UNIVERZITA PAVLA JOZEFA ŠAFÁRIKA V KOŠICIACH

BIOLOGY OF CRYPTOGAMS

Practical Lessons

Michal Goga, Marko S. Sabovljević
Martin Bačkor, Dajana Kecsey
Deepti Routray

Prírodovedecká fakulta

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FACULTY OF SCIENCE
INSTITUTE OF BIOLOGICAL AND ECOLOGICAL SCIENCES
DEPARTMENT OF PLANT BIOLOGY

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(Practical lessons)

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BIOLOGY OF CRYPTOGAMS (practical lessons)

Course Notes

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Preface

Welcome to Biology Of Cryptogams (Practical lessons), a comprehensive guide that introduces you to the fascinating diversity and systematics of lower plants. These practical lessons are designed to provide hands-on training, allowing students to engage directly with the interesting study of Cryptogams by observation, analysis, and identification and experimentation. Beginning with the overall idea of cryptogam in a field trip near by the vicinity of Botanical garden then followed by the basics of microscopy in, will help to gain essential skills in understanding the components and proper usage of a microscope, a fundamental tool in botanical studies.

The journey progresses to the study of cyanobacteria, the photosynthetic prokaryotes vital to Earth's ecosystems, followed by red algae (Rhodophyta), known for their distinctive pigmentation and ecological significance. Further, the manual explores the diverse Heterokontophyta, including diatoms and brown algae, before delving into green algae (Chlorophyta), which share close evolutionary ties with land plants.

As the chapters advance, you will investigate groups like Myxomycota, Oomycota, and Zygomycota, focusing on their unique life cycles and ecological roles. The study of fungi continues with Ascomycota and Basidiomycota, where you will observe their reproductive structures, life cycles, and significance in ecosystems. This practicals also dedicates a section to lichens, symbiotic organisms that play crucial roles as bioindicators and resources in nature. Finally, it culminates with Bryophytes, examining the non-vascular plants like mosses and liverworts, emphasizing their role in water regulation, ecological balance and importance.

This practice book provides detailed step-by-step instructions, supported by illustrations and explanations, to help you understand the structural and functional attributes of lower plants. By completing these exercises, you will acquire a foundational understanding of the diversity and complexity of these organisms, setting the stage for advanced studies in botany. This book encourages curiosity and a scientific approach to exploring the intricate world of lower plants and microorganisms, aiming to deepen your appreciation of their role in the natural world.

Acknowledgment:

We would like to express our sincere gratitude to our PhD. Students Mgr. Martina Gavurová, Mgr. Anastasiia Tomliak, Mgr. Richard Frenák, whose contributions have significantly enriched this practice book. Their insights and collaborative spirit have been instrumental in shaping its content and enhancing its quality.

We also extend our heartfelt thanks to the Department of Plant Biology at Institute of biological and ecological science for their support, resources, and encouragement. The department's nurturing academic environment and emphasis on excellence have been a foundation of this collaborative effort.

This practice book is the result of a collective effort, and we deeply appreciate the dedication and hard work of everyone involved. Thank you for helping make this practice book a success.

Authors

Content:

A. Organization of the Exercise

In the classroom, students work independently and complete the tasks in the order specified by the exercise tutor.

The student is obligated:

1. Arrive for the exercise on time and stay in the classroom until the assigned tasks are completed, and the exercise tutor checks their workbook.
2. The student is obligated to understand in advance the principles and procedures of the tasks to be performed during the exercise.
3. Justify any absence from the exercise. Each missed task needs to be made up. The date for a makeup session will be determined through mutual agreement with the exercise tutor.
4. Students are required to maintain order at their workstations. After completing the exercise, they must clean the glass, tidy up plant materials, and prepare the table to its original state.
5. Students can leave the classroom only with the knowledge and consent of the exercise tutor.

General Rules of Occupational Safety:

Before starting work in the Botanical Practice Classroom, the student must be familiar with the basic rules for working with chemicals, electrical equipment, and must understand the principles, functions, and operation of the instruments used.

Among the stated rules, we emphasize mainly these principles:

1. Eating, drinking, and smoking are prohibited in the laboratory. Laboratory tools, containers, and equipment must not be used for eating, drinking, or storing food.
2. Only clearly labeled chemicals and undamaged laboratory tools should be used during work.
3. When working with glass (e.g., slides and cover slips), protect yourself from cuts. Any leftover pieces of broken glass should be removed from the table with a brush (not by hand) and placed in a waste bin with a solid bottom.
4. Alcohol burners must not be left unattended while burning.
5. Corrosive substances and toxins must not be inhaled orally; safety pipettes must be used.
6. Work with flammable and explosive substances should be done carefully and only after informing other workers. Be cautious of open flames!
7. Immediately flush spilled acids with water, neutralize with powdered soda, and flush again with water.
8. Students must not interfere with electrical components of devices under voltage, use or repair faulty and damaged electrical equipment.
9. Students are obligated to operate equipment according to the instructions provided by the manufacturer and the exercise tutor's guidance.
10. Before starting work with tools, instruments, and machines, students must ensure they are not damaged, wet, or otherwise compromised. Any malfunctions, damage, or improper functioning of tools and equipment must be reported immediately.

11. In case of a fire, a fire extinguisher should be easily accessible nearby. Electrical devices under voltage should never be extinguished with water, water-based, or foam fire extinguishers.
12. Treat consumables, chemicals, energy, and devices with care.

Basic Principles of Providing First Aid:

In case of accidents, only first aid is provided in the classroom. For more serious injuries, in addition to necessary treatment, it is essential to ensure that the injured person receives professional medical assistance. Every injury must be reported to the exercise tutor.

Basic Principles of Providing First Aid:

a) Mechanical Injuries (cutting, tearing, and puncture wounds):

- Typically caused by carelessness while handling glass or working tools (scalpel, dissection needle).
- Minor injuries and abrasions are treated directly in the laboratory (clean the area around the wound with a 1.5-3% hydrogen peroxide solution).
- For larger injuries, cover the wound with a sterile dressing and seek medical assistance.

b) Inhalation Poisoning:

- Prevent the harmful substance from entering the body; remove the affected person from the room and carefully remove clothing (which may contain toxic substances).
- Ensure basic life functions through artificial respiration.
- Offer tea or coffee.

c) Ingestion Poisoning:

- Rapidly eliminate the toxin from the body, induce vomiting by drinking about 1.5 liters of lukewarm water, administer crushed activated charcoal as an antidote.

d) Skin Irritation by Corrosive Substance:

- Immediately flush the affected area with a stream of water for 5-10 minutes.
- Use a neutralizing solution and apply a sterile dressing.

e) Eye Irritation by Corrosive Substance:

- Immediately rinse the eye with a stream of water, use boric water, and rinse the eye again.
- Report immediately to the exercise leader.

f) Injuries Caused by Electricity:

- Remove the affected person from the reach of electric current by switching off the main switch at the entrance to the classroom.
- Move or pull the affected person away with a dry object.
- Start artificial respiration immediately.
- **Important Phone Numbers**

Fire Emergency: 150
Emergency Medical Service: 155
Police: 158
Emergency (SOS): 112

1. Introduction to the Study of Cryptogams

Task No. 1: Field survey of cryptogams in the botanical garden, including greenhouses, with a focus on their areas of use (ecological, biological, aesthetic, cultural, and pharmaceutical significance)

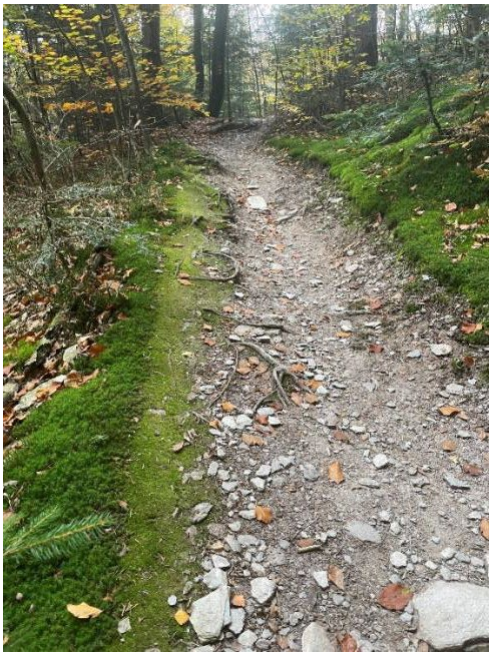
In 1883, German botanist August Wilhelm Eichler introduced a phylogenetic classification system of the plant kingdom, dividing it into two fundamental subkingdoms: **cryptogams and phanerogams**. Although these terms are rarely used in modern taxonomy, we will use them in this workbook for simplicity to avoid listing all taxonomic groups.

Cryptogams (from the Greek kryptos = hidden, gamos = marriage) represent a diverse group of the plant kingdom and plant-like organisms, with their main characteristic being reproduction via spores rather than seeds. This means that, unlike higher plants that form seeds, their reproductive organs are not visible. A synonym for cryptogams might be "lower plants." In contrast, phanerogams are more evolutionarily advanced plants that reproduce by seeds. Unlike cryptogams, they form reproductive structures – flowers – in which seeds are developed as part of their reproductive cycle.

Cryptogams can be divided into the following major groups: cyanobacteria (blue-green algae), algae, bryophytes (mosses, hornwort and liverworts), fungi (including lichenized fungi), ferns, clubmosses, and horsetails. One of the key features of cryptogams is the absence of a cuticle – a waxy layer that provides higher plants with protection and prevents water loss. While this might seem like a disadvantage, the lack of a cuticle actually makes cryptogams excellent organisms for biomonitoring due to their sensitivity to environmental changes. Their inability to retain water and moisture also makes them well-suited for living in humid and wet environments. Since they lack a developed system of conductive elements (with the exception of bryophytes, which have a primitive conductive system) and root systems, the absence of a cuticle allows them to absorb water and nutrients directly through the surface of their thallus.

Thanks to this feature, it's possible to monitor how factors like temperature, humidity, or pollution affect these organisms. For many years, all these organisms were overlooked and did not receive much public attention, often due to their small size or perceived insignificance. However, today we know that cryptogams are **primary producers** in both aquatic and terrestrial ecosystems. They include autotrophic organisms capable of producing organic matter from inorganic sources. Additionally, bacteria and archaea that generate biomass chemically (e.g., by oxidizing organic compounds) can also fall into this category.

Another important fact is that cryptogams are not only primary producers but also **pioneer organisms**. This means they play a key role in primary succession – particularly cyanobacteria, algae, lichens, and bryophytes. This process is crucial for the development of new ecosystems. Initially, we refer to microecosystems, where interactions between biotic and abiotic factors, along with the microorganisms involved, occur within a small, limited area. In principle, they function similarly to full ecosystems but are more confined.



To better understand this, we can consider examples such as a decaying tree trunk in the forest, a drop of water from a stagnant pond placed under a microscope, a moss community growing on a rock, or a lichen growing on a branch or other surface. The study of these microecosystems allows researchers to examine ecological interactions in a "controlled environment" and contributes to a better understanding of local biodiversity, microbial ecology, and adaptation strategies.

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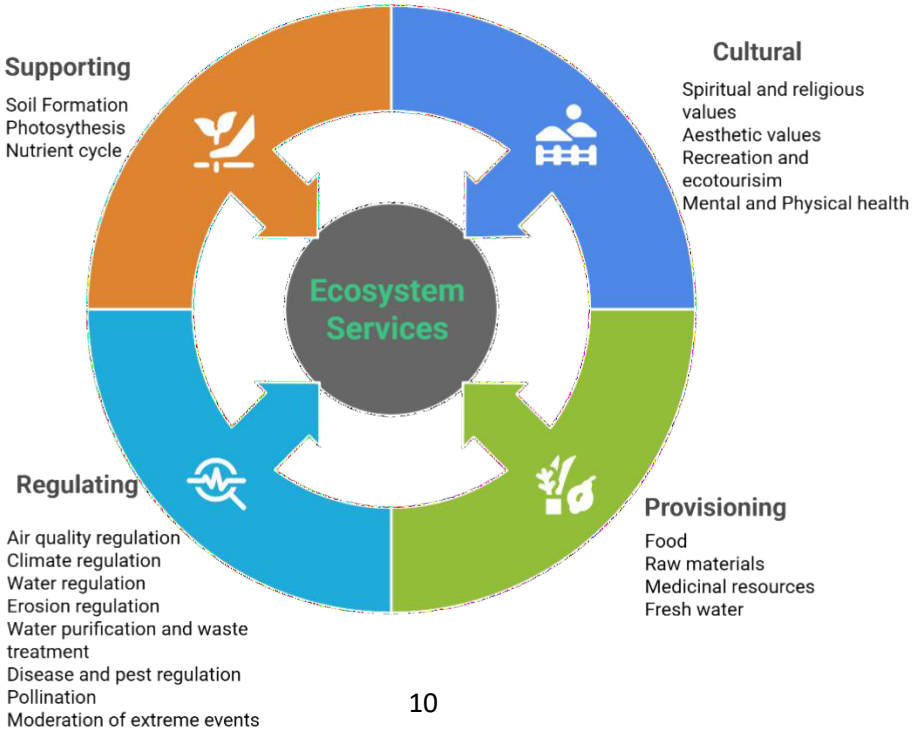

Cryptogams also play an important role in soil stabilization, where they form a soil crust and bind small soil particles using fungal mycelia, moss protonema, rhizoids and filaments of free-living algae. In this way, they protect the soil from erosion caused by wind or water, improve soil structure, and enhance water retention. This also contributes to the proper nutrient cycle by facilitating nitrogen fixation. These properties of cryptogams are especially important in arid regions, where they improve soil conditions and support the growth of higher plants.

