sessile or sub-sessile, sepaerated from the filament by a transverse septum.

6. Each oogonium has a lateral beak, a receptive spot and a large ovum. The ovum bears single large egg nucleus and many chromatophores.

Position of Sex-organs: According the position of sex-organs, the monoecious species may be of two types. Position of sex-organs in V. sessilis: The sex-organ are directly formed on the main filament. The antheridia and oogonia are formed close to each other, but they are sessile.

Position of sex-organs in V. geminate: The sex-organs are borne on certain special branches. These branches are short and bear terminal antheridium and lateral group of oogonia. Identification Sub- division – Algae.

(1) Filamentous thallus,

(2) Presence of chlorophyll,

(3) Cell wall of cellulose.

Class – Xanthophyceae.

(1) Chromatophores yellow-green,

(2) Photosynthetic reserve-oil droplets,

(3) Motile cells with unequal flagella.

Heterosiphonales.

(1) Thallus multinucleate, unicellular and siphonaceous. Family – Vaucheriaceae.

(1) Thallus branched, filamentces tabular and coenocytic,

(2) Zoospores multiflagellate

(3) Sexual reproduction oogamous. Genus – Vaucheria.

(1) Branching irregular or lateral,

(2) Sex organs without constriction at the basal septum.

2. Oscillatoria

Division- Cyanophyta

Class - Cyanophyceae

Order - Nostocales

Family - Oscillatoriaceae

Genus - Oscillatoria

The genus Oscillatoria is very common. It grows abundantly in dirty stagnant and polluted water channels forming a blackish blue-green mass. Besides, it also occurs on moist rocks, temporary water pools, ditches, drains, streams, sewers and muddy banks of rivers.

Vegetative structure

The thallus:

1. The plant body is filamentous. The filamentous occurs singly or large numbers of them are interwoven to form extensive flat stratum or spongy sheets.

2. The filaments are unbranched.

3. They are long or short, usually straight.

4. Usually, sheath around the trichomes is absent. However, an inconspicuous delicate sheath is present in some species so that they are slippery in touch.

5. All the cells alike except the terminal one which may be conical, convex, rounded, pointed, bent or coiled.

6. In most of the species the cells are usually shorter than the breadth of the trichome.

7. Freshly mounted filaments (in water) show a characteristic oscillating movement, like the movement of pendulum in a clock.

8. The cell structure is typically similar to myxophycean cells. The cellular protoplasm is differentiated into outer colored chromoplasm and central hyaline centroplasm.

9. The cells are prokaryotic.

Reproductive structure:

1. The reproduction in Oscillatoria takes place by the formation of hormogones.

2. The hormogones are small piece of trichomes which separate from parent filament and grow separately into new thalli.

3. They are formed as a result of the death of intercalary cell which becomes empty and acts as biconcave separation disc.

Identification

Sub-division – Algae. (1) Thallus simple, (2) Presence of chlorophyll, (3) Cell wall of cellulose.

Class – Myxophyceae. (1) Chromatophore not organised, pigments diffused, blue-green, (2) Photosynthetic reserve cyanophycean starch, (3) True nucleus absent, (4) Sexual reproduction absent.

Order – Nostocales. (1) Thallus with trichomes, unbranched, or with false branching, (2) Hormogones, heterocysts, exospores and endospores generally present.

Family – Oscillatoriaceae. (1) Trichomes uniseriate, sometimes tapering at the ends, (2) Heterocysts and spores absent, (3) Sheath absent or diffluent.

Genus – Oscillatoria. (1) Trichomes not in bundles, (2) Trichomes without a sheath (3) Trichomes straight and cylindrical.

3. Chlaymydomonas

Division- Chlorophyta

Class - Chlorophyceae

Order - Volvocales

Family - Chlamydomonadaceae

Genus - Chlaymydomonas

The alga occurs as free swimming in fresh water ponds, lakes and ditches. A few species grow on moist damp soil. It forms green surface layer on water.

