PRACTICAL PART

In the laboratory:

1. Open the envelope. You will be given the text of the tasks and the answer sheet.

Fill your **name, surname and code on the cover page** and only the **code on**

each page of the answer sheet.

2. Only questions concerning materials and equipment are allowed.

3. After one hour **the tasks and answer sheets will be collected** by assistants.

ONLY ANSWER SHEET WILL BE CHECKED.

GOOD LUCK!

4.1.1 Laboratory «PLANT PHYSIOLOGY, MORPHOLOGY AND ANATOMY».

Dear Participants!

In the laboratory "**PLANT PHYSIOLOGY, MORPHOLOGY AND ANATOMY"** you will be given the following three tasks:

Task 1. The study of physical and chemical properties of photosynthetic pigments.

Task 2. The study of angiosperm flowers structure.

Task 3. The study of anatomic structure of a plant organ on a cross section.

Duration of the lab work is **60 minutes.**

Maximum number of points – **68**.

You have to write down your results and answers into the **ANSWER SHEET** which will be collected by an assistant when the time elapses. It is not necessary to write anything in the task sheets.

Result sheets taken away from the laboratory will not be accepted!

Please be careful when performing reactions and do not let the reagents and solutions come in contact with your skin and clothes! Use gloves when necessary!

Contact the assistant in case of any unforseen situations!

Good luck!

Country_____

First name_____ Family name _____

Code_____

<u>Task 1.</u> (35 points) The study of physical and chemical properties of photosynthetic pigments.

The conversion of the energy of light into chemical energy occurs in plants with the help of pigment-protein complexes of chloroplast membranes. These complexes include photosynthetic pigments which determine the activity of the primary photosynthetic processes. An understanding of photosynthesis is impossible without knowledge of photosynthetic pigment properties. Chlorophyll and other photosynthetic pigments have several specific properties: absorption of different wavelengths of light, ability to participate in redox reactions, solubility in different types of solvents, etc.

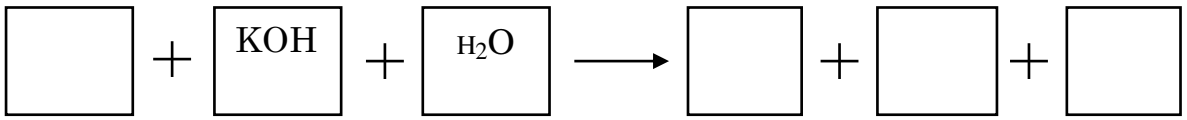
You have to study several of these properties of photosynthetic pigments during this task.

Materials and equipment

1.	A stand with tubes.	1
2.	Pipettes.	5
3.	Ethanol extract of photosynthetic pigments (Flask A).	1
4.	20 % KOH solution (Flask B).	1
5.	Distilled water (Flask C).	1
6.	Petrolic (petroleum) ether (Flask D).	1
7.	A sheet of white paper.	1
8.	A water bath.	1
9.	A tube holder.	1
10.	10 % HCl solution (Flask E).	1
11.	Saturated $(CH_3COO)_2Zn$ solution (Flask F).	1
12.	Saturated ascorbic acid solution (Flask H).	1

1.1. (8 points) Transfer 3 ml of pigment solution from **flask A** into tube № 1 and also 3ml into tube № 2.
Add five drops of 20% KOH from **flask B** and 1 ml of H_2O (from **flask C**) to the tube № 1 and to the tube № 2 - only 1 ml of H_2O.

Fill in the missing components of the chemical reaction you have just observed in scheme 1.1 of the answer sheet. Please use the number corresponding to the appropriate formulae from the list below.



1. $C_{55}H_{72}O_5N_4Mg$ – chlorophyll.
2. $C_{34}H_{30}O_5N_4\ MgK_2$ – potassium salt of the chlorophyllic acid.
3. $C_{55}H_{74}O_5N_4$ – pheophytin (phaeophytin).
4. $C_{20}H_{39}OH$ – phytol.
5. CH_3OH – methanol.
6. C_2H_5OH – ethanol.
7. $MgCl_2$ – magnesium chloride.
8. KCl – potassium chloride.

1.2. (4 points) Add 1 ml of the petrolic (petroleum) ether (from the **flask D**) to the tubes № 1 and № 2, shake well and leave to stand until the fractions separate completely.

Determine the colour of each fraction in the tubes № 1 and № 2. Write down the results in the appropriate cells of the table 1.2 of the answer sheet. Please use single letter colour codes as shown below.

A. violet;
B. blue;
C. green;
D. yellow;
E. red;
F. olive brown;
G. black;
H. colourless;

Tube №	Reagent	Experiment 1.1.	Experiment 1.2.
		ethanol fraction colour	**petrolic ether fraction colour**
1	KOH		
2	**H_2O**		

1.3. (4 points) Which pigments are responsible for the colour of the petrolic fraction on the tubes № 1 and № 2? Write down in the answer sheet (1.3) single letter codes for the compounds from the list below:

№ 1: _____
№ 2: _____

A. anthocyanins;
B. carotenoids;
C. phycobilins;
D. chlorophylls;

1.4. (2 points) Add 3 ml of the pigment extract to the tube № 3 (**flask A**) and add 5 drops of HCl (**flask E**). Mix the tube contents thoroughly by shaking. Record the new colour. Add 1 ml of the saturated $(CH_3COO)_2Zn$ solution (from the **flask F**) to the same tube. Heat the solution on the water bath. Mix by shaking andrecord the new colour of the solution.

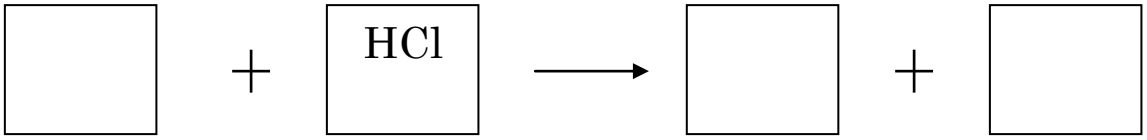
Write the results down in the table 1.4 of the answer sheet. Please use single letter colour codes as shown below.