The **symbiotic relationships** of cryptogams play a key role in many ways. Almost all cryptogams engage in such relationships. For instance, approximately 90% of all terrestrial vascular plants are in symbiosis with mycorrhizal fungi. Another example includes cyanobacteria, which form symbiotic relationships with bryophytes or lichens. In aquatic environments, algal symbioses with other organisms are common—coral reefs are a perfect example of this mutualism. Algae living in coral reefs perform photosynthesis to produce oxygen and organic substances that nourish corals. In return, corals provide algae with carbon dioxide and shelter from predators. This symbiosis is crucial for coral reef growth and calcification, thereby supporting the biodiversity of these unique ecosystems.

Another example is the symbiosis between cyanobacteria and bryophytes. Cyanobacteria can fix atmospheric nitrogen in nutrient-poor areas, enabling bryophytes to colonize habitats where no other plant could survive. Cyanobacteria convert atmospheric nitrogen into forms usable by mosses, while the mosses provide moisture and a stable environment for the cyanobacteria. Lichens are another example of a self-contained symbiosis, consisting of at least two partners—a photobiont and a mycobiont. The photobiont produces organic substances via photosynthesis for the mycobiont, which in return supplies water and inorganic nutrients. Though opinions about the mutual benefits of lichen symbiosis sometimes differ, this relationship is a prime example of mutualism.

Thanks to their sensitivity to changes in air, water, and soil quality, cryptogams serve as excellent environmental indicators. Lichens and bryophytes are the most commonly used bioindicators, as they lack a protective cuticle, do not have a developed root system, and absorb moisture and nutrients directly from the environment through their entire body. This makes them especially vulnerable to pollution and thus reflective of environmental quality. In biomonitoring, besides qualitative analysis, the presence or absence of certain cryptogam species can indicate changes in environmental quality.

https://www.sciencedirect.com/science/article/abs/pii/B9780443132315000040 (Occurence of cynobacteria symbiosis in many species)

Beyond these ecological indicators, cryptogams have a much broader significance. Due to their biochemical properties and practical uses, **cyanobacteria** possess remarkable biological potential. Some species, such as *Spirulina*, are rich in proteins and vitamins and are strong antioxidants, making them popular dietary supplements. Cyanobacterial extracts are also used in cosmetics and skincare products for their anti-inflammatory and moisturizing properties. As cyanobacteria such as *Nostoc* often grow in areas with high UV radiation, they produce protective pigments (e.g., scytonemin), which give them a natural SPF (Sun protection factor) that is far from negligible (Kecsey et al., 2023). Furthermore, some strains produce bioactive compounds with antibacterial, antiviral, or anticancer properties, offering promising avenues for pharmaceutical drug development. Ecologically, due to their nitrogen-fixing ability, cyanobacteria are promising candidates for biotechnological applications such as biofertilizers and biofuels.

Phaeophyceae represent an important group of brown algae rich in bioactive substances and minerals. For example, the seaweed kelp (order Laminariales) is a source of iodine, which is used as a dietary supplement for proper thyroid and metabolic function. Some brown algae, also known as sea vegetables, are commonly consumed—added to salads, cooked into Italian-style pasta, soups, or made into protein-rich crunchy snacks (Kandale et al., 2011). **Alginic acid**, extracted from brown algae, is widely used in the food industry as a thickener or stabilizer in products such as ice cream or baked goods. In pharmaceuticals, substances such as **fucoxanthin**, **phlorotannins**, and **sulfated polysaccharides** (fucoidan) help neutralize free radicals, protect cells from oxidative stress, and possess anti-inflammatory, antibacterial, and neuroprotective properties (Kelman et al., 2012; Jeong et al., 2015; Heo et al.) 2005).

Rhodophyta (red algae) also form a significant group, found mostly in saltwater. They are among the most important producers of bioactive compounds (Khan et al., 2009), with applications in cosmetics, pharmaceuticals, and the food industry. The most widely cultivated red algae for commercial purposes include *Porphyra, Eucheuma, Kappaphycus*, and *Gracilaria* (Lüning et al., 2003). Their cell walls contain polysaccharides such as floridean starch, sulfated galactans, porphyran, and agar, which offer health benefits and strong antimicrobial, antioxidant, and therapeutic effects. Additionally, red algae contain the omega-3 fatty acid EPA (eicosapentaenoic acid) and various sterols such as desmosterol, cholesterol, fucosterol, and chalinasterol (Sánchez-Machado et al., 2004). These features make red algae a valuable natural supplement for both human and animal nutrition. They also contain specific pigments such as α- and β-carotene, lutein, and zeaxanthin, known for their potent antioxidant activity and their role in immune system support (Aziz et al., 2020).

Green algae or **Chlorophyta**, form the largest group of algae and are used in food, medicine, and ecology. Due to their rich content of vitamins, minerals, and omega-3 and omega-6 fatty acids, they are found in cuisines worldwide and serve as a vegan alternative to fish oil. Green algae are commonly used in various nutritional supplements, such as *Chlorella* or *Ulva lactuca* (sea lettuce), which have detoxifying and antioxidant effects. They also support immune function, digestion, blood pressure regulation, and help combat infections. In medicine, they are valued for their antibacterial, antiviral, antioxidant, and anti-inflammatory properties, making them useful in cosmetics, particularly in anti-aging products. Ecologically, green algae absorb CO_2, help produce biofuels, treat wastewater, and form the base of aquatic food chains. They are also used as animal feed, organic fertilizers, bioplastic materials, and textile dyes. These properties make green algae a vital contributor to food security, human health, and environmental sustainability.

Lichens are a unique and diverse group formed through a symbiosis between a fungus and a photosynthetic partner. They are often considered a part of the fungi kingdom. They grow in virtually every terrestrial environment—from polar regions and deserts to Antarctica. Many lichens are highly sensitive to anthropogenic emissions and often disappear completely in response, making them effective environmental indicators (Cislaghi et al., 1977). Traditionally, people used lichens in folk medicine to treat coughs, wounds, inflammation, and infections. For example, Iceland moss (*Cetraria islandica*) was commonly used as a decoction for bronchitis and lung inflammation. Thanks to their antibacterial, antioxidant, and antiproliferative properties, lichens have gained scientific attention as sources of bioactive compounds with high potential for pharmaceutical use (Ślusarczyk et al., 2021; Goga et al., 2020).

Parmelia pastillifera *Parmelia sulcata*

Menegazzia terebrata *Collema tenax*

Fungi constitute a kingdom of their own, like plants or animals. They feed on decomposing organic matter and thus play a critical role in ecosystems as saprotrophs, returning nutrients to the soil. Some species are edible and widely used in cuisine (e.g., mushrooms, boletes), others have medicinal properties or are used in biotechnology (e.g., for antibiotic production like penicillin). However, some are poisonous and pose risks to humans and animals. Arguably the most important fungal symbiosis is mycorrhiza, the mutualistic relationship between fungi and the roots of higher plants. Here, the fungus helps the plant absorb nutrients and water by increasing its absorptive surface, while the plant provides the fungus with organic substances, such as carbohydrates. About 90% of all terrestrial plants engage in a symbiotic relationship with mycorrhizal fungi.

Bryophytes are a very ancient group of land plants, considered the first true terrestrial plant group, and occur in nearly all habitats, except seas. This poikilohydric group of small green thalloid plants includes three big divisions: Marchantiophyta (liverworts), Bryophyta (mosses), and Anthocerotophyta (hornworts). Bryophytes play a key ecological role as pioneer species in soil formation and stabilization, moisture retention, hydrological cycle regulation, and carbon sequestration (especially species of the genus *Sphagnum*). They also provide habitats for microorganisms and invertebrates (Glime, 2007) and contribute to nutrient cycling in forest ecosystems.

Bryophytes are rich in bioactive compounds such as phenolic compounds, terpenes, and other aromatic substances with significant medicinal potential. They are used in biotechnology to produce compounds for the pharmaceutical, cosmetic, and perfumery industries, as well as insect repellents. Additionally, they contain phytochemicals with anti-inflammatory, antioxidant, and antimicrobial properties, making them a valuable resource for drug development. *In vitro* cultivation allows for their large-scale production. Historically, bryophytes were traditionally used by indigenous communities to treat various ailments. All these uses highlight the **ethnobotanical and therapeutic importance** of bryophytes (Das et al., 2022). For instance, compounds found in liverworts, including terpenes and bis(bi)benzyls, exhibit a wide range of effects—antimicrobial, antifungal, antioxidant, anti-inflammatory, and even anticancer (Asakawa, 2007). The moss *Bryum cellulare* shows antifungal effects, *Sphagnum palustre* exhibits anticancer activity, and *Polytrichum pallidisetum* has anti-inflammatory effects (Das et al., 2022).

Fig description: Different types of Bryophytes (Büdel et al. 2024).

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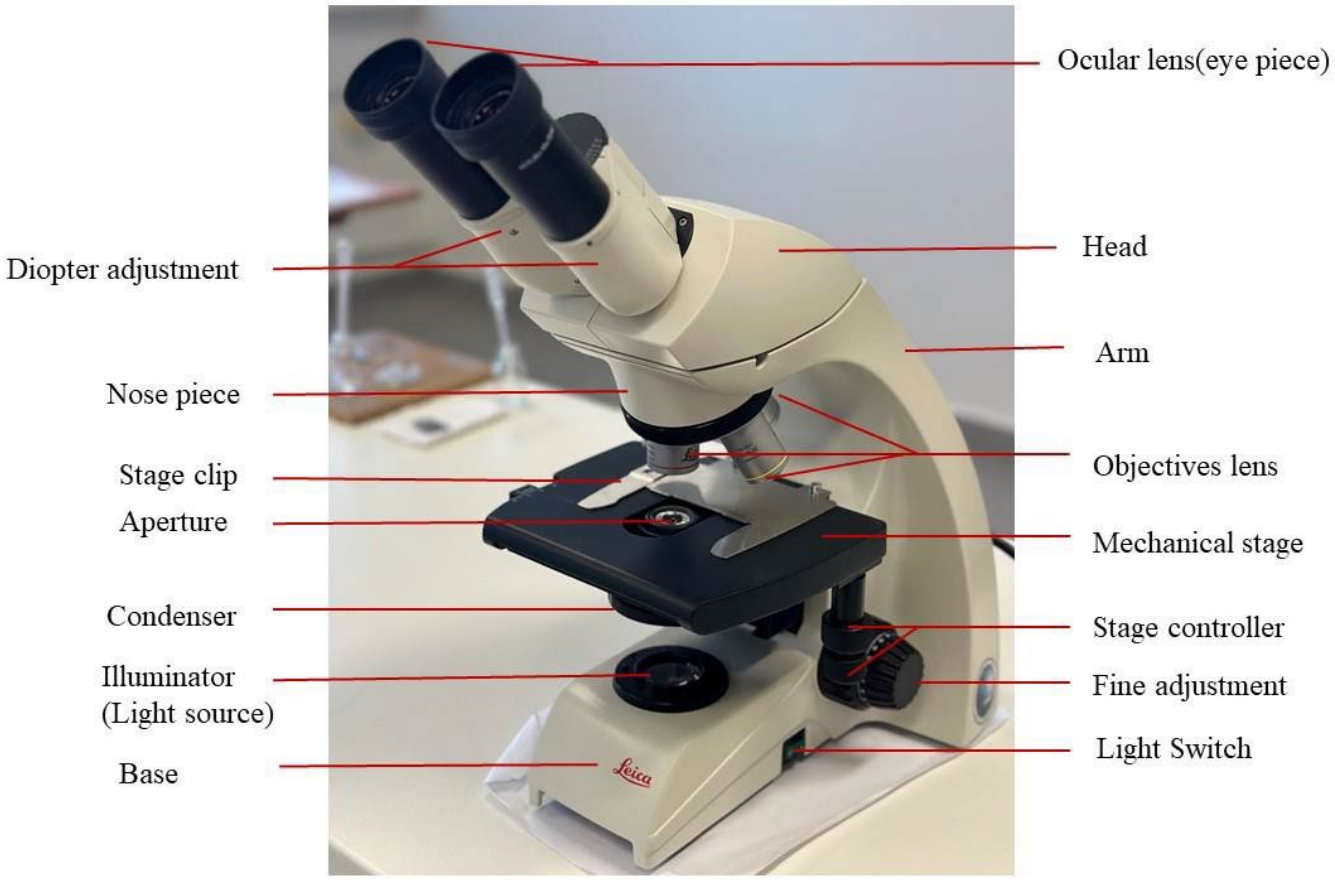
2. BASICS OF MICROSCOPY

LIGHT MICROSCOPE



Microscopy procedure:

- Set the lowest magnification, slightly adjust the stage, and insert the specimen.
- Bring the stage closer to the objective and slowly move the coarse adjustment knob until the observed object appears.
- Refine the focus with the fine adjustment knob.
- For observation at higher magnification, adjust the observed object to the center of the field of view and switch the objective with the turret changer and refine the focus with the fine adjustment knob.
- For better observation, also work with the condenser – adjust the light intensity.