The thallus:

1. The plant body is unicellular, motile and the cells occur singly.

2. The shape of cell may be oval, spherical or oblong. Size is approximately 20 µ in length.

3. The anterior end of the cell usually shows papillate projection to which two whiplash type of flagella are attached.

4. The cell possesses a firm, two layered cell wall which encloses protoplasm.

5. Each cell possesses single, large, cup-shaped chloroplast. Single large nucleus is situated in the cavity of chloroplast. The cup-shape of chloroplast can be seen only in side view.

6. Each cell is characterized by the presence of single pyrenoid on the broad portion of chloroplast.

7. The pyrenoid body shows central protein surrounded by starch grains.

Palmella stage:

1. Stage asexual reproduction of chlamydomonas that resembles a genus palmella is called palmella stage.

2. This stage results under unfavorable conditions.

3. The group of daughter cells (two, four or eight) remains embedded in a common mucilaginous envelope of parent cell.

4. The cells are non-motile but as soon as they get moisture they develop flagella and escape from envelop.

Identification Sub-division –

Algae. (1) Presence of a simple thallus, (2) Chlorophyll present, (3) Cell wall made of cellulose

Class – Chlorophyceae.

(1) Presence of a difinite nucleus,

(2) Chloroplast present, grass green colour,

(3) presence of starch

(4) Reproductive structure motile and flagella equal in length. Order – Volvocales.

(1) Thallus motie,

(2) Protoplast with contractile vocuoles.

Family – Chlamydomonadaceae.

Genus – Chlamydomonas.

(1) Oval or Pyriform shape of the thallus which in unicellular,

(2) Cup-shaped chloroplast,

(3) Presence of an eye spot,

(4) Fornation of Palmella stage.

Results:

2. Comparative study of vegetative and reproductive parts of various fungi mentioned in theory.

Aim: To study of vegetative and reproductive parts of various fungi mentioned in theory.

Materials and Methods:

- Plant Material Needed for the Entire Laboratory

  Chrysanthemum (insect-pollinated)

- Dissecting Microscope
- Compound Microscope
- Dissecting Instruments such as scales, probes, scissors
- Prepared slide of mixed pollen, whole mount (Wards/Turtox 91W7001)
- Mixed pollen whole mount key to pollen
- Microscope slides
- Microscope slide covers
- Pasture pipette
- Water
- Materials needed for calibration of the ocular micrometer
- Compound Microscope
- Ocular micrometer
- Stage micrometer
- Dissecting Microscope
- Incandescent Lamp
- White Paper

Procedure:

Obtain and examine one thallus of plant.

Results:

3. Study and section cutting and lectophenol mount of plant disease materials studied in theory.

Aim: To identify common disease-causing organisms and the symptoms of the diseases

Principle: There are quite a large number of organisms that are parasitic/pathogenic to humans. These organisms substantially damage the human body and cause diseases, which may even be fatal sometimes. These organisms exhibit characteristic features in their external morphology. Symptoms of the diseases caused by them are also specific.

Materials and Methods: Preserved specimens/permanent slides/photographs of Ascaris, Entamoeba, Plasmodium, Ring-worm fungus and compound microscope Procedure Observe the preserved specimens/slides/photographs and note down the features in the practical record book. Take care to observe all the minute details and draw labelled diagrams of the pathogens.

Procedure :

The lactophenol cotton blue (LPCB) wet mount preparation is the most widely used method of staining and observing fungi and is simple to prepare. The preparation has three components:

Phenol: kills any live organisms;

Lactic acid : It preserves fungal structures, and

Cotton blue : It stains the chitin in the fungal cell walls.

Preparation of lactophenol cotton blue (LPCB) slide mounts

1. Place a drop of seventy percent alcohol on a clean microscope slide.
2. Material from cultures of filamentous fungi should be removed using a stiff inoculating wire not the loop used for manipulations with bacteria or yeasts.
3. Flame the wire by holding it upright in the hottest part of the Bunsen flame, just above the blue cone, until the whole length of the wire glows red hot.