A. violet;
B. blue;
C. green;
D. yellow;
E. red;
F. olive brown;
G. black;
H. colourless.

Reagent	New colour in the tube
HCl	
$(CH_3COO)_2$ Zn	

1.5. (6 points) In the scheme 1.5 of the answer sheet, please write the possible components of the reaction in the tube № 3 after addition of hydrochloric acid to the

pigment extract. Please use the number corresponding to the appropriate formula from the list below.



1. $C_{55}H_{72}O_5N_4Mg$ – chlorophyll.
2. $C_{34}H_{30}O_5N_4MgK_2$ – potassium salt of the chlorophyllic acid.
3. $C_{55}H_{74}O_5N_4$ – pheophytin (phaeophytin).
4. $C_{20}H_{39}OH$ – phytol.
5. CH_3OH – methanol.
6. C_2H_5OH – ethanol.
7. $MgCl_2$ – magnesium chloride.
8. KCl – potassium chloride.

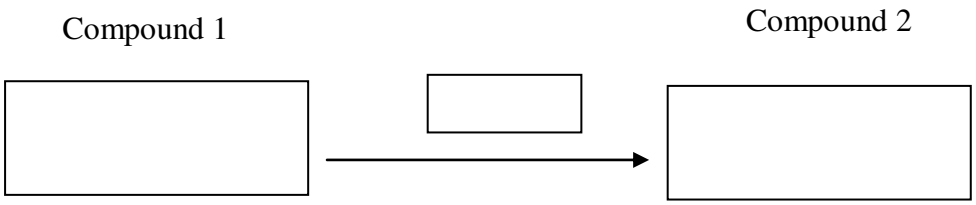
1.6. (1 point) Add 2 ml of the pigment extract and 2 ml of ascorbic acid (**flask H**) to the tube № 4. Mix by shaking until the colour changes.

Please note the colour change. Put the results in the table 1.6 in the answer sheet. Please use the single letter colour codes shown below.

A. violet;
B. blue;
C. green;
D. yellow;
E. red;
F. olive brown;
G. black;
H. colourless.

Extract colour before reaction	Solution colour after reaction
C	

1.7. (6 points) Complete the scheme of this reaction (1.7 in the answer sheet) using compound and condition numbers from the two lists below:



Compounds:

1. chlorophyll;

2. pheophytin (phaeophytin);

3. ascorbic acid;

Conditions:

4. electrons involved;

5. protons involved;

6. light involved.

1.8. (4 points)

Write the results down in the table 1.8 of the answer sheet. Please use single letter colour codes shown below.

A. violet;
B. blue;
C. green;
D. yellow;
E. red;
F. olive brown;
G. black;
H. colourless.

Compound №	Colour before reaction	Colour after reaction
1		
2		

Task 2. (12 points) The study of angiosperm flowers structure.

Materials and equipment

1.	Fixed flower preparations (A, B, C).	x 3
2.	Forceps.	x 1
3.	Dissecting needles.	x 2
4.	A magnifying glass.	x 1

2.1. (6 points) Study the morphology of flowers A, B, C. Using formula numbers (1-14) from the list below, indicate the correct formula for each flower in the answer sheet.

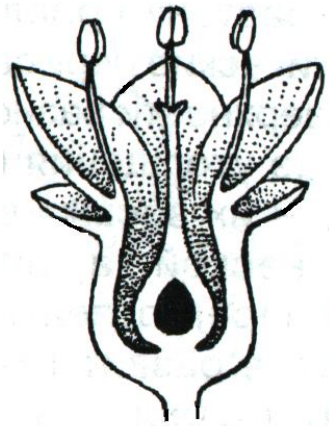
1. $* \; K_5 \; C_5 \; A_\infty \; \underline{\underline{G_\infty}}$
2. $* \; P_5 \; A_\infty \; \underline{G_\infty}$
3. $* \; K_5 \; C_5 \; A_{5+5} \; G_{(\underline{3})}$
4. $* \; K_{(5)} \; C_5 \; A_{5+5} \; G_{(\underline{5})}$
5. $* \; K_5 \; C_5 \; A_\infty \; G_{\underline{1}-}$
6. $* \; K_{(5)} \; C_5 \; A_\infty \; G_{\overline{(5)}}$
7. $\uparrow \; K_{(5)} \; C_{1,2,2} \; A_{(5+5)} \; G_{\underline{1}}$
8. $\uparrow \; K_{(5)} \; C_{1,2,2} \; A_{(9)1} \; G_{\underline{1}}$
9. $* \; K_0 \; C_5 \; A_5 \; G_{\overline{(2)}}$
10. $* \; K_{2+2} \; C_4 \; A_{2+4} \; G_{(\underline{2})}$
11. $\uparrow \; K_{(5)} \; C_{(2,3)} \; A_{2,2} \; G_{(\underline{2})}$
12. $* \; K_{(5)} \; C_{(5)} \; A_5 \; G_{(\underline{2})}$
13. $\uparrow \; K_0 \; C_{(5)} \; A_{(5)} \; G_{\overline{(2)}}$
14. $* \; P_{3+3} \; A_{3+3} \; G_{(\underline{3})}$

* = polysymmetrical

↑ = monosymmetrical

A	B	C

2.2. (3 points) The diagrams show the types of ovaries characteristic of angiosperm flowers. Using the numbers (1-4) from the table below, record the types of ovaries for the flowers A, B and C in the answer sheet.