3. CYANOPHYTA (Blue-green algae)

Task No. 1: Observing heterocysts and akinetes in selected cyanobacteria samples.

Theoretical Introduction:

We classify the Cyanophyta division into the kingdom *Eubacteria*. Together with another kingdom - *Archaebacteria*, they form *Prokaryota*, which means they are organisms without a single nucleus.

The "Age of Cyanobacteria" refers to the period between 2.5 and 0.6 billion years ago. It was a time when cyanobacteria, as dominant phototrophs, had an impact on the Earth's atmosphere by gradually increasing the amount of oxygen. This led to the transition to an environment where oxygen was present, creating conditions for the existence of organisms, including humans, as we know them today.

Anatomy: Cyanobacteria form a monophyletic, morphologically diverse, and ecologically significant group of organisms. They are prokaryotes, less complex than eukaryotes due to the absence of a nucleus and some organelles. Besides, they are also crucial oxygen producers and efficient fixers of atmospheric nitrogen. Their nitrogen fixation makes them important contributors to the nitrogen cycle, particularly in tropical oceanic regions or terrestrial environments.

Morphology: Cyanobacteria exhibit a unique cell morphology. They can vary in size and color (Singh and Montgomery 2011). There are three basic morphological forms: single-celled, colonial, and multicellular filamentous forms.

Characteristics of specialized cells (gas vesicles, heterocysts, akinetes):

Vesicles primarily function in planktonic species, providing them with a mechanism to adjust their vertical position within the water column. **Heterocysts** are cells with thick cell walls formed in response to nitrogen deficiency in nature. These cells constitute a monophyletic group of filamentous cyanobacteria. They differ in size from vegetative cells, being larger and forming strong outer layers. They can be easily distinguished under a microscope. Intracellular hyaline bodies are also found at the junction of vegetative cells and heterocysts. These are cells capable of nitrogen fixation. **Akinetes** are brown thick cells of cyanobacteria that form under limited light, sometimes even in the absence of phosphates. They differ in size from vegetative cells, being larger, with thick cell walls and additional tegumentary layers. They are resistant to various factors such as desiccation or low temperatures. They are often formed in senescent populations or in response to various phenomena such as nutrient deficiency, habitat desiccation, or lack of solar energy. Usually, they are formed by filamentous cyanobacteria containing heterocysts.

Pigments of cyanobacteria: The color of cyanobacteria is mediated by a combination of chlorophyll pigment either with blue accessory pigments, including phycocyanin and allophycocyanin, or with red pigments, such as phycoerythrin. Chemically, these are structures called phycobilins (phycobiliproteins).

Systematics of cyanobacteria: Cyanobacteria are a group of organisms particularly challenging to classify (Komárek 2014). Today, this group comprises approximately 2000 species of cyanobacteria divided into 150 genera (Vincent 2009). The exact number of taxa is difficult to determine, but based on the structure of the filament and the presence of specialized cells (heterocysts), they are divided into four main orders:

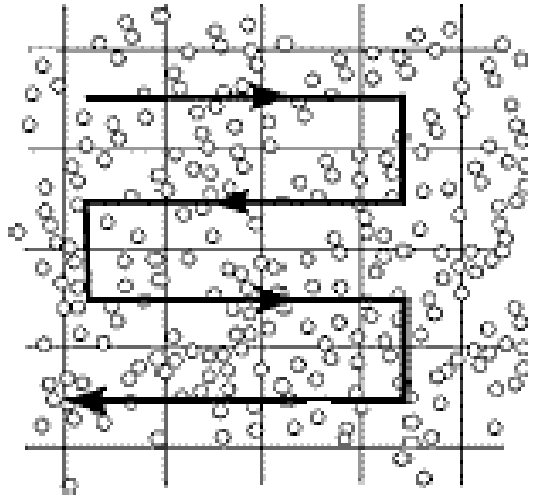
1. Order Chroococcales
2. Order Oscillatoriales
3. Order Nostocales
4. Order Stigonematales (Poor 2011)

Materials and tools:

Samples of cyanobacteria, Pasteur pipette PE, microscope

Procedure:

1. Thoroughly resuspend the prepared cell suspension.
2. Apply approximately 10 µl of the cell suspension onto a microscope slide and gently cover it with a cover slip. Avoid introducing air bubbles into the field of view.
3. Place the prepared slide with the sample under the microscope and use the lowest magnification (5x or 10x) to locate the image, gradually increasing the magnification.
4. Traverse the field of view from the upper left corner diagonally (see figure 1) and attempt to count heterocysts and akinetes (see figure 2) in our sample.
5. After completing the observation, carefully rinse the slide and cover slip with water and dry them thoroughly.



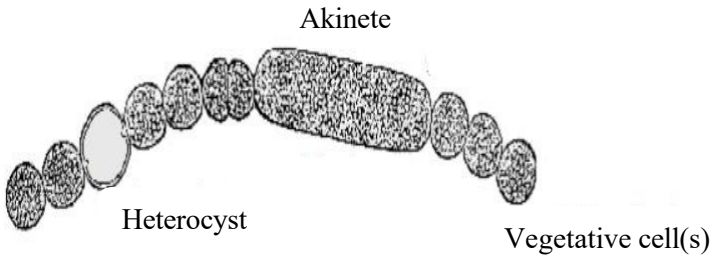


Fig.1: Procedure of counting heterocysts and akinetes, **Fig.2:** Pictures of cell types .

Evalution:

Let us count how many heterocytes and akinetes we observed in our sample.

Drawing and answer:

Task no. 2: Photosynthetic pigments of cyanobacteria.

Theoretical introduction:

Photosynthetic organisms - plants, algae and some bacteria - rely on various pigments to capture light energy from the sun. These colored compounds are particularly diverse in algae, and their importance in algal biology is obvious because photosynthetic pigments can reveal much about the physiology of these organisms and their likely evolutionary relationships. Cyanobacteria have probably lost the gene expressing the enzyme chlorophyllide and oxygenase (CAO) and are therefore unable to synthesize chlorophyll b. Cyanobacteria lack a light-harvesting complex (LHC) and instead use phycobilisomes to harvest light.

We can divide/separate pigment mixtures using thin layer chromatography (TLC). Organic solvents such as acetone, methanol, ethers, DMSO...) are usually used for their extraction. In our case, we will use thin aluminum plates coated with silica gel. A sample of the compound mixture is applied to the starting line of the plate and the plate is then placed vertically into a suitable mobile phase. When the mobile phase passes through the sample, it starts carrying compounds with it. The passage of compounds adsorbed on silica gel is slowed down to varying degrees depending on their polarity; as polarity increases, the rate of retardation (retention) increases. (Note: adsorption means interaction between substances without forming a chemical bond.) As a result, some compounds (more polar) remain near the bottom of the plate, while others (less polar) are carried by the solvent (mobile phase) closer to the top. Using a ruler, we measure the distances of individual pigments and calculate the retention factor. The retention factor (Rf) is used to measure the movement of compounds along the TLC plate. Rf is defined as the distance traveled by an individual component divided by the total distance traveled by the solvent. Its value is always between zero and one.

Material and tools:
Acetone extract of cyanobacteria and green algae, TLC plates, glass vials with caps, tweezers, scissors, mobile phase (60% petroleum ether/16% cyclohexane/ 10% ethyl acetate/ 10% acetone/ 4% methanol), capillaries, automatic pipette

Procedure:
1. Take the cyanobacteria and algae, dry them with paper towels and place them separately in a rubbing bowl, pour 1 ml of acetone.

2. We extract pigments by spreading. The extraction is sufficient when we obtain a deep green acetone extract.

3. Using a thin glass capillary, apply the acetone algae extract to the starting line of the TLC plate and place it in a glass vial containing 1 ml of mobile phase and cover the vial with a lid. The principle shown in Fig. 1.

4. After dividing the mixture of pigments, we identify them according to their position on the plate and their color.

5. Using a ruler, we measure the distances of individual pigments within the plate and calculate the Rf factor according to the principle shown in Fig. 2.

6. Draw a chromatogram in the protocol and write the results in a table.

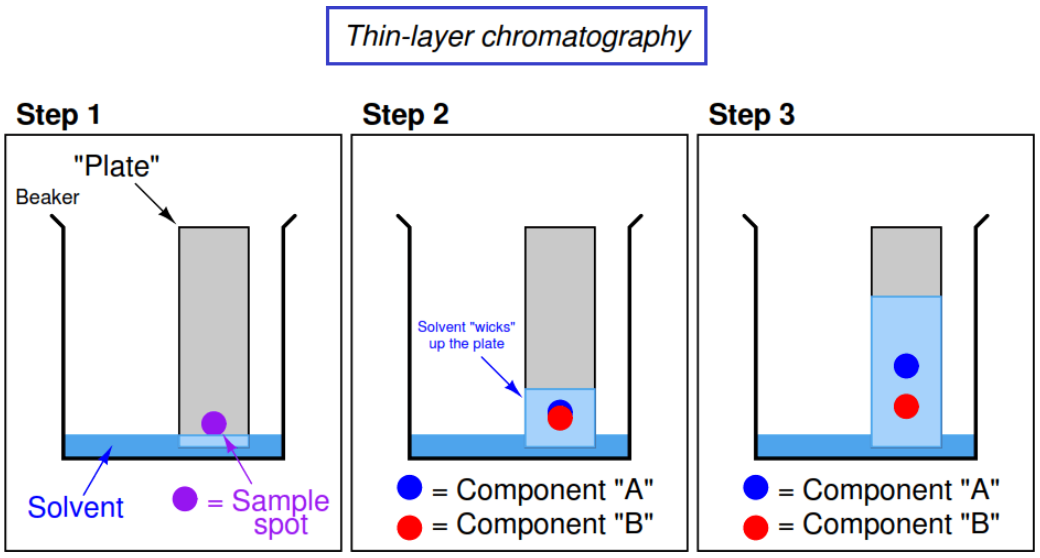


Fig. 1 Principle of division on a TLC plate. Solvent - mobile phase, component A, B - components/compounds.

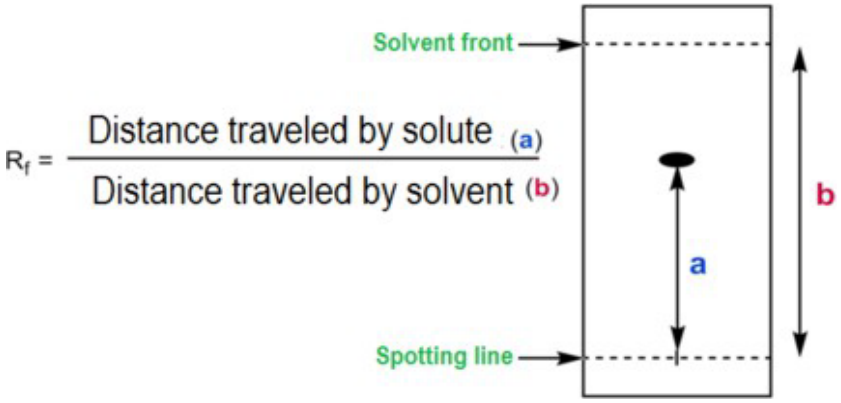


Fig. 2 Calculation of the retention factor. Spotting line - the place where we apply a mixture of pigments with a capillary (start position 1 cm from the bottom edge). Solvent front- position as long as the mobile phase comes out (0.5 cm from the upper edge). a- the distance traveled by the component. b- total distance traveled by the solvent-mobile phase.

Evaluation and Response:

Pigment	**Color**	**Position on the TLC plate** **Rf / Rentention factor**
Carotenoid		
Pheophytin a		
Chlorophyll a		
Chlorophyll b		
Xanthophyll		

Task no.3: Preparation of slides with description.

Theoretical Introduction:

Cyanobacteria are prokaryotic **autotrophic organisms** that have a characteristic **unicellular or filamentous thallus**. They form self-growing cells that are undifferentiated into tissues. Unicellular cyanobacteria known as cocci can often form colonies. Observed, for example, in the genus *Synechocystis*. Filamentous cyanobacteria, such as the genus *Nostoc*, form filaments that are not often branched.

Material and tools:

Permanent preparations of cyanobacteria: *Synechocystis aquatilis*, *Nostoc commune*

Procedure:

1. We will prepare a native preparation. Using a Pasteur pipette, take a small amount of cyanobacteria from the medium, drop it on a glass slide and cover it with a coverslip.