You must ensure that the inoculating wire has cooled before placing it in a fungal culture – it should have cooled sufficiently after approximately ten seconds.

4. Remove the cap from the tube but do not put it on the bench. Kill any contaminating microorganisms by flaming the neck of the tube.
5. Remove a small amount of the culture. For fungal cultures, it is often useful to take a little of the agar medium together with the fungus. In any case, the material should be disturbed as little as possible when being transferred to the slide.
6. Flame the neck of the tube once more and replace the cap.
7. Immerse the fungal material in the drop of seventy percent alcohol. This drives out the air trapped between the hyphae.
8. Tease out the material very gently with mounted needles.
9. Do not forget to sterilise the inoculating wire and the needles after use by heating to red heat in a Bunsen flame
10. Fungal structures are readily visualised after staining with a lactophenol cotton blue dye preparation.
11. Before the alcohol dries out add one or at most two drops of the stain. A common fault is to add too much to the preparation. Holding the coverslip between your index finger and thumb, touch one edge of the drop of stain with the edge of the coverslip.
12. Lower the coverslip gently onto the slide, trying to avoid air bubbles. Your preparation is now ready for examination.
13. Make the initial examination using a low power objective lens. The thinner parts of the preparation, generally around the edges of the mounted material, will yield the best images.
14. Switch to a higher power 40X objective for more detailed examination of spores and other structures.

Observation

A. Entamoeba

Observe the following features of the parasite in the slide or photograph:

(i) It is unicellular.

(ii) Shape of the cell is irregular due to pseudopodia.

(iii) A single nucleus is present eccentrically in the cell.

(iv) In the nucleus a peripheral ring of granule of nucleoprotein and central karyosome are observed. Rest of the space in the nucleus looks empty (Fig. 14.1).

(v) A few food vacuoles may be seen in the cytoplasm. Contractile vacuoles are absent. Mature quadric nucleated cysts may be present. Fig.14.1 An Entamoeba

B. Ascaris The external features of round worm are as follows: (i) Body long (20 to 40 cm), cylindrical (5 to 6 mm diameter) with no segmentation (Fig. 14.3). (ii) Sexes are separate; the females are longer than the males. (iii) Both the ends are pointed; posterior end of male is ventrally curved. (iv) Mouth is situated at the anterior end, and is surrounded by three lips, one present middorsally and rest two lips are situated ventrolaterally (for viewing these lips a magnifying lens is needed). (v) Single longitudinal lines are present on the dorsal, ventral and on the two lateral sides, all along the length of the body. Out of these the lateral lines are comparatively more distinct than the others lines. (vi) Excretory pore is present on the ventral surface slightly behind the anterior end. (vii) In addition to the ventrally curved posterior tip, the male worm has a pair of penial spicules very close to the cloacal opening. (viii) In case of female specimen a female genital aperture is present mid-ventrally about one third distance from the anterior end. Systematic position Phylum – Aschelminthes Class – Nematoda Type – Ascaris lumbricoides Note: Round worm or Ascaris is one of the common parasite found in the intestine of human beings. Symptoms: (a) Irregular bowel, (b) Occasional vomiting, (c) Anaemia

C. Results:

4. Study of various types of lichens.

Introduction

The word ‘lichen' has a Greek origin, which denotes the superficial growth on the bark of olive trees. Theophrastus, the father of botany, introduced the term ‘lichen' and this group of plants to the world.

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. The phycobiont and the mycobiont loose their original identity during the association and the resulting entity (lichen) behave as a single organism, both morphologically and physiologically. Hence the lichen is called as a composite organism. In lichen thallus (body) the mycobiont predominates with 90% of the thallus volume and provides shape, structure and colour to the lichen with partial contribution from algae. Whatever visible from out side in a lichen thallus is fungal part, that holds algal cell inside. Hence the lichens are placed in the Kingdom – Mycota (Fungi). The fungi present in lichens are called as lichenized fungi. Among the 20,000 lichen species known in the world 95% belongs to the Ascomycetes group of fungi while Bacidiomycetes and Deuteriomycetes groups are represented by only 3% and 2% of species respectively.