 **1**.	 **2**.	 **3**.	 **4**.
Superior ovary (Hypogynous flower)	Middle ovary (Perigynous flower)	Half-inferior ovary (Half -epigynous flower)	Inferior ovary (Epigynous flower)

A	B	C

2.3. (3 points) Please indicate in the answer sheet to which family the plants with flowers A, B and C belong. Use the numbers (1-10) from the list below.

1. Ranunculaceae (buttercups)
2. Oleaceae
3. Rosaceae.
4. Leguminosae (Fabaceae), Papilionaceae.
5. Fagaceae
6. Cruciferae (Brassicaceae).

7. Labiatae (Lamiaceae).

8. Solanaceae.

9. Compositae (Asteraceae).

10. Liliaceae.

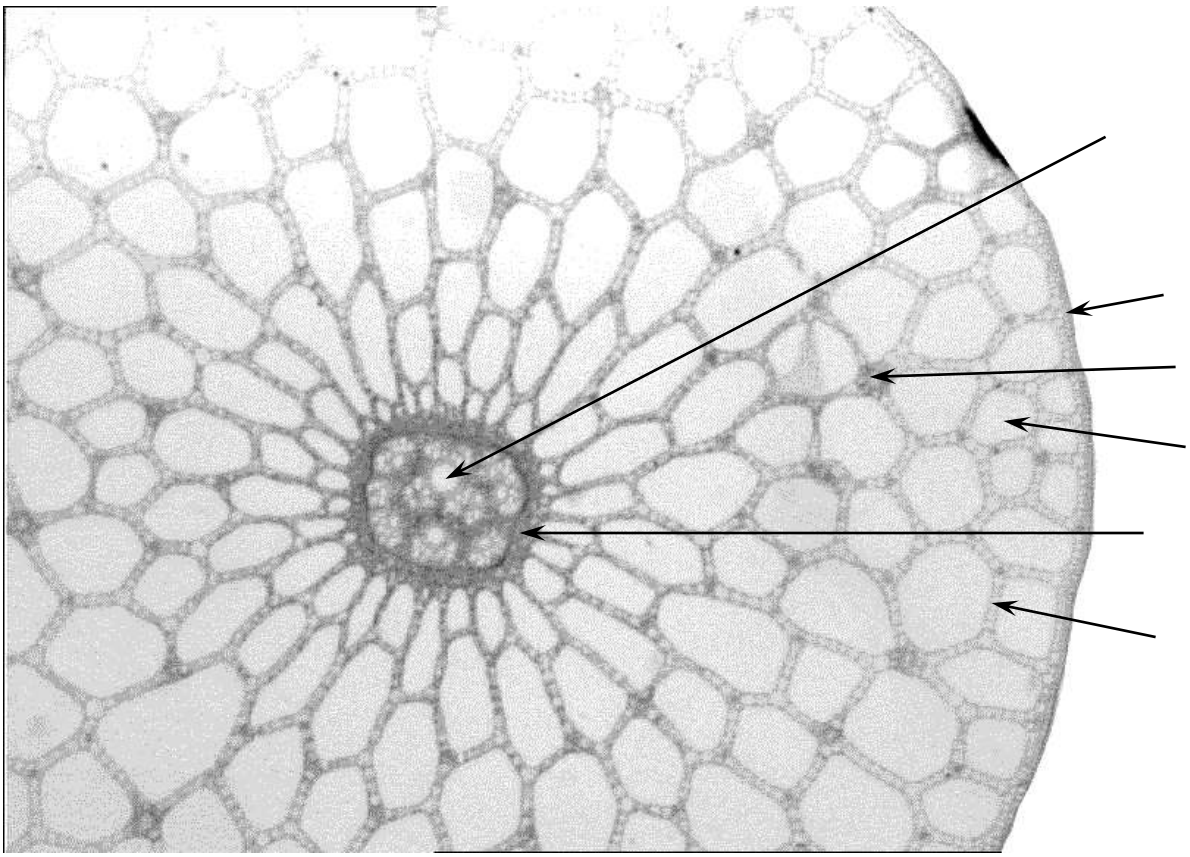
A	**B**	**C**

Task 3. (21 points) The study of anatomic structure of a plant organ on a cross section.