2. We try to capture the cell structures as accurately as possible.

3. We will then describe the individual pictures.

Evaluation:

Observe the culture with the genera *Nostoc* and *Synechocystis*. Draw and describe the preparations. Focus on the difference in cell morphology, as well as the aforementioned specialized cells - heterocytes, akinets.

Drawing and answer:

Task 4: Observation of permanent slides of selected red algae species using a stereo microscope. We observe the following species:

- *Oscilatoria*
- *Chroococcus*
- *Spirulina*
- *Nostoc*

Oscilatoria, filamentous algae

Chroococcus, large single cell

Spirulina,unicellular spirals

Nostoc,multi cellular colonies

References:

1. Singh, S. P., & Montgomery, B. L. (2011). Determining cell shape: adaptive regulation of cyanobacterial cellular differentiation and morphology. *Trends in microbiology*, *19*(6), 278-285.
2. Komárek, J. (2014). Taxonomic classification of cyanoprokaryotes (cyanobacterial genera), using a polyphasic approach. *Preslia*, *86*, 295.
3. Vincent, W. F. (2009). Cyanobacteria.
4. Poór et al. 2011 https://www.sjf.tuke.sk/transferinovacii/pages/archiv/transfer/19-2011/pdf/094-097.pdf

4. RHODOPHYTA (Red algae)

Theoretical Introduction:

It is assumed that red algae have several characteristic features and, as stated by Campbell and Woelkerling (1990), they do not occur in any other group of organisms and are as follows:

- Eukaryotic cells
- Anatomically without flagella
- Storage substance is floridean starch (amylopectin)
- Nutrient reserves stored in the cytoplasm, not in chloroplasts
- Phycoerythrin, phycocyanin, and allophycocyanins as accessory pigments
- Chloroplasts with unstacked (unfolded) thylakoids
- Chloroplasts without outer endoplasmic reticulum

Relatively few representatives of the division ***Rhodophyta*** live in freshwater environments. Most red algae live attached to rocks near marine coasts. Here, they form part of **coral reefs because their cell walls are encrusted with** $CaCO_3$. The cell wall can be **fibrillar (composed of cellulose) or amorphous**, where polysaccharides such as **agar** and **carrageenan** predominate.

These substances are well soluble in water after heating, with agar solidifying into a firm gel upon cooling. Agar is used in microbiology for preparing solid culture media. Additionally, agar is also used in the food industry for making puddings, gelatin, and various fruit and meat jellies. Red algae can form **unicellular**, **filamentous**, or **tissue-like thalli**. Within the division, we distinguish two classes: *Bangiophyceae* and *Florideophyceae*.

In more than 90% of all red algae species, **chlorophyll a**, **chlorophyll b**, and several **carotenoids** occur together with **phycoerythrin** and **phycocyanin** (Wehrmeyer 1983), which provides a much wider range of wavelengths for photosynthesis (Fig. 1). Different ratios of these pigments can result in red, blue, brown, black, yellowish, or greenish thalli. However, most marine species are red or pink, while most freshwater species are bluish to dark grey. **Phycoerythrin** allows them to utilize shorter wavelengths of light for photosynthesis, enabling them to live at depths of up to 200 meters in the ocean.

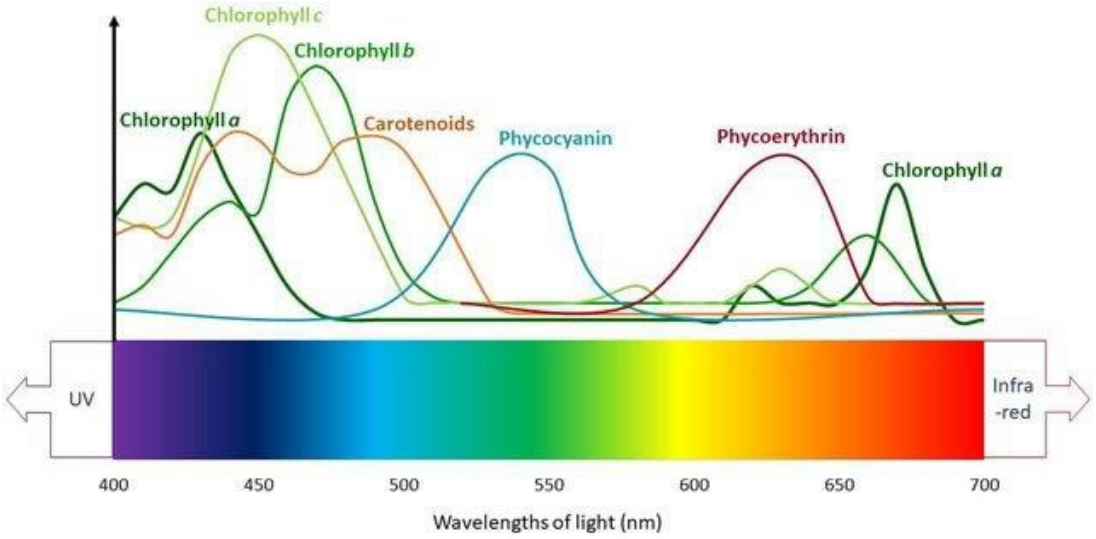


Fig. 1 Spectrum of wavelengths showing the absorption spectra of chromoproteins present in red algae; chl-a = chlorophyll a; chl-b = chlorophyll b, carotenoids and phycobilliproteins (adapted from Hagman C.H.C 2020).

Task 1: What is Phycobilliproteins? Why is it important?

Theoretical Introduction:

In addition to chlorophylls and carotenoids, red phycoerythrin and blue-green phycocyanin, along with allophycocyanin, are present in the chloroplasts. In this task, we will find out which of these pigments is most abundant in our sample of algae. NORI is the Japanese name for various edible seaweeds from the genus *Porphyra*, most commonly referring to the alga *Porphyra tenera*.

Materials and tools:

NORI sheet (algae), mortar, pestle, ethanol, scales, test tubes, pipette, cuvette, spectrophotometer.

Procedure:

1. Use the scales to weigh 0.5g of the NORI algae sample.

2. Using a mortar and pestle, crush the sample with a small amount of ethanol.

3. Transfer the sample into a plastic test tube and use a pipette to add ethanol, bringing the total volume to 5ml.

4. Transfer the samples into glass cuvettes and measure the absorbance of the pigments (phycoerythrin, phycocyanin, and allophycocyanin) in the sample at different wavelengths using a spectrophotometer (A=650; 615; 560).

5. Use the formulas to calculate the pigment content in the algae sample: PE – phycoerythrin, PC – phycocyanin, APC – allophycocyanin.

Formula:

$$APC = \{ A_{650} - 0.208(A_{615})\} / 5.09 \text{ mg mL}^{-1}$$

$$PC = \{ A_{615} - 0.474(A_{650})\} / 5.34 \text{ mg mL}^{-1}$$

$$PE = \{ A_{560} - 2.41(PC) - 0.849(APC)\} / 9.62 \text{ mg mL}^{-1}$$

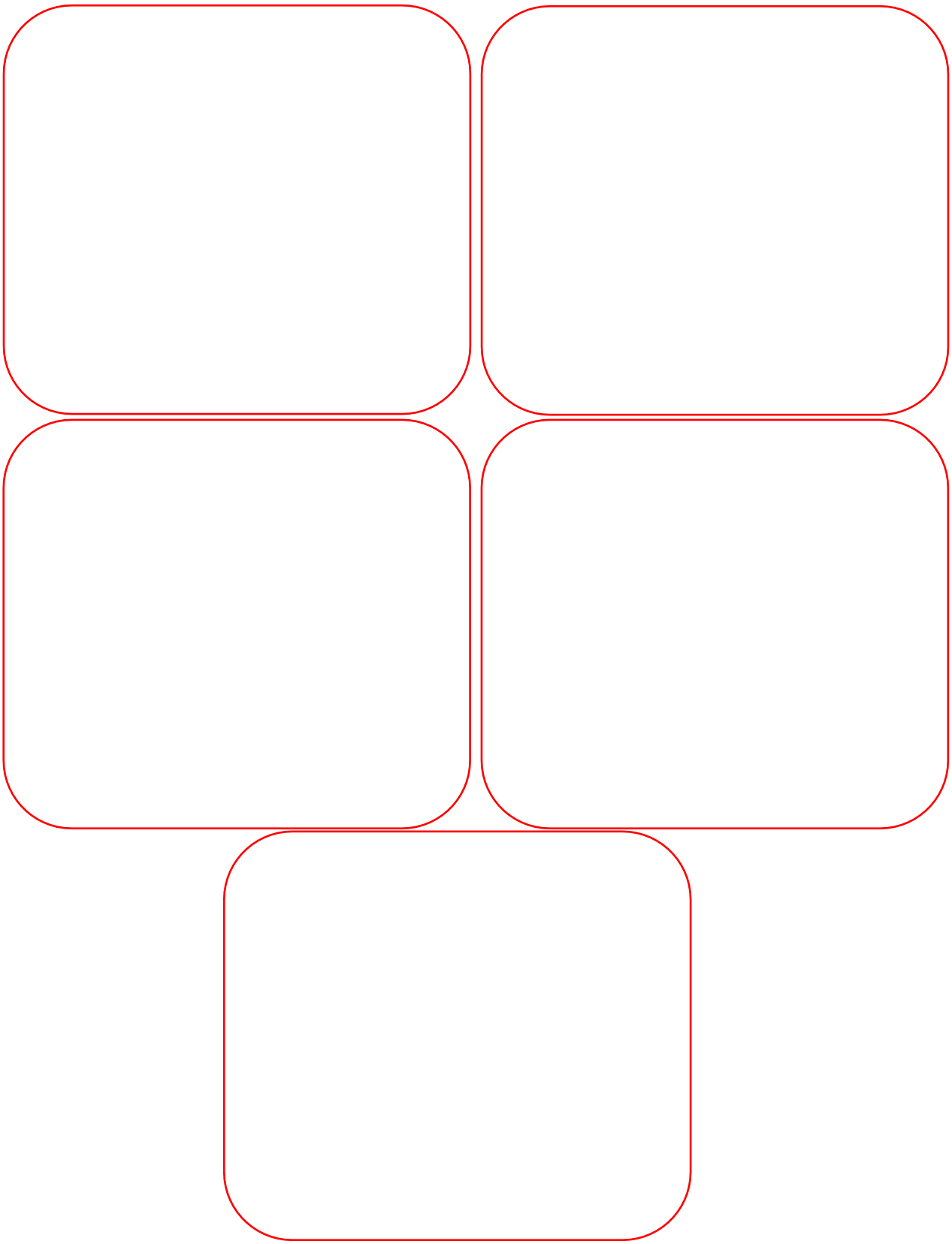
Evaluation and response:

Task 2: Observation of permanent slides of selected red algae species using a stereo microscope. Observe the following species:

Laurencia	*Nemalion helminthoides*
Ceramium sp	*Pterocladia pinnata*
Ceramium sp.	*Lomentaria clavellosa*
Gracilaria	*Nitophyllum punctatum*
Polysiphonia sp.	*Gastroclonium clavatum*
Rytiphloea tinctoria	*Chylocladia vertictillata*
Gigartina acicularis	*Corallina officinalis*

Procedure:

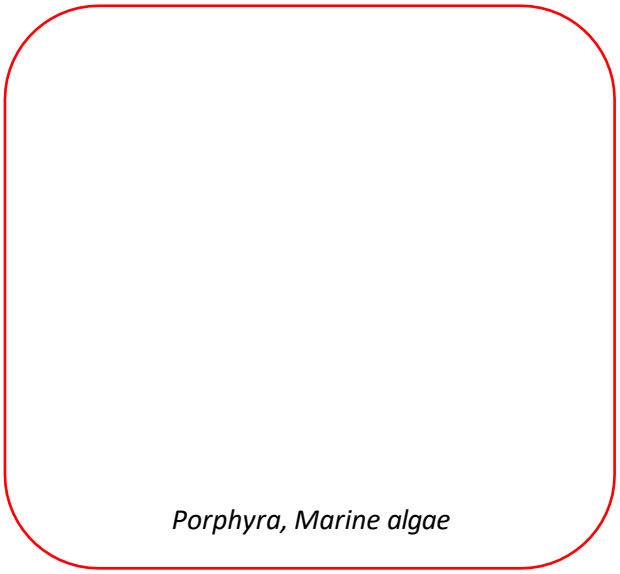
1. Observe permanent slides of selected species of macroscopic red algae, draw, and describe the observed structures. Select 5 species.



Task 3. Observation of permanent slides of selected red algae species using a microscope. We observe the following species:

- *Lemanea sp.*
- *Porphyra,* seaweed

Lemanea, freshwater algae



Porphyra, Marine algae

References:

1. Campbell, S. J., & Woelkerling, W. J. (1990). Are Titanoderma and Lithophyllum (Corallinaceae, Rhodophyta) distinct genera?. Phycologia, 29(1), 114-125.
2. Wehrmeyer, W. (1983). Phycobiliproteins and phycobiliprotein organization in the photosynthetic apparatus of cyanobacteria, red algae, and cryptophytes. In Proteins and nucleic acids in plant systematics (pp. 143-167). Berlin, Heidelberg: Springer Berlin Heidelberg.
3. Hagman, C. H. C. (2020). Phytoplankton in humic and colored Nordic lakes.