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 – foliicolous (epiphyllous). In general any lichen growing on other plant is called as epiphytic . The lichens can grow on underwater rocks, but not freely in water or on ice. The lichens are widely distributed in almost all the phytogeographical regions of the world. Sufficient moisture, light and altitude, unpolluted air and undisturbed, perennial substratum often favour the growth and abundance of lichens.

By their appearance the lichens can be grouped into three main categories of growth forms,

• Crustose lichens: The thallus in crustose lichen is closely attached to the substratum without leaving any free margin. The thallus usually lacks lower cortex and rhizines (root like structure). Such lichens are collected along with their substratum for the detailed study.

• Foliose lichens: 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 lichens: Here the lichen 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.

There are few intermediate categories of growth forms such as,

• Leprose lichens: The leprose lichen is powdery or granular and does not form smooth thallus.

• Placodioid lichens: In this case the lichen thallus is closely attached to the substratum at centre and lobate or free at the margin, but lack rhizines.

• Squamulose lichens: Here the lichen thallus is in the form of minute lobes, having dorsiventral differentiation. The rhizines may be present or absent. This is a form intermediate between crustose and foliose.

• Dimorphic lichens: In case of dimorphic lichens single thallus has the characters of both foliose/ squamulose and fruticose lichens. The squamules are the primary thallus, which bears erect body of fruticose lichen, the secondary thallus.

Differentiating lichens from other groups of plants

The non-lichenized fungi, algae, moss, liverworts (bryophytes) are the plants, which grow on rocks, bark and on soil and can be confused for lichens to the beginners. However, lichens

can easily be differentiated from these plants in the field. The lichens are never greener as algae, liverworts and mosses. Foliose lichens in the moist places or in wet condition may look greener, but have thick, leathery thallus while liverworts have non-leathery and slimy thallus. The dimorphic forms of lichens such as Cladonia may confuse with the leaf liverworts and mosses. However, leaf liverworts and mosses have dense small leaf like structures throughout the central axis of the plant, while in case of dimorphic lichens the squamules of semicircular shape usually present at the base of the central axis or sparse throughout. Algal mat are usually found in water-flooded habitat and slimy. The beginners may confuse the dried algal mat on rocks and bark for lichens. By spraying some water on these mat one can make out whether it is algal mat or lichen.

Collection and preservation:

Both the micro and macrolichens are visible to naked eye in the field. However, a hand lens, preferably of 10x magnification, is necessary to examine the fine structure of the thallus while collecting. A sharp, flat edged chisel (1 to 2 inch wide edge) and a hammer (1 kg weight) are the tools required for collecting lichens from the bark. A pointed or stout flat edged chisel can be used to collect lichens growing on rocks. Polythene packets (smaller (6 x 12 inch) and bigger sized), rubber bands, labeling stickers, a field book, notebook, pen, pencil, plant press, knife, secateur (twig cutter), hand lens, old news papers or blotters, (nylon) ropes, collection bags, herbarium packets are the other necessary items required during a lichen collection trip. An altimeter, Global Positioning System (GPS), camera and few other instruments can be carried as per the objectives of the study (Fig. 13). The field book is different from the notebook in having printed columns for entering required data, such as date, locality, altitude, collector's name and remarks. Every page of field book has serial number. The numbers are also printed several times, one below the other on the free (right) side of the filed book. These numbers are for placing along with the specimen in the field.