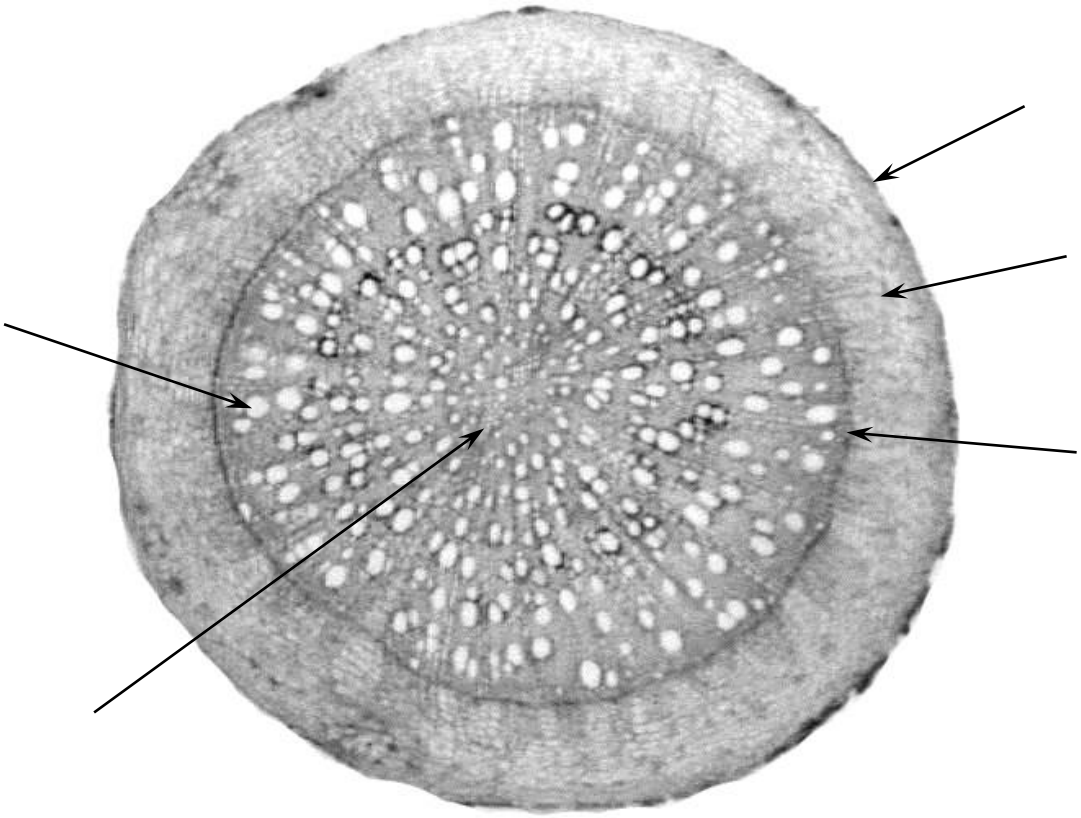
Materials and equipment

1.	Fixed parts of a plant organ.	1
2.	Microscope «Axiostar».	1
3.	Forceps.	1
4.	Dissecting needles.	2
5.	Blade.	1
6.	Glass slides.	2
7.	Cover slips.	4
8.	Dropping bottle with phloroglucin solution.	1
9.	Pipette.	1
10.	10 % HCl solution (Flask E).	1
11.	Distilled water (Flask C).	1

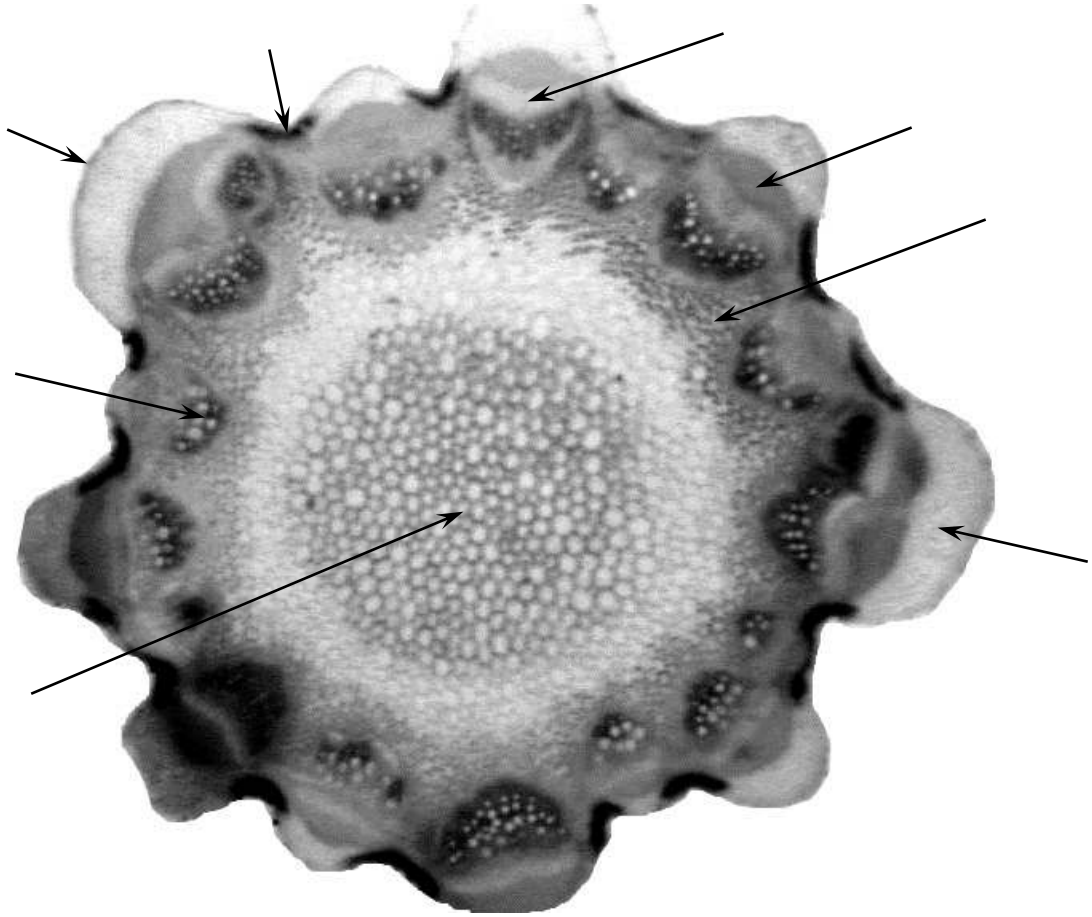
Prepare a cross section of the object you are given. Stain this cross section with phloroglucin and add several drops of HCl. Wash the preparation thoroughly with water for 2-5 minutes and then cover it with a cover slip. Observe the preparation under the microscope. Compare the cross section you have just prepared to the schemes 1-6 below and determine which scheme it corresponds to.