5. Heterokontophyta:
a. Unicellular (Bacillariophyceae - diatoms)
b. Multicellular algae (Phaeophyceae - brown algae), and (Xanthophyceae- yellow-green algae)

Theoretical Introduction:

In heterokont (different-flagella) algae (Williams 1991), the presence of **unequal-length flagella** is the basic characteristic. Movement is provided by the longer **pleuronematic** flagellum, which is formed by two rows of specific hairs (**mastigonemes**). The shorter flagellum is characterized by a basal part enlargement, where a stigma (eyespot) can be observed inside the chloroplast. It is composed of a red-orange pigment and allows the detection of light, which the alga seeks for photosynthesis.

The practical tasks focus on the class of diatoms (*Bacillariophyceae*), belonging to the division *Heterokontophyta*. **Bacillariophytes**, also known as Diatoms, are unicellular or forms colonies. The cell wall of each diatom is composed of a **unique silica coat (frustule)**. Using the analogy of a Petri dish, the **lower (smaller) part** is called the **"hypotheca"** and is covered by the **upper part "epitheca**," with the flat, free part called the **"valve"** and the overlapping side band called the **"pleura".** According to the shape of the shells, diatoms are divided into radially symmetric (centric) and bilaterally symmetric (pennate) types. An important taxonomic feature on the surface of the shells is the slit called the **"raphe",** which is related to the flow of cytoplasm and plays an important role in diatom movement.

Task 1: Observation of three different kinds permanent slides of the Bacillariophyceae – diatoms.

Procedure:

1. Observe permanent slides of diatom mixed species (From freshwater, marine and fossil marine) draw and describe the observed different structures.
2.

Drawing:

Diatoms, freshwater mixed species

Diatoms, marine species

Diatoms, fossil marine mixed species

Task 2: Observing permanent slide from the Class Phaeophyceae (Brown Algae) -

- *Fucus vesiculosus*, female conceptacle with oogonia
- *Fucus vesiculosus*, seaweed, male concenptacle with anthredia

Procedure:

1. Observe permanent slides of brown algaes, draw and describe the observed difference between the male and female structure of *Fucus vesiculosus* .

Drawing:

Fucus vesiculosus, female conceptacle

Fucus vesiculosus, male conceptacle

Task 3: Observation of Herbarium Specimens of Selected Species of Brown Algae Using a Stereo Microscope. Choose 2 Species each form the below list:

Collection (Adriatic Sea) 2 thalli

Padina pavonia

Halopteris scoparia

Punctaria latifolia

Dictyota dichotoma

Cystoseira compressa

Dictyopteris membranacea

Dictyota sp.

Cystoseira barbata

Scytosiphon lomentaria

Collection (Norwegian Sea) 2 thalli

Saccharina latissima

Ascophylum nodosum

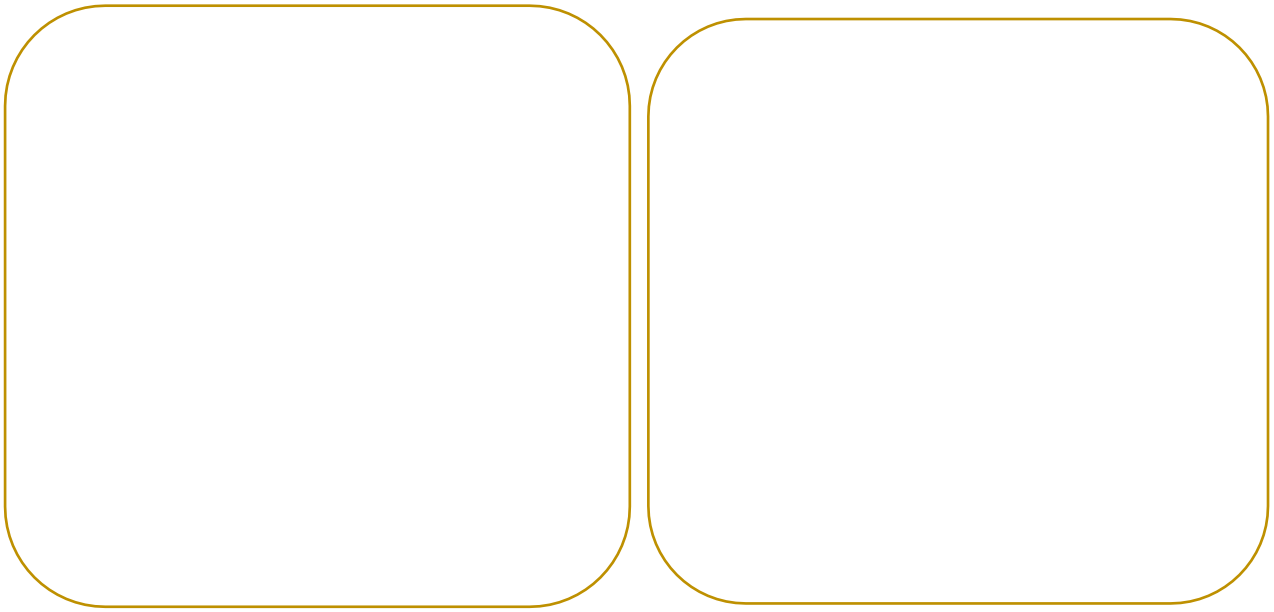
Fucus vesiculosus

Fucus serratus

Fucus spiralis

Pelvetia canaliculata

Drawing:



Task 4: Observation of Permanent Slides of Algae from the Class Xanthophyceae (Yellow-Green Algae)- *Tribonema sp.*

Procedure:

1. Observe permanent slides of yellow-green algae, draw and describe structure of *Tribonema*, filamentous golden algae.

Drawing:

References:

Williams, D.M. 1991. Phylogenetic relationships among the chromista: a review and preliminary analysis. *Cladistics* 7:141-156. [a cladistic analysis primarily of morphological data]

6. CHLOROPHYTA (Green Algae)

Theoretical Introduction:

Green algae (Chlorophyta) are a diverse and ecologically important group of photosynthetic organisms found in various environments, including freshwater, marine, and terrestrial habitats (Van den Hoek et al 1981). Their green color arises from the dominance of chlorophyll pigments, primarily chlorophyll a and b, within their chloroplasts, which enable efficient photosynthesis (Green and Durnford 1996). These algae exhibit a wide range of cellular organization, from unicellular forms like Chlamydomonas to large multicellular species like *Ulva* (sea lettuce).

The chloroplasts in green algae are highly diverse in shape and serve as a distinguishing feature among species. They can take various forms, such as cup-shaped in *Chlamydomonas*, spiral in *Spirogyra*, star-shaped in *Zygnema*, or reticulate (net-like) in *Hydrodictyon*. This diversity reflects adaptations to their environment and photosynthetic needs. Green algae play a critical role in ecosystems as bioindicator, primary producers, contributing to oxygen production and forming the base of aquatic food webs. Additionally, their potential use in biotechnology, particularly in biofuel production and wastewater treatment, highlights their significance beyond natural ecosystems.

Task 1: Algae as bioindicator test

Green algae, such as *Spirogyra*, are photosynthetic organisms that occurs in freshwater environments. They play a crucial ecological role and are sensitive to changes in their surroundings, making them effective **bioindicators**—organisms used to monitor the health of an environment. One key environmental parameter that affects and is affected by algae is **pH**, the measure of acidity or alkalinity of water.

Photosynthesis and pH Relationship :

During photosynthesis, green algae absorb carbon dioxide (CO_2) and release oxygen. In water, CO_2 reacts with water to form **carbonic acid (H_2CO_3)**, which dissociates to release **hydrogen ions (H^+)**, lowering the pH and making the water more acidic. When algae perform photosynthesis and remove CO_2, the formation of carbonic acid is reduced, causing the pH to rise—making the water more **alkaline**.

This natural mechanism allows *Spirogyra* to **influence** the pH of its environment, and that pH change can be used as an **indicator of photosynthetic activity**. Because *Spirogyra* reacts visibly to changes in pH, nutrient levels, and light conditions, it can serve as a simple yet powerful model for observing environmental changes. A healthy, active growth in neutral to mildly basic pH and reduced activity in acidic conditions can highlight pH stress. Moreover, its effect on pH through photosynthesis can demonstrate biological processes occurring in aquatic ecosystems. The experiment has two parts.

In this experiment, *Spirogyra* is used to:

1. Observe its tolerance and growth in different pH conditions (acidic, basic, neutral).
2. Investigate its photosynthetic effect on the pH of water under light and dark conditions, using litmus paper as an indicator.

Part 1: pH Tolerance of *Spirogyra*

Objective :

To observe how *Spirogyra* grows in acidic, basic, and neutral pH environments.

Materials:

- Three beakers or erlenmeyer flasks (100–250 mL)
- Cultured *Spirogyra* (equal amounts)
- Litmus papers
- Distilled water
- Acidic medium (acid)
- Basic medium (base)
- Droppers or pipettes

Procedure:

1. Label three beakers: **Acidic**, **Neutral**, and **Basic**.
2. Fill each beaker with equal volumes of distilled water.
3. Adjust the pH of each beaker :

    - Add a few drops of acid to the *Acidic* beaker.
    - Leave the *Neutral* beaker with just distilled water.
    - Add a small amount of base to the *Basic* beaker.

4. Dip pH paper in each beaker to confirm the pH condition:

    - Acid: Blue litmus turns red.
    - Base: Red litmus turns blue.
    - Neutral: No color change.

5. Add an equal quantity of *Spirogyra* to each beaker.
6. Place the beakers in a well-lit area and observe growth and colour over several days.

We observe

- Changes in pH of all three mediums.
- Chlorophyll pigment changes due to different pH.

Part 2: Effect of Photosynthesis on pH

Objective :

To examine how *Spirogyra* photosynthesis affects water pH in light (open beaker) vs. dark (covered beaker) conditions.

Materials:

- Two beakers with equal volumes of distilled water
- Cultured *Spirogyra*
- Transparent lid or plastic wrap
- Aluminium foil or opaque cover
- Litmus paper or pH indicator
- Light source (sunlight or lamp)

Procedure:

1. Add equal amounts of *Spirogyra* to two beakers filled with distilled water.
2. Cover one beaker completely with foil to block light (dark condition).
3. Leave the second beaker uncovered or cover it with a transparent lid (light condition).
4. Place both beakers in the same environment with consistent temperature.
5. Allow the setup to sit for 1hour
6. After the incubation period, test the pH of both beakers using litmus paper or pH strips.

We observe

- In the **light-exposed beaker**, *Spirogyra* is expected to perform photosynthesis, consume CO_2, and raise the pH (turning litmus paper blue or showing alkaline pH).
- In the **dark-covered beaker**, photosynthesis will not occur, and CO_2 may accumulate from respiration, lowering pH (turning litmus paper red or showing acidic pH).

Task 2: Observation of Various Chloroplast Shapes in the Division Chlorophyta.

Theoritical Introduction:

The class Zygnematophyceae is characterized by genera with various chloroplast shapes, which aid in their identification. Their structure also includes pyrenoids.

Materials and Tools:
Culture of algae from the genus *Spirogyra* and *Zygnema*, 50 ml test tube, tweezers, microscope slide, cover slip, microscope.

Procedure:

- Take a small amount of algae into a 50 ml test tube.
- Using tweezers, place the algae onto a microscope slide and add a drop of water.
- Cover with a cover slip and observe under the microscope, aiming to identify individual genera based on chloroplast shapes.

Evaluation:

During the observation, draw and describe the characteristic chloroplast shapes of this genus.

Drawing:

Task 3: Observation of Permanent Slides and herbarium species

Permanent slides (All 4 species) :	Herbarium (2 species):
• *Hematococcus* • *Hydrodictyon* • *Ulothrix* • *Volvox*	• *Kirchnerielle contorta* • *Halimeda tuna* • *Codium fragile* • *Ulva rigida* • *Cladophora prolifera* • *Cladostephus verticullatus*

Drawing:

References:

1. Van den Hoek, C., Lobban, C. S., & Wynne, M. J. (1981). Chlorophyta: morphology and classification. *The biology of seaweeds. University of California Press, Berkeley*, 86-132.
2. Green, B. R., & Durnford, D. G. (1996). The chlorophyll-carotenoid proteins of oxygenic photosynthesis. *Annual review of plant biology*, *47*(1), 685-714.

7. Division MYXOMYCOTA, OOMYCOTA, ZYGOMYCOTA

Theoretical Introduction:

The group **"Slime Molds"** includes several divisions – Myxomycota, Plasmodiophoromycota, Dictyosteliomycota, Acrasiomycota, and Labyrinthulomycota (sometimes classified under Stramenopila), reflecting their diversity and unclear classification. Slime molds can be more easily divided based on the structure of their thallus into plasmodial (Myxomycota, Plasmodiophoromycota), cellular (Dictyosteliomycota, Acrasiomycota), and net-like (Labyrinthulomycota) slime molds. The fundamental shared features of slime molds are the absence of cell walls and **phagotrophic** nutrition.