The lichens are usually collected along with their substratum irrespectively of their growth form. Only the lichens that are very loosely attached to substratum are scraped out and collected. The corticolous lichens growing on tree trunk at reachable height (up to 2 – 3 m

from ground) are usually collected and canopy lichens can be found fallen on ground. Special tree climbing methods can be adopted for studying the canopy lichens. Superficial bark should be removed with the help of chisel or knife in order to avoid damage to the trees. The ramicolous lichens are collected by cutting twig with secateur. In case of saxicolous lichens smaller pieces of the rocks should be collected in order to avoid over weight. The lichens on the edges or crevices of rock are collected by breaking the rock. Sufficient amount of specimens (at least 2 thallus or patch of 5-10 cm) should be collected, as the material will be consumed for chemical analysis (TLC) and microscopic study. The bulk collection also helps to designate it as various types (Holotypes, Isotype, Partype) in case it is new taxa. Further, it will also be convenient to distribute it to other herbaria as exsiccates or voucher specimens. However, unnecessary or repeated collection of same material should be avoided in order to conserve this group of plants. For beginner different lichen specimens can look same or specimens of same species may look different. Till one gains experience, samples looking different on careful observation can be collected. The collected lichen samples are transferred to the polythene packets, labeled and closed with the help of rubber bands. Several such packets are then transferred to larger polythene or collection bags. Or one can also keep the collected material in newspaper or blotter packets. It will be better if the different specimens are kept in different packets to avoid mixture. Otherwise, all the collections from a single tree can be kept together, or even collections from several same species of tree in a study area can also be put together in bigger polythene bag. The lichen specimens should not be kept in polythene packets for longer duration as it gets spoiled due to fungal attack when wet or changes in colour as it dries. While collecting the lichens the field data required should be noted in the field book and its respective number is cut and put in the packet along with the specimen. After returning from field all the specimens should be transferred to newspaper or blotter packets along with their labels for drying. The lichen specimens on wet barks should be kept in plant press and tied tightly. Otherwise the bark gets curled up as it dries, makes uncomfortable to preserve in herbarium packets and also gives shabby look. Much dried and curled specimens can be stretched using water and by spreading on blotters. The specimens can be dried under sun. If the specimen-processing place is moist, damp or if it is winter or rainy season, the materials can be dried with the help of heater or hot-air oven. If insects are

seen in a collection they should be killed either by drying the specimens openly in hot sun or by placing sealed specimen polythene in deep freezer (- 20°C) for three days.

The lichen herbarium packets should be made up of thick, white or brown hand made acid free paper . The paper sheet of dimension 13.5x11.5 inches is folded lengthwise twice and then side ways to produce the packets of dimension 7x5 inches with upper flap of 3.5 inches to stick the label (Fig. 15). The herbarium label should contain the information of name and family of the lichen (which can be written after the identification), detailed locality and altitude from where it has been collected, date of collection, a reference number, collectors name and notes on its substratum and any other interesting observation. After the identification name of the person who identified (determined) the specimen can also be mentioned. The label should be written legibly with black, waterproof, permanent ink pen and not with ballpoint or gel pen. Alternatively A4 sized paper can also be used for making herbarium packets. If it is used, the dimensions of the packet change slightly. Using A4 papers has the advantage of saving time, avoiding messy gum, ink and shabby look due to bad handwriting by printing the details on the paper. A label template can be designed in the word processor for this purpose. If all the information regarding a species is available in computer, it is even possible to programme PC to print large number of label within a short period of time. The black laser print should be taken and not inkjet prints.

No poisoning methods are available for lichen preservation. The sufficiently dried lichen specimen should be pasted on thick, hard paperboard of dimension 6.5x4.5 inches (little less than the total packet size) and then placed inside the herbarium packet. The board also should have the same reference number as on the label. In case of terricolous lichens gum (Quick Fix, Fevicol, paint) should be applied all around the soil cluster in good amount (without spoiling the lichen), so that the soil does not get powdered. Later on such specimens can be pasted on the board. Small or delicate samples can be kept within a paper packet pasted to the board, or in small boxes. A piece of tissue paper is kept over the lichen specimen pasted on the board while keeping it within the herbarium packet. A herbarium packet should have only the specimen belonging to single species and mixtures should be avoided. If the material is in large amount they can be distributed in two or more packets to avoid spoiling of specimen

due to crushing or shabby look of the packet. However, all these packets should have same number, one packet with good specimen can be treated as original while others are marked as duplicate. Several herbarium packets are then kept upright in drawers or cupboard / hardboard boxes of dimensions approximately 8x5x12 inches (like shoe box). The boxes are then placed in almirahs.