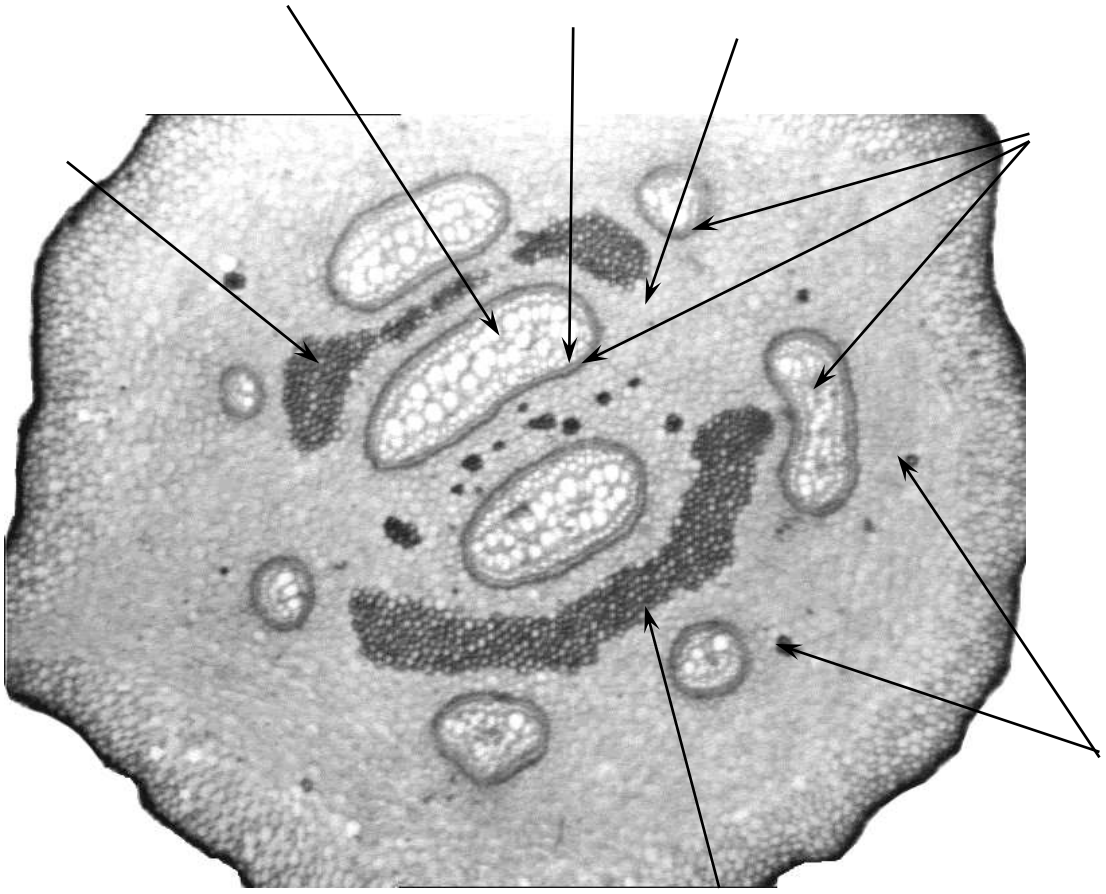
1



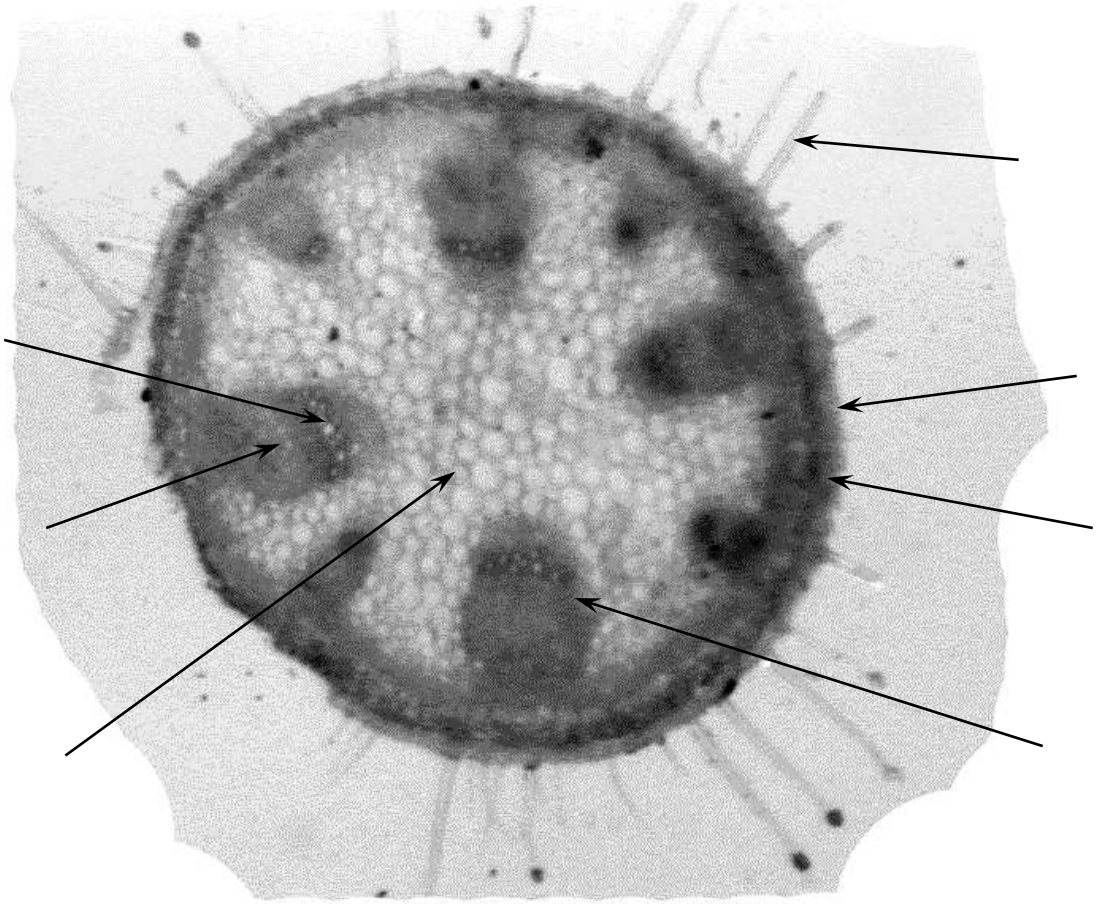
2



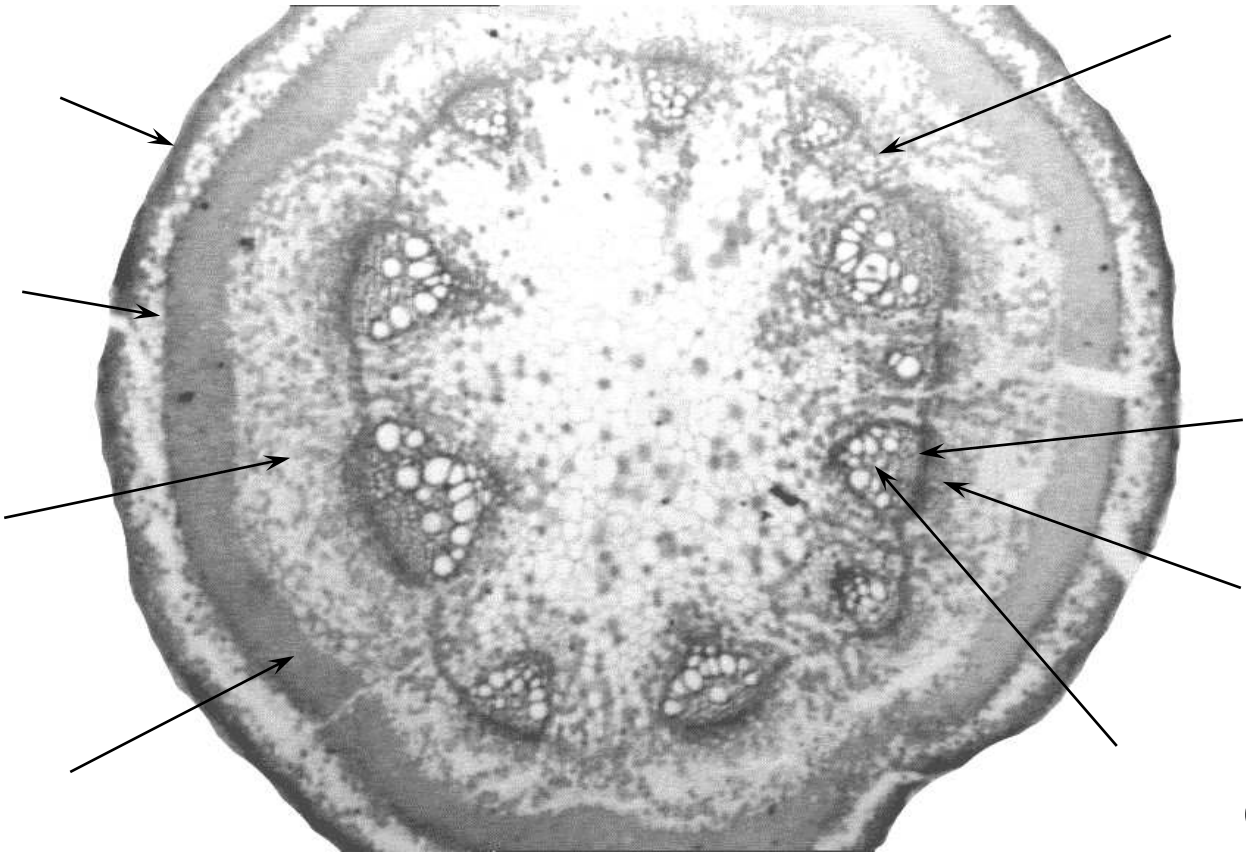
3



4



5



6

3.1. (8 points) Please label (using the numbers from the list below) the tissue elements pointed to by arrows on the scheme corresponding to **your** cross section in the answer sheet.

1. Endodermis.
2. Phloem elements.
3. Phellogen (Cork cambium).
4. Collenchyma.
5. Phelloderma.
6. Chloroplasts.
7. Epidermis.
8. Exodermis.
9. Core (Pith, Medulla).
10. Aerenchyma.
11. Periderm.
12. Sclerenchyma.
13. Pericycle.
14. Xylem elements.
15. Stoma.
16. Chlorenchyma.
17. Cambium.
18. Medullary ray (Pith ray).
19. Interfascicular cambium.
20. Fibrovascular bundle.

3.2. (9 points) What elements (1-18) are coloured by phloroglucin in the presence of HCl? Please, mark with "+" correct answer in the answer sheet.

1. Endoderm cells.
2. Elements of phloem.
3. Cells of phellem (Cork).
4. Cells of collenchyma.
5. Tracheids.
6. Vessel cells.
7. Epidermis.
8. Trichomes.
9. Stomata guard cells.
10. Root hair.
11. Cells of phellogen (Cork cambium).
12. Sclerenchyma fibers.
13. Pericycle cells.
14. Xylem elements.
15. Rhizoids.
16. Cells of parenchyma.
17. Cambium cells.
18. Satellite cells.

3.2.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18

3.3. (1 point) What compounds are coloured by phloroglucinin the presence of HCl? Write the corresponding number (1-6) from the list below into the answer sheet.

1. Cellulose.
2. Pectin.
3. Lignin.
4. Suberin.
5. Cutin.
6. Hemicellulose.

3.3.:_____

3.4. (1 point) Determine which organ the cross section was made from. Write the corresponding number (1-6) from the list below into the answer sheet.

1. Root.
2. Stem.
3. Leaf stalk (Petiole).
4. Flower stalk.
5. Runner.
6. Rhizome.

3.4.:_____

3.5. (1 point) Determine which division of higher plants the plant you study belongs to. Write the corresponding number (1-4) from the list below into the answer sheet.