Slime molds of the division Myxomycota are not fungi (see below; fungi have cell walls and different modes of nutrition). They also differ in their life cycle, which includes two main stages. It begins with the germination of a spore, releasing haploid protoplasts into the environment. These protoplasts can be flagellated (**myxomonads**) or amoeboid (**myxamoebae**) and are motile. These forms can divide or form dormant microcysts, depending on environmental conditions. Two haploid myxomonads or myxamoebae fuse to form a diploid zygote, which is the foundation of a **plasmodium**. The plasmodium is a multinucleate structure, functioning as one large cell with hundreds of nuclei. When the plasmodium reaches a certain size or under specific conditions, it transforms into one or more fruiting bodies (This is another distinction from fungi, where fruiting bodies grow from a persistent mycelium). Based on the structure of the fruiting body, they can be categorized as **sporangium**, **aethalium**, **pseudoaethalium**, or **plasmodiocarp**. Other divisions have different life cycles, fruiting bodies, and other characteristics (Jabur 2023).

The **Kingdom of Stramenopila** includes the divisions Oomycota (water molds), Hyphochytriomycota, and Labyrinthulomycota, as well as algae from the division Heterokontophyta. A distinguishing characteristic of water molds is their mode of asexual reproduction, carried out through **biflagellate zoospores**, where one flagellum is smooth and the other hairy (= heterokont). Their life cycle includes primary and secondary asexual spores. Primary spores have apical flagella, while secondary spores have lateral flagella. Sexual reproduction in Oomycota occurs via oogamy. The thallus of water molds can be unicellular or filamentous; if filamentous, the hyphal strands are **coenocytic**. They feed saprotrophically (order Saprolegniales) or parasitically (order Peronosporales).

The **Kingdom of Fungi** (Jabur 2023) is a unique group of organisms. They are more closely related to animals than to plants, with both belonging to the domain Opisthokonta. The body of fungi is composed of **hyphae**, which form a **mycelium**. Fungi have cell walls and are heterotrophic, reproducing via spores that can be produced sexually or asexually (e.g., conidiospores). Some groups do not form mycelium but produce unicellular thalli in a so-called yeast stage. Fungi play irreplaceable roles in ecosystems, acting as decomposers of organic

matter, parasites, and symbionts with higher plants (mycorrhiza), influencing plant growth worldwide.

The fungi kingdom includes the divisions Chytridiomycota (chytrids), Zygomycota (zygomycetes), Ascomycota (sac fungi), and Basidiomycota (club fungi).

The division Zygomycota has two classes: Zygomycetes (molds) and Trichomycetes. Key characteristics of this division are coenocytic mycelium and the formation of thick-walled, non-motile, but highly durable zygospores. They reproduce asexually using conidia and feed saprotrophically (order Mucorales), parasitically (order Entomophthorales), or through endomycorrhizae (order Glomales).

Task 1: Observe permanent slides of the division Myxomycota. Draw and describe the fruiting body of these species, including the following structures: (hypothallus, spores, capillitium).

Drawing

a) *Stemonitis*

Stemonitis capilithium with spores

b) *Fuligo*

Fuligo Fruiting body

Task 2: Why are slime molds used as model organisms?

.....
.....
.....

Task 3: Observe, draw, and describe the permanent slides of the division Oomycetes (conidia, infected tissues, sporangia in different host tissues).

- *Plasmopara viticola*
- *Phytophthora infestans*
- *Peronospora tabacina*

Drawing:

Plasmopara viticola

Phytophthora infestans

Peronospora tabacina

References:

1.Jabur, Sanaa. (2023). Basic forms of fungi (structure and classification). 10.13140/RG.2.2.27343.46241.

8. Division ASCOMYCOTA

Theoretical Introduction:

The phylum **Ascomycota** (Wijayawardene et al 2018), also known as sac fungi, constitutes a well-defined group of organisms. The thallus of sac fungi is composed **of hyphae with septa with simple pores**, forming a mycelium or existing as single-celled (yeasts). The mycelium may be organized into fungal tissue. Based on the type of hyphal organization, we distinguish between plectenchyma**,** prosenchyma**,** and pseudoparenchyma**.**

Reproduction in sac fungi occurs sexually through the production of **ascospores** or asexually via **conidiospores** and other mechanisms (e.g., budding in yeasts). Sexual reproduction requires the presence of two compatible nuclei within a single cell—a stage referred to as the **dikaryotic stage**. Ascospores are formed within **asci** (sacs, from which the group derives its name), usually in sets of 8, resulting from the meiotic division process. However, exceptions are common, and different species may have varying numbers of spores in their asci, ranging from 1 to many. Asci with spores are typically protected by clusters of sterile hyphae—**paraphyses**—which, together with the asci, form the spore-bearing layer (**hymenium**) within the fruiting body (**ascocarp**). Depending on morphology, three types of fruiting bodies are distinguished: cleistothecium**,** perithecium**,** and apothecium. In **cleistothecia**, spores are entirely enclosed, and the fruiting body opens by disintegration. **Perithecia** have a similar structure, but the spores are arranged more regularly, and a specialized opening called an ostiole facilitates spore release. **Apothecia** are cup-shaped, with hymenia exposed to the environment.

In ascomycetes, asexual reproductive stages (**anamorph**) are distinguished from sexual stages (**teleomorph**) within the same species. Some species reproduce exclusively asexually, forming only anamorphic stages. These two forms of the same species can be morphologically very different; in the past, they were often assigned two separate names (e.g., *Botrytis cinerea* and *Botryotinia fuckeliana*). This dual naming system was discontinued only in 2013. Based on the presence or absence of sexual reproduction, an artificial group called Deuteromycota was created to include species forming only anamorphic stages. However, this group is no longer supported by the latest fungal taxonomy research.

Sac fungi exhibit diverse ecology and nutritional strategies, including coprophilous**,** lignicolous**,** hypogeous**,** lichenized**,** saprophytic**,** and parasitic species.

Task 1: Prepare a native slide from a culture of *Saccharomyces cerevisiae*, observe, and describe the dividing cells and buds.

Drawing

Task 2: Observe the permanent slides of *Penicilium,* blue mold and *Taphrina deformans* infected peach leaf curl and describe the Conidia, conidiophores.

Drawing

Penicillum mycelium and conidiophores

Taphrina deformans, asci and ascospores

Task 3: Observe the permanent slides.

- *Claviceps purpurea*
- *Tuber rufum*
- *Morchella edulis*

Drawing

Claviceps purpurea, sclerotium

Tuber rufum, hymenium and asci

Morchella edulis, asci and spore

Task 4: Observe the pathogenic infection and describe what you see.

Drawing:

References:

1. Wijayawardene, N. N., Hyde, K. D., Lumbsch, H. T., Liu, J. K., Maharachchikumbura, S. S., Ekanayaka, A. H., ... & Phookamsak, R. (2018). Outline of ascomycota: 2017. *Fungal diversity*, *88*(1), 167-263.

9. Division BASIDIOMYCOTA

Theoretical Introduction:

The **Basidiomycota** phylum, or basidiomycete fungi, is a sister group to the Ascomycota phylum. The thallus of most basidiomycetes consists of **hyphae with septa containing dolipores**, forming mycelium. Some species have a **unicellular thallus**, similar to the yeasts of the Ascomycota phylum. During development, the thallus of basidiomycetes passes through three stages: **primary mycelium** (homokaryon), **secondary mycelium** (heterokaryon), and **tertiary mycelium** (basidiocarp or fruiting body). Like Ascomycota, basidiomycetes also exhibit a dikaryotic stage.

Reproduction in basidiomycetes occurs sexually via **basidiospores** or asexually through budding, fragmentation, conidia, and other methods. Basidiospores are formed on specialized structures called basidia, typically in sets of 4, within the hymenium. In addition to basidia, the hymenium of basidiomycetes contains sterile components such as **basidioles** and **cystidia**. Basidiospores are usually unicellular and haploid.

The hymenium in the **Hymenomycetes** class can take on various forms, most commonly **lamellate** (gilled), **tubular**, **spiny**, or **dispersed**, and the fruiting bodies exhibit diverse shapes and structures. The Gasteromycetes class (puffballs and related fungi) have closed fruiting bodies, with spores maturing in a tissue called the **gleba**. The classes Urediniomycetes (rust fungi) and **Ustilaginomycetes** (smut fungi) do not form basidiocarps but produce **teliospores** during their life cycle. Teliospores often serve as overwintering structures and develop into the future basidium, upon which basidiospores form.

Type of Fruiting Bodies in different classes:

Class Hymenomycetes: fruiting bodies have hymenia of various shapes

- **Order Agaricales**: stalk, cap, ring, volva
- **Order Aphyllophorales**: various shapes

Class Gasteromycetes: closed fruiting body — gleba within the peridium

- **Order Lycoperdales**: powdery gleba
- **Order Phallales**: foul-smelling, initially hypogeous (underground)
- **Order Nidulariales**: “bird’s nests”

Class Urediniomycetes: do not form fruiting bodies; form aecium, uredinium , telium

Class Ustilaginomycetes: do not form fruiting bodies; form sorus

A unique phenomenon in basidiomycete fungi (but not only them) is mycorrhiza. It is a symbiotic association between plant roots and fungal hyphae. This coexistence is beneficial for both parties for several reasons. For the plant, the absorptive surface area for water and minerals increases, while the fungus obtains sugars from photosynthesis. The result is a plant that is more resistant to drought, pH changes, and other environmental factors, and for mycorrhizal fungi, this is often the only source of nutrition.

Besides mycorrhiza, basidiomycete fungi also obtain nutrients saprotrophically, parasitically, and some species are known to be lichenized.

Task 1: Observe the permanent slides from class Hymenomycetes, determine the type of hymenium and draw its parts (basidia, cystidia, spores).

Drawing:

a) *Boletus edulis*

b) *Polyporus sp.*

Task 2: Based on the permanent slides observe and draw telia (black rust infection in wheat).

Drawing:

Task 3: Observe the permanent slide from class Urediniomycetes (*Gymnosporangium sabinae*)and draw aecidia and pycnidia.

Drawing:

Task 4: Observe the permanent slide from class Ustilaginomycetes (*Ustilago zeae*)and draw spores.

Drawing:

Task 5: Choose 4 mushroom models (2 edible and 2 nonedible), draw and describe their anatomical structure.

Drawing:

10. LICHENES

Theoretical Introduction:

Lichens represent a **symbiotic association** between a fungal partner (**mycobiont**) and a photosynthetic partner (**photobiont**) (Lakatos 2011). This so-called coexistence between the photobiont (cyanobacterium or alga) and the fungus (from the divisions Ascomycota or Basidiomycota) brings **benefits** that would not exist if they functioned independently. However, the nature of this symbiosis is still under discussion, as recent research has discovered **another partner** in this relationship—**yeasts**. Lichenologists also agree that **bacteria** play a **significant role** in this symbiosis.

The vegetative part of the lichen is formed by a **thallus** (thallus*, pl.* thalli), from which morphologically diverse structures may develop. These structures can be filamentous, branched, leaf-like, variously clustered, thin crusts, powders, or even amorphous coatings. The morphology and anatomy of lichen thalli are **adaptations** to their environment, where the mycobiont (fungal part) acts as the exhabitant, and the photobiont (photosynthesizing part) is the inhabitant. There are several subtypes of thalli, but most can be categorized into three main types: crustose, foliose, and fruticose.

In terms of the distribution of the photosynthetic partner (green algae/cyanobacteria), thalli can be categorized as either **homoiomerous** (*Collema* sp.), where the photobiont is more or less evenly distributed within the thallus, or **heteromerous** (*Xanthoria parietina*), where the thallus is differentiated. In the latter, a cross-section often reveals a distinct **upper cortex**, **a layer of photobionts**, a **medulla**, and a **lower cortex**. In some cases, fruticose thalli may also include a **central cord** (*Usnea* sp.), or in certain species, this central cord may be **absent** (*Cladonia* sp.).

Lichens are also pioneer organisms that inhabit extreme habitats worldwide. They can be found in polar regions, tropical and subtropical areas, temperate zones, and even deserts. Their ability to survive in such **specialized conditions is partly due to the production of secondary metabolites**, which provide them with sufficient protection. Recently, these products have garnered significant interest from the scientific community across various fields, as these compounds were long neglected due to research limitations. Today, they are the subject of study in chemistry, biology, and other interdisciplinary sciences.

Secondary metabolites are **organic compounds** that are not directly involved in the growth, development, or reproduction of lichens. They are **predominantly produced by the mycobiont** and are stored on fungal hyphae, **extracellularly in the form of crystals**. Approximately 1,100 secondary metabolites (including their derivatives) are currently known, often produced in response to changes in environmental conditions or climate. Their content ranges from 0.1-5% of the dry thallus, and some species can produce up to 30%. These metabolites are synthesized via three biosynthetic pathways: the mevalonate, shikimate, and acetylmalonate pathways. Their significance and applications are wide-ranging. The biological and ecological activity of secondary metabolites includes anti-herbivory, antioxidant, anti-

inflammatory, allelopathic, antibacterial, and antiproliferative effects, among others. Lichens, due to their excellent accumulation of heavy metals, are also used in biomonitoring to assess air quality. Additionally, secondary metabolites protect the photobiont from excessive UV radiation, further demonstrating photoprotective activity.