The storage place of herbarium should be dry to prevent fungus growth on lichens. Booklice are the common insects which quietly damage packets and lichen specimen in short time during storage. However, booklice can be kept away by placing naphthalene balls in herbarium boxes and in almirah. The lichen specimens within the boxes as well as in almirah can be arranged in alphabetical order. As in flowering plants, no particular classification system is followed for lichen specimen arrangement.

Identification of lichens

The collected lichen specimens are initially segregated according to their growth form. Within the growth forms the specimens can be further grouped according to the type of their fruiting bodies (apothecia, perithecia, or sterile).

Before starting the examination of specimen for identification, one should have a tool or pencil box containing few items needed for handling the specimens. The simple but necessary items needed are razor or snapper blades, plastic-handled needles, pointed and flat-tipped forceps, injection syringes (2 ml capacity) or capillary tubes or glass rods, pencil, sharpener, eraser, small transparent scale, round brush of 0 – 1 size, Quick Fix, permanent ink pen, etc. The syringes are used for keeping and applying chemical reagents during identification. One syringe will always have distilled water in it. The syringes are kept capped while not in use.

Lichens are identified by studying the morphology, anatomy and chemistry of the specimen. The micro and macrolichen keys of Awasthi (1988, 1991) are the important literature referred for identification of lichens. The beginner should have a glossary of technical terms in lichenology while identifying the lichens. Illustrated glossary or identifications manuals are rare for Indian lichens. A botany student or one with mycological background can follow

the terminology and key. All the observations made on a specimen can be written on a piece of paper (description slip) and kept inside the herbarium packet to avoid repeated observation or section cutting. The description slip should contain the specimen number.

The morphological and anatomical characters to be observed in lichen specimens differ from one group of lichen to the other. However, some common characters to be noted are given below.

Morphology

The morphological characters of a lichen specimen is studied under dissection or stereomicroscope. Type of thallus (leprose, crustose, foliose, squamulose, dimorphic, fruticose), its shape (irregular, circular) and size is noted.

Upper surface : The colour of the thallus, texture (smooth, rough, warty), presence of finger like projections (isidia), granular powder in groups (soredia), fine powder (pruina), black dots (pycnidia, Fig. 19) and whitish decorticated areas (pseudocyphellae, Fig. 20) are to be noted in case of crustose and foliose lichens. The branching pattern, length and breadth of marginal lobes, presence of hair like structures (cilia, Fig. 21) in case of foliose lichens has to be noted.

The morphoology of fruiting bodies have to be studies separately. In case of apothecia, shape (rounded or stretched or lirellate apothecia), size, the mode of attachment (stalked or not), colour and texture of the apothecial margin and disc, presence or absence of powder (pruina, Fig. 22) on the disc, shape of the disc (convex or concave) are necessary characters to be observed. In case of lirellate apothecia the branching pattern and colour of slit can be noted. Sometimes apothecial disc become loose, powdery or hazy and such cups are usually held by long stalk. Such apothecia are called as mazaedium (Fig. 23). The colour of the mazaedium and length of the stalk can be noted. In case of perithecia, its colour, shape, size, the position of the opening (apical or lateral), whether single or grouped are to be noted. Some

crustose lichen does not form definite fruiting body. However, fruiting body structures group together and visible as bulged or flat structures with various shapes usually black, brown or grey coloured. Such structures are called unorganized ascocarp (Fig. 24) or fruiting body.

The lichen substances are identified by performing colour spot test or thin layer chromatography (TLC) or by high performance liquid chromatography (HPLC). Standardized methodology is available (Walker and James 1995) for performing lichen chemistry.