1. Lycopodiophyta.
2. Equisetophyta.
3. Polypodiophyta.
4. Pinophyta.
5. Magnoliophyta.

3.5.:_____

3.6. (1 point) Using the cross section you have just prepared, determine which ecological group (relative to water availability) the plant belongs to. Write the corresponding number (1-4) from the ecomorph list below into the answer sheet.

1. Hygrophyte.
2. Hydrophyte.
3. Mesophyte.
4. Xerophyte.

3.6.:_____

4.1.2 Laboratory «ANIMAL MORPHOLOGY, ANATOMY AND SYSTEMATICS».

Dear Participants!

In the laboratory "ANIMAL MORPHOLOGY, ANATOMY AND SYSTEMATICS" **you will be given the following three tasks:**

Task 1. Detaching pedes (extremities) of crayfish (*Astacus*) and determination of their function.

Task 2. Test for knowledge of animal taxa.

Task 3. Determination of species name of freshwater gastropod molluscs. molluscs.

The duration of the lab work is **60 minutes.**

Maximum number of points – **66.**

You have to write down your results and answers into the **ANSWER SHEET** which will be collected by an assistant when the time elapses. It is not necessary to write anything in the task sheets.

Result lists taken away from the laboratory will not be accepted!

Please note that the results from the task 1 must be shown to the assistant BEFORE the time limit!

Please do not forget to put zoological objects and instruments in their original positions when finished, as these will be used by the next group.

Should the mollusc shells become damaged, you can ask for a replacement.

Good luck!

Country_____

First name_____ Family name _____

Code_____

Task 1. (36 points) Detaching pedes (extremities) of crayfish (*Astacus*) and determination of their function.

Material, instruments and equipment

1.	*Astacus leptodactylus* (♂).	1
2.	A set of instruments (2 forceps, scissors, scalpel, dissecting needles).	1
3.	Dissecting tray	1
4.	A magnifying glass.	1
5.	Cotton sheet.	1
6.	Latex gloves.	1
7.	Pins marked 1 to 18.	18
8.	Foam plate for pins.	1

The narrow-fingered crayfish (*Astacus leptodactylus*) is quite common in fresh water bodies in temperate climates which are characterised by a relatively high content of dissolved oxygen and mineral salts. A magnifying glass is sufficient to study the structure of pedes (appendages) of crayfish.

You need to observe the details of animal's segmentation, to find its body parts and sequentially detach the pedes (appendages excluding the first (antennuales or smallest) pair of antennae) from one side of animal's body, assembling them in order on a plate with the help of pins. Then it is necessary to determine the function of each ped and write it down in the answer sheet.

Description of the techniques.

1. Take the animal in your hand, abdominal (ventral) side up. It is recommended to use a cotton sheet and latex glove. Beware of small spicules ***on the carapace!*** Carefully study the pedes of all body parts (with the help of a magnifying glass if necessary).

2. Using forceps sequentially detach all pedes from one side of animal's body. To do this, hold the ped at its base with the forceps and pull away from the crayfish. You can also use scissors and/or scalpel if necessary.

3. Assemble the pedes on pins with the corresponding numbers (1, 2, 3, etc.). Start umbering from the head. Put the pedes on the foam plate in the correct order.

Attention! The practical results of task 1 must be registered by an assistant on a special control sheet. The correctness of pedes preparation and numbering is scored. If a ped is damaged in the process of preparation to such an extent that it cannot be recognized, the points for this ped are not scored.

Please raise your hand when finished with the first task so that your work can be checked. If the assistant is busy with another participant, you should continue with the next task, but please note that the results of task 1 are not counted if they were shown to the assistant after the total time limit (60 minutes).

In the answer list of **task 1** each ped has 3 variants of its possible function. Study the table, determine the function for each ped, then mark the correct function for each ped in the table with a circle (●). **Note: a participant gets 1 point for every correct answer and loses 0.5 point for every wrong answer.**

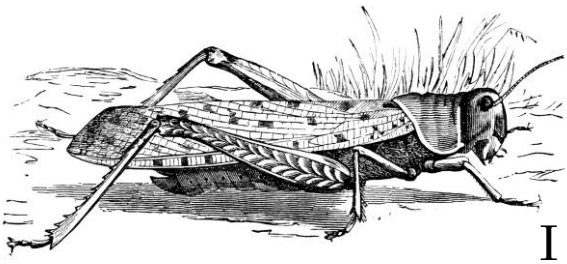
Pedes (extremities)			
№	Functions		
1.	○ sensory	○ respiratory	○ reproductive
2.	○ swimming	○ food grinding	○ respiratory
3.	○ transferring food to mouth	○ respiratory	○ reproductive
4.	○ reproductive	○ transferring food to mouth	○ sensory
5.	○ transferring food to mouth	○ walking	○ defence/attack
6.	○ defence/attack	○ transferring food to mouth	○ reproductive
7.	○ reproductive	○ swimming	○ respiratory
8.	○ swimming	○ capturing and holding food	○ reproductive
9.	○ reproductive	○ respiratory	○ defence/attack
10.	○ reproductive	○ walking	○ sensory
11.	○ reproductive	○ transferring food to mouth	○ walking
12.	○ walking	○ food grinding	○ sensory
13.	○ walking	○ reproductive	○ defence/attack
14.	○ walking	○ respiratory	○ reproductive
15.	○ defence/attack	○ swimming	○ walking
16.	○ swimming	○ food grinding	○ respiratory
17.	○ reproductive	○ sensory	○ swimming
18.	○ swimming	○ transferring food to mouth	○ respiratory

Task 2. (10 points) Animal taxonomy test.