Task 1: Observation different types of lichen thalli (Anatomical and Morphological Perspectives).

Materials and Tools:

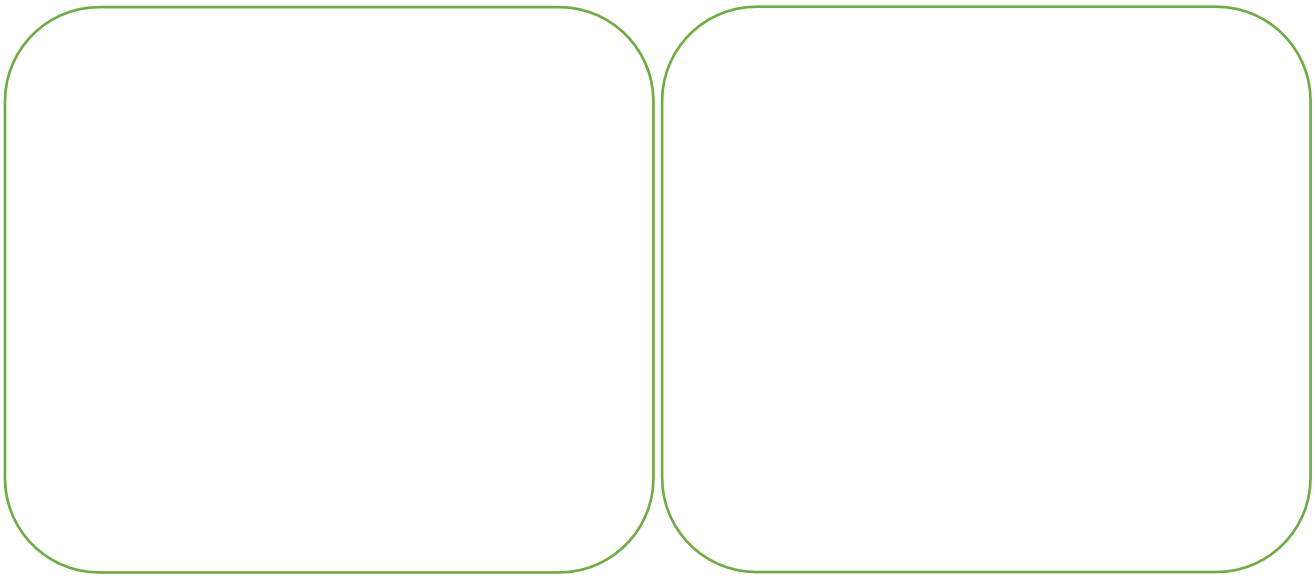
Lichen thalli, paper towels, distilled water, blade, microscope slides and coverslips, dropper

Procedure:

1. Moisten the thallus in distilled water for 5–10 minutes.
2. Remove the thallus, lightly dry it using a paper towel, and prepare a thin section of the thallus using a razor blade.
3. Place the thallus section on a microscope slide, cover it with a coverslip, and add water to the edge of the coverslip.
4. Observe the native specimen under a microscope.

It is important to make the section as thin as possible to ensure the better visualization of specimen, making it easier to distinguish the distribution of the photobiont within the thallus. Sketch the observed object and anatomically describe the parts of the thallus. Arrangement of photobiont, mycobiont.

Drawing:



Task 2: Recrystallization of Secondary Metabolites of Lichens.

Theoretical Introduction:

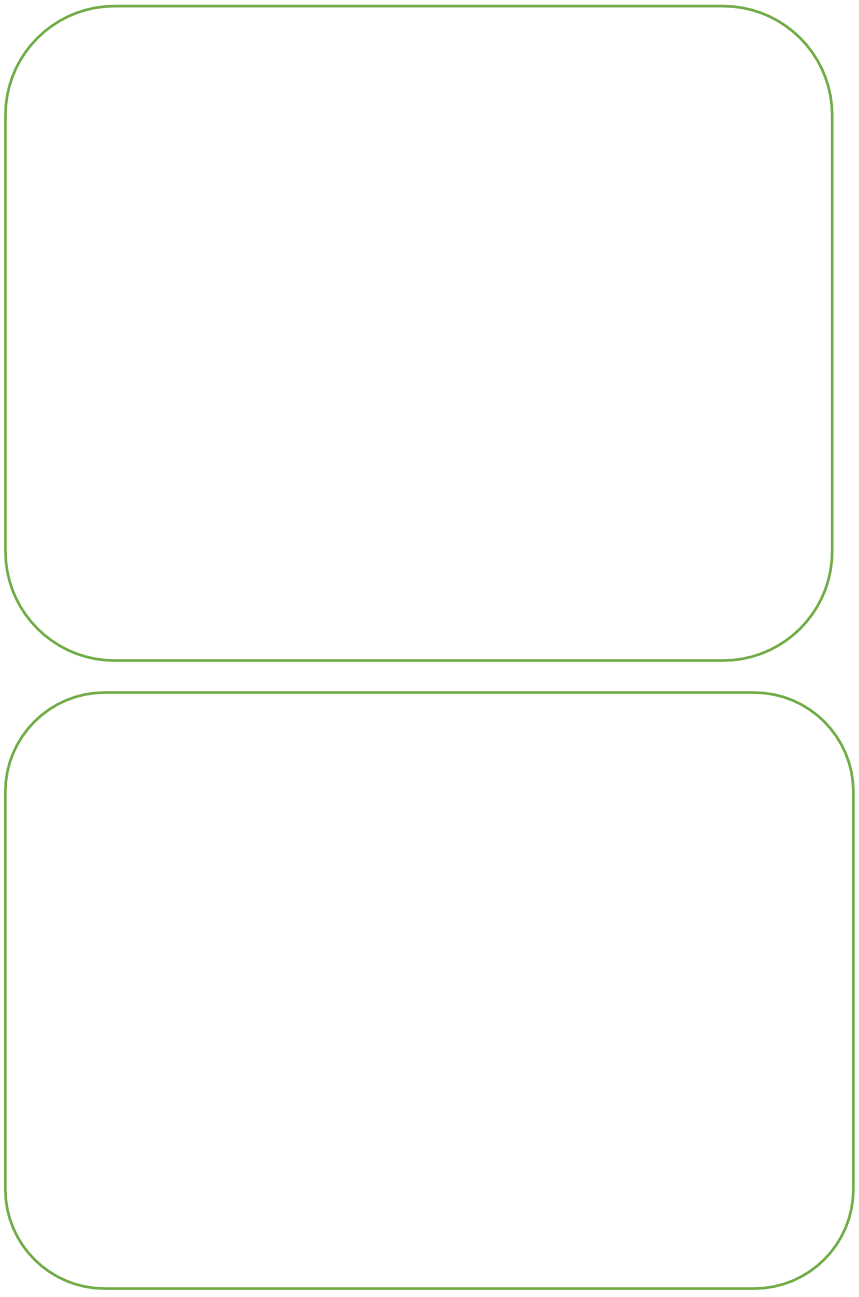
Secondary metabolites are phenolic compounds synthesized primarily by the fungal component (mycobiont) of lichens. They are also known as lichen acids or lichen metabolites. Their synthesis occurs through three biosynthetic pathways: the acetyl-malonate pathway, the shikimate pathway, and the mevalonate pathway. More than 1,000 lichen metabolites have been described. The content of secondary metabolites in dry thallus ranges from 0.1% to 10%, and in some species, even up to 30%. Their solubility in water is very low or almost negligible. Organic solvents such as acetone or methanol are used to dissolve them. Secondary metabolites occur as crystals on the surface of the mycobiont hyphae, from where they can be washed off with solvents. The extract contains several secondary metabolites with similar polarities, making separation sometimes challenging. Thin-layer chromatography (TLC) is commonly used for the identification of secondary metabolites. With the correct selection of the mobile phase and the appropriate ratio of polar to non-polar phases, it is possible to effectively separate the extract containing major secondary metabolites.

Procedure:

1. Take small pieces of two different lichen species and place them on a microscope slide.
2. Using a dropper, apply a drop of acetone to the lichens (work in a well-ventilated area) and let the drop evaporate. Repeat the process 3–4 times until a visible coating is observed on the thallus surface.
3. Gently remove the thallus with tweezers, leaving the extract on the slide.
4. On the area with the crystallized metabolites, apply a drop of a mixture of glycerol and glacial acetic acid (1:1), cover with a coverslip, and gently heat to dissolve the metabolites.

5. Examine the prepared slide under a microscope and sketch the shapes of the secondary metabolites.

Drawing:



Task 3: Separation of Secondary Metabolites Using TLC Plate in a Developing Chamber.

Procedure:

1. Place a small piece of the thallus into an Eppendorf tube with acetone to wash out secondary metabolites.
2. Prepare a chamber with the mobile phase and a TLC plate.
3. Mark the start and end points on the TLC plate, and use a small capillary to apply the extract from the Eppendorf tube onto the plate.
4. Allow the plate to develop, then observe how the extract separates under a UV lamp.
5. Mark the spots representing secondary metabolites with different polarities.
6. Dip the plate into a mixture of sulfuric acid and vanillin, burn it, mark the secondary metabolites, and calculate the Rf factor.

Evaluation:

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11. Division BRYOPHYTA

Theoritical introduction

The division Bryophyta is one of the oldest group (Chopra 2005) and exhibits **progressive** features, similar to higher plants. Some species of bryophytes have a simple system of conducting elements called **hydroids** and **leptoids.**

The morphology of bryophytes is distinguished by the alternative of the life cycles. The dominant life cycle is called the **gametophyte (n)**, and the stage that produces spores, called the **sporophyte (2n)**. In bryophytes, the sporophyte is dependent on the gametophyte (the opposite of higher plants). The photosynthetic part is represented by the gametophores, which is anchored to the substrate using rhizoids. **Rhizoids** are simple hair-like extensions growing from the epidermal cells of bryophytes. Their primary role is anchoring and attachment to the substrate, but depending on the species, bryophytes may also use rhizoids for water and nutrient uptake, but not for extensive transport. The male reproductive organ is called the **antheridium** and produces reproductive cells known as spermatozoids. The female reproductive organ, the **archegonium**, has a flask-like shape and contains a single reproductive cell known as the **oosphere**. Bryophytes also reproduce **vegetatively** through **fragmentation** of the thallus or the formation of various structures (for example : **gemmae)** .

On the gametophyte, the following structures can be identified: rhizoids (root-like structures), cauloids (stem-like structures), and phylloids (leaf-like structures). The gametophyte (namely gametophore – green part of it) represents the photosynthetic part and is anchored to the substrate. The sporophyte consists of the following parts: the seta, sporangium, peristome (mouthpart), and operculum (cap). Spores are formed in the sporangium. In young sporophytes, the calyptra (cap) can also be observed. Young sporophytes can also assimilate and do the photosynthesis.

In terms of the importance of organelles, vacuoles play a significant role in bryophytes. Besides basic functions such as cell expansion, storage of soluble substances, and water balance, they play a key role in the transport and storage of more than 200,000 known to date secondary metabolites, including phenolic, terpenoid, and alkaloid compounds. Most of these metabolites are considered as a defence mechanism against biotic and abiotic stress.

Mosses are classified as **acrocarpous** and **pleurocarpous**, with the basic differences as follows:

- Acrocarps are usually unbranched and upright, forming a mound-like colony.
- Acrocarpous mosses grow more slowly than pleurocarpous mosses.
- The sporophytes of acrocarpous mosses emerge from the tips of the gametophores, and the gametophore growth stop
- Pleurocarpous mosses branch freely and sprawlingly in a various pattern.

- The sporophytes of pleurocarpous mosses appear laterally in the middle of the stem (seta), and the moss apex continue to grow.
- Due to their growth speed, pleurocarpous mosses better colonize hard substrates, including rocks.

Liverworts (Marchantiophyta), are a division of spore-producing plants belonging to bryophytes. They contain **oil bodies**—intracellular organelles bounded by a single membrane containing lipophilic globules. These are distinct organelles uniquely found in liverworts. They play a dynamic role in cellular metabolism and serve as sites for the accumulation and sequestration of essential oil. Oil bodies are widely used in taxonomy and chemosystematics, and many of their secondary metabolites are known to be bioactive, considered potential sources of medicinal compounds.

Task 1: Prepare a native specimen from representatives of the division Marchantiophyta (*Marchantia polymorpha*). In the specimen, identify and draw the following cellular structures: air chambers, air pores, epidermis, chlorenchyma, parenchyma, and vacuoles.