Colour spot test: Three chemical reagents commonly used for colour spot test are aqueous potassium hydroxide (K), bleaching powder or aqueous solution of calcium hypochlorite (C) and aqueous solution of paraphenyldiamine (Pd). K-test is performed either on upper surface of thallus (cortex) or on medulla by exposing it with blade, or on both. A drop of K solution is taken with the help of syringe, capillary tube or with glass rod and then placed on the cortex or medulla and colour reaction is noted. Similarly, C and Pd test are performed and colour changes are recorded. KC-test is performed by applying K solution first and then applying C solution over earlier K solution drop immediately. The colour of the cortex or medulla changes due to presence of particular lichen substances in lichen thallus.

TLC: Sometimes, many lichen substances are undetectable in colour spot test or it may not give proper result. In such cases TLC have to be performed. In TLC various lichen substance presents in a lichen thallus get separated as spots on plate. The spots are identified as different lichen substances by noting its colour and measuring the distance moved by it, with the help of TLC manuals

Results:

5 Study of external features & anatomy of vegetative and reproductive parts of Marchantia and Funaria

PROCEDURE

1. From the classroom culture provided, obtain some of the specimen and place it in a small culture dish partially filled with water.
2. Observe the specimen with a dissecting microscope. Note that the stoneworts resemble what we would think of as a plant. They are divided into "stems" and "branches" (Figure 21-4).
3. Search for flask-shaped and spherical structures along the stem. These are the sex organs. If none is present on the specimen, observe the demonstration slide (Figure 21-5) that has been selected to show these structures.
4. The flask-shaped structures are **oogonia**, each of which contains a single, large egg (Figure 21-6). Notice that the oogonium is covered with cells that twist over the surface of the gametangium. Because of the presence of these cells, the oogonium is considered to be a *multicellular* sex organ. Multicellular sex organs are present in all land plants.

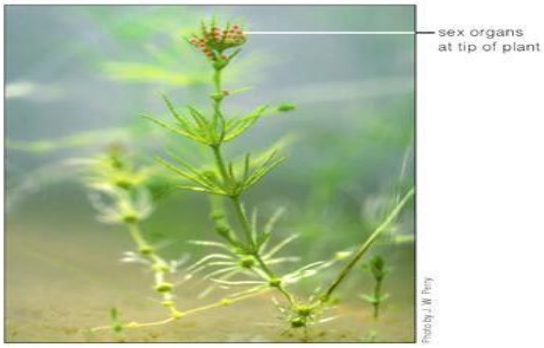

Figure 21-4 The stonewort, *Chara*, with sex organs (2×).

Based on your study of previously examined specimens, would you say the egg is motile or nonmotile?

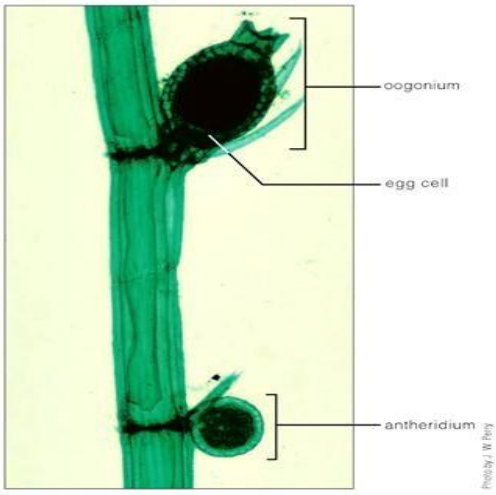

Figure 21-5 *Chara*, showing sex organs (30×).

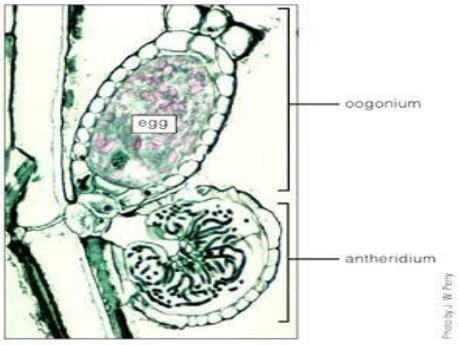

Figure 21-6 Antheridia and oogonia of *Chara* (80×).