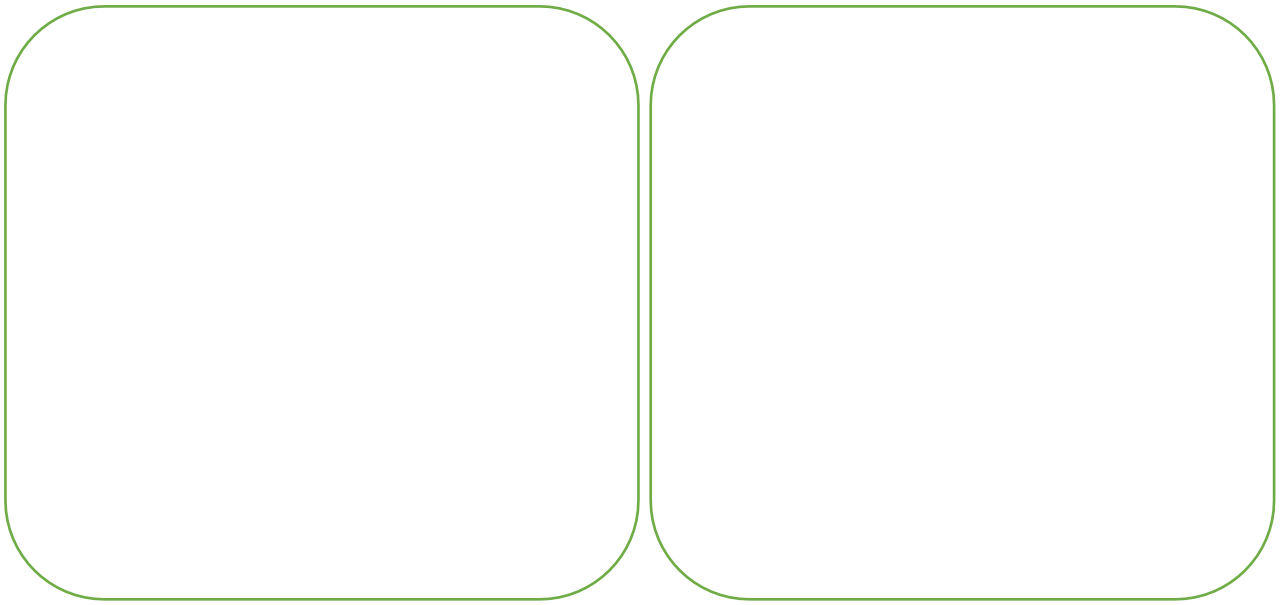
Drawing:

a) *Marchantia polymorpha*

b)

Task 2: Observe, draw and describe one representative of mosses that forms a pleurocarp and one representative that forms an acrocarp.

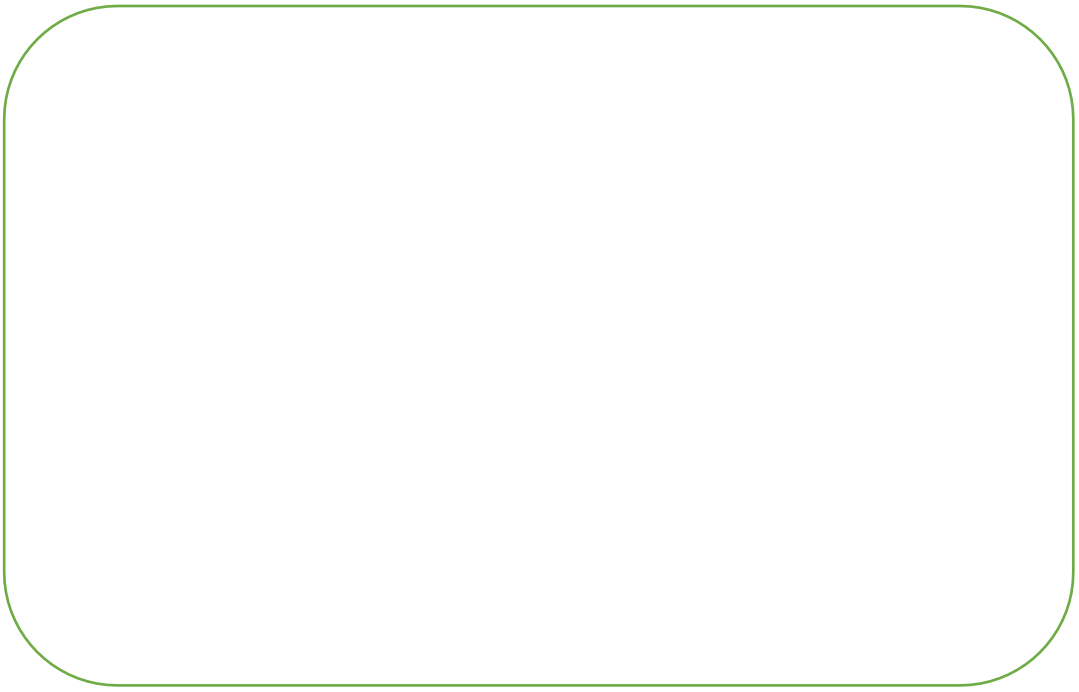
Drawing:



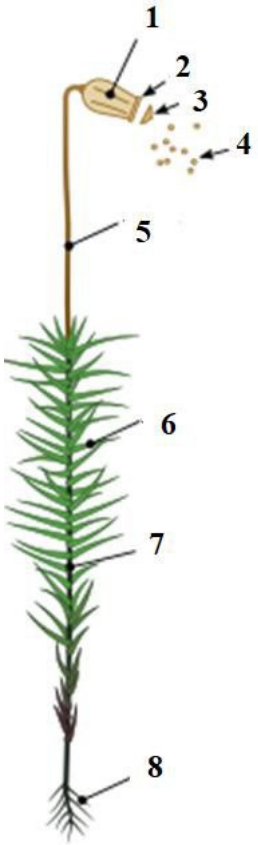
Pleurocarp Acrocarp

Task 3: Observe the liverwort *Marchantia polymorpha*, the gemmae and prepare a specimen. Draw the specimen, identify, and describe the oil bodies.

Drawing:



Task 4: Describe the following image.



1 .....

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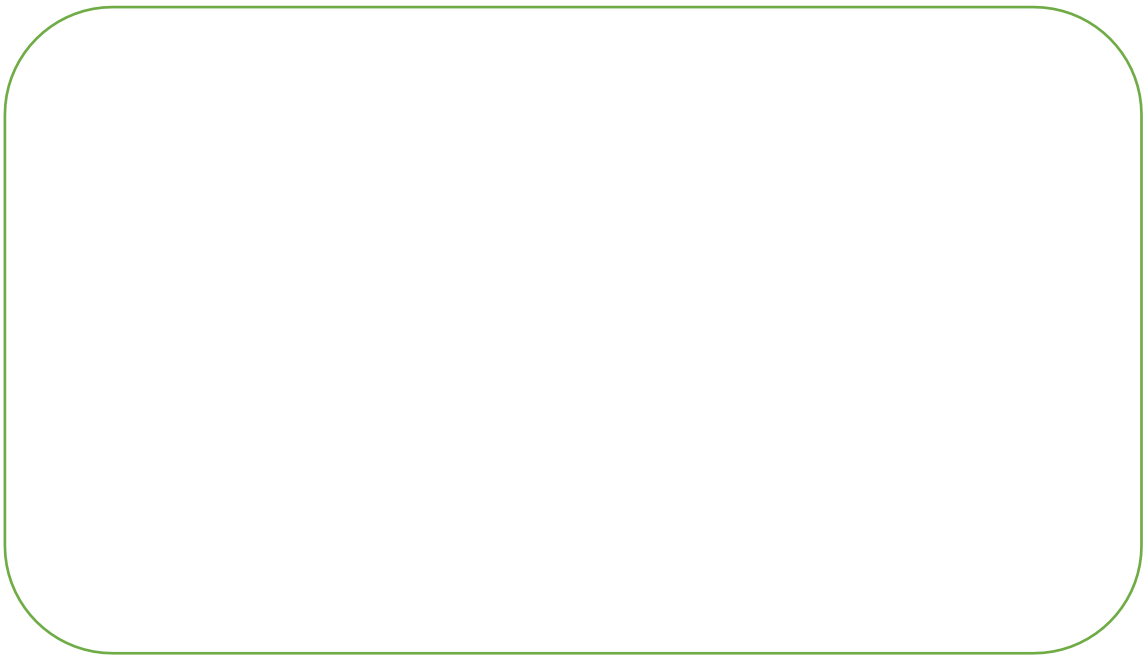
Task 5: Prepare a specimen of peat moss (*Sphagnum sp.*) and observe the hyalocytes/chlorocytes.

Drawing:



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Glossary of terms:

Amoeboid – a cell without a cell wall that forms pseudopodia, which it uses for movement or to capture food.

Apical – growing or extending at the apex

Apex – the peak of a structure or the end of a filament.

Apothecium – a cup-shaped fruiting body of ascomycete fungi.

Ascocarp- the fruiting body of ascomycete fungi.

Ascospores – spores of ascomycete fungi that arise sexually.

Ascus – see "sac."

Ascus – Site of meiotic division and ascospore formation.

Basidiocarp – the tertiary mycelium of basidiomycete fungi; the fruiting body that arises from secondary mycelium.

Basidiole – sterile cells in the hymenium of basidiomycete fungi.

Basidium – a specialized structure in the hymenium where basidiospores are formed.

Cenocytic – without septa between cells; a multinucleate cell often in a tubular shape.

Capillitium – sterile threads in the fruiting body of slime molds that bear spores.

Cleistothecium – a closed fruiting body of ascomycete fungi.

Coprophilous – growing on excrement.

Conidiospores – conidia, spores produced asexually.

Cyst – a thick-walled cell resistant to adverse conditions.

Cystid – sterile cells in the hymenium of basidiomycete fungi.

Dikaryotic stage – a stage with two genetically compatible (but different) haploid nuclei within one cell.

Diploid – having two sets of chromosomes.

Dolipore – a pore in the septa of basidiomycete fungi characterized by a more complex thickened structure.

Ecium – a spore-producing structure of rust fungi in the tissue of the secondary host.

Etalium – a fruiting body of slime molds with variable shape and size that develops from the entire plasmodium.
Flagellated – a cell with one or more flagella.

Fragmentation – a method of asexual reproduction in lower plants that occurs through the breaking off of a part of the thallus.

Gleba – the hymenium in the basidiocarp of the class Gasteromycetes, enclosed by a peridium.

Haploid – having one set of chromosomes.

Heterokaryon – secondary mycelium of basidiomycete fungi formed after the interaction of two compatible homokaryotic mycelia; it contains genetically distinct nuclei and is haploid.

Heterokont – having flagella of unequal lengths and structures.

Heterotrophic – feeding on organic substances synthesized by other organisms.

Homokaryon – primary mycelium of basidiomycete fungi characterized by the presence of identical nuclei in the cells.

Hypha – a filament of fungal mycelium.

Hymenium – the spore-producing layer of fungi.

Hypogeous – living below the surface.

Hypothallus – a thin layer of plasmodium at the base of the fruiting body in slime molds.

Isodiametric – having a regular shape, with properties that are the same in every direction of the cell.

Lateral – growing on the side of a structure.

Lignicolous – growing on wood.

Lichenized – living in symbiosis with algae/cyanobacteria.

Meiosis – a reduction division of cells, 1 diploid → 4 haploid daughter cells.

Mitosis – cell division, 1 diploid → 2 diploid.
Mycelium – a collection of hyphae; the thallus of fungi.

Mycorrhiza – a symbiosis between fungi and the roots of plants.

Oogamy – A sexual process where a non-motile female gamete (oosphere) is fertilized by a motile male gamete (spermatozoid).

Ostiolum – Opening of the perithecium.

Paraphysis – Sterile hypha of fungi.

Perithecium – Flask-shaped fruiting body of sac fungi.

Phagotropic – a mode of nutrition in which the organism surrounds its prey (often bacteria and small algae) by rearranging cytoplasm and forming pseudopodia to engulf and digest it.

Plasmodium – Naked multinucleated thallus of slime molds.

Plectenchyma – Fungal tissue where hyphae grow apically and are secondarily aggregated.

Protoplast – Cell enclosed by the cytoplasmic membrane.

Prosenchyma – Fungal tissue with hyphae arranged in parallel.

Pseudoparenchyma – Fungal tissue made up of isodiametric cells.

Sorus – Spore-bearing structure of smut fungi.

Sporangiophore – Specialized hypha bearing sporangia.

Sporangium – Spore case; site of spore formation; in slime molds, a specific type of fruiting body with uniform structures in shape and size.

Peridium – Various types of firm tissue enclosing/protecting the fertile part of the fruiting body.

Pseudoetallium – Compound fruiting body of slime molds formed from several or many sporangia.

Plasmodiocarp – Indeterminate structure retaining the shape of the original plasmodium.

Teliospore – Durable thick walled spore of rusts and smuts where karyogamy occurs.

Telium – Cluster of dikaryotic cells in the tissue of the primary host that give rise to teliospores.

Uredinium – Cluster of dikaryotic cells in the tissue of the primary host from which urediniospores are produced.

Yeast Stage – Single-celled stage of the thallus.

Zoospore – Motile spore.

Zygospore – Thick-walled, non-motile spore of the Zygomycota phylum.

Zygote – Cell formed by the fusion of two haploid gametes.

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BIOLOGY OF CRYPTOGAMS (practical lessons)
Course Notes

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