Results:

6. Collection of algae, fungi, plant diseases materials and bryophytes available locally.

OVERVIEW

Plants are not always large and found living in soil. Many forms are microscopic and live in water. Regardless of their size or where they might live, all plants have one characteristic in common: they are all capable of making their own food through photosynthesis. In order to use this process, plants need a green pigment called chlorophyll. The plants you are about to study are no exception, however, other pigments like brown and red often mask their green colour.

PURPOSE

To observe two different species of green algae.

To diagram and compare these green algae to each other.

To observe an example of brown and red algae and compare them to green algae.

MATERIALS

*Wherever possible, collect specimens from the ocean for use in this lab.

• *Ulothrix, preserved • Microscope

• *Spirogyra, preserved • Glass slides

• *Zygnema, preserved • Cover slips

*Brown algae • Eye dropper

• *Red algae

PROCEDURE

Part A: Green Algae

Ulothrix

1. Prepare a wet mount of preserved Ulothrix for viewing under the microscope.

2. Observe the algae under both low and high power objective lenses.

3. Note the following parts shown in Figure 1.

a) Green, horseshoe shaped chloroplasts

b) Nucleus

c) Cell wall

d) Filament

OVERVIEW

In the material presented in the Student Guide, you were introduced to a number of microorganisms that inhabit water. These organisms may be either photosynthetic (producers) called phytoplankton or herbivores (primary consumers) called zooplankton. If you remember some of them have the capacity to swim but it is over-shadowed by their dependence on the movement of water currents to move them about. In this lab, you will collect and identify some of these organisms.

PURPOSE

To find out what plankton are found in water.

To observe what they have in common.

To collect and identify marine and/or freshwater plankton.

Materials and Methods

• Microscope • Eye dropper

• Slides • 1.5% methyl cellulose solution

• Cover slips • Paper towels

• Lens paper • Collecting nets

• Collecting bottles

Prepared Slides

Phytoplankton

• blue-green algae

• algal protists (flagellates, dinoflagellates, diatoms)

• other algae

Zooplankton

• protozoan protists (ciliates, flagellates, sarcodinans)

• rotifers

• crustaceans

Procedure

1. Use the eyedropper to get a drop of the water that contains an organism.

2. Prepare a wet mount of the water drop but do NOT include a cover slip. If the organism is too large and / or too motile for a plain microscope slide, then a deep welled slide should be used.

3. Use the low power objective lens first to observe the organism. If the organisms are swimming too fast then add a drop of the methylcellulose solution. This will reduce the activity on the slide without killing the organism.

4. Now add a cover slip and repeat the observation under, low, medium, and if possible, high power.

5. Next decide the group to which it belongs and record on the data table.

6. Use the identification guide on the next page to find the formal name of the organism. Record this also on the data table.

7. Sketch a careful diagram of the species to illustrate the characteristics of the organism you are viewing.

Purpose: To observe the parts of a typical mushroom

Materials:

- Mushroom
- Forceps/Fingers
- Microscope Slide
- Dissection Microscope
- Paper Towels
- Lab Sheet
- Light Microscope

Procedure:

The mushrooms used in today's lab activity are not clean. You are NOT to taste or eat the mushrooms at any time. This is a lab activity and any behavior that does not comply with the rules will result in a zero.

- Obtain a mushroom. On the back of this sheet, (a) draw a diagram of the mushroom you see. Label the cap, stipe, and gills. (see diagram in data section)
- Grasp the cap firmly with one hand and the stipe with the other. Gently wiggle and/or twist the stipe until it breaks away from the cap.
- Peel away some strips from the stipe (like string cheese). The thin, hair-like filaments are called hyphae. Put this under the dissection scope. (b) Examine them and describe them in the data section)
- Next, look under the cap and observe the gills. (c) Draw what the underside of the cap/gills look like on the data sheet
- Using your finger or forceps, peel away a gill. Make a wet mount slide of the gill and examine it under high power on the compound light microscope. Look for spores. (d) Describe what you see on the data sheet
- After you are done, clean off your slide and place it back in the box where you found it. Throw mushroom pieces away and properly put away microscopes.
- **Results:**