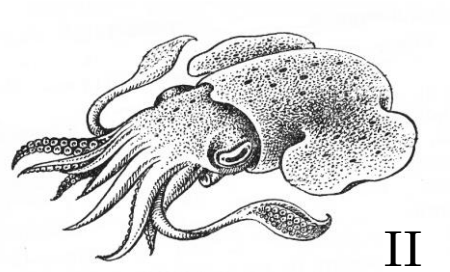
Page 7 has pictures of ten animals numbered with roman numerals. The table below has the names of animal phyla (A–K), subphyla or classes (a–k) and genera (1–10).

Phylum		Subphylum/Class		Genus	
A.	***Annelida.***	**a.**	***Anthozoa.***	**1.**	***Araneus.***
B.	***Arthropoda.***	**b.**	***Cephalopoda.***	**2.**	***Asterias.***
C.	***Chordata.***	**c.**	***Chelicerata.***	**3.**	***Corallium.***
D.	***Cnidaria.***	**d.**	***Crustacea.***	**4.**	***Cyclops.***
E.	***Echinodermata.***	**e.**	***Hydrozoa.***	**5.**	***Fasciola.***
F.	***Mollusca.***	**f.**	***Insecta.***	**6.**	***Hydra.***
G.	***Nematoda (Nemathelminthes)***	**g.**	***Polychaeta.***	**7.**	***Locusta.***
H.	***Platyhelminthes.***	**h.**	***Scyphozoa.***	**8.**	***Musca.***
J.	***Porifera.***	**j.**	***Asteroidea (Stellaroidea)***	**9.**	***Nereis.***
K.	***"Protozoa".***	**k.**	***Trematoda.***	**10.**	***Sepia.***

Please label the taxonomic position of each animal using the information from the table – put the corresponding code for phylum, subphylum/class and genus next to animal picture in the answer sheet.



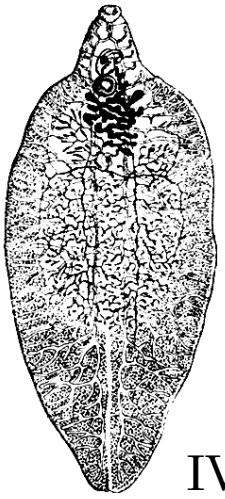
I



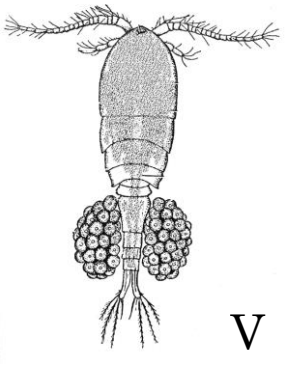
II



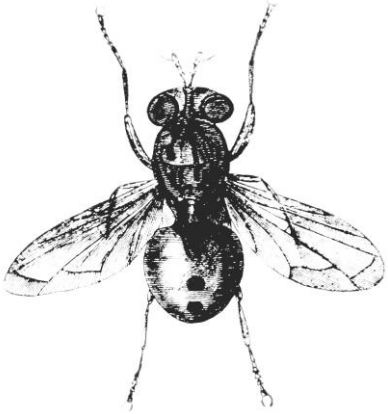
III



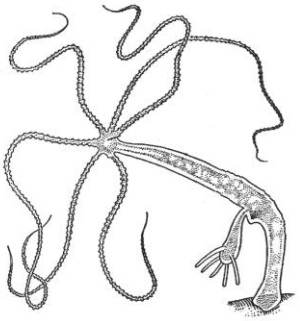
IV



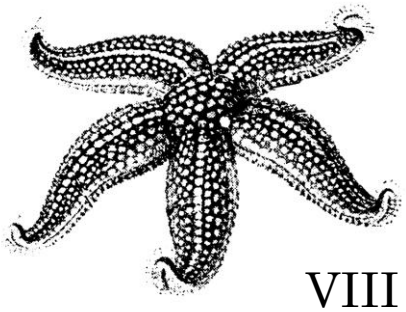
V



VI



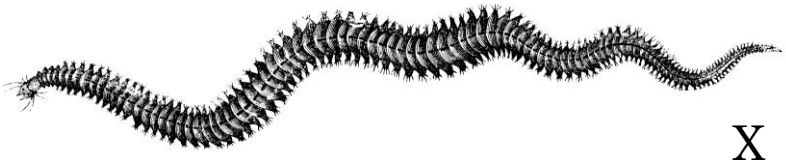
VII



VIII



IX



X

Task 3. (20 points) Determination of species name of freshwater gastropod molluscs.

Materials, instruments and equipment

1.	A tray with 10 shells of gastropod molluscs to be classified.	1
2.	An accessory tray for used shells.	1
3.	A ruler.	1
4.	A set of instruments (forceps, dissecting needles).	1
5.	A magnifying glass.	1

Many species of gastropod molluscs live in fresh water. They play an important role in water ecosystems. Many are specific intermediate hosts of helminthes– parasites of humans and domestic animals. In this connection taxonomic identification of freshwater gastropod molluscs as not only theoretical, but also applied value.

The tray has 10 numbered shells of gastropod molluscs. The classification key below allows the identification of species names and includes illustrations explaining the details of shell structure and measurements. Classify the molluscs you are given and place the numbers written on their shells next to species names in the table in the answer sheet.

Species name	Shell number
Viviparus contectus	
Bithynia tentaculata	
Physa fontinalis	
Aplexa hypnorum	
Radix ovata	
Radix auricularia	
Lymnaea stagnalis	
Planorbarius corneus	
Planorbis planorbis	
Segmentina nitida	

CLASSIFICATION KEY

1a. Shell aperture (opening) has an operculum (lid)……(2)

1b. Shell aperture without an operculum (lid)……..(3)

2a. Shell is at least 20 mm high, green-brown, sometimes with three dark stripes on the last turn of the whorl……. *Viviparus contectus*

2b. Shell is not more than 15 mm high, uniformly brown without stripes……..*Bithynia tentaculata.*

3a. Shell is like a tower or a cone with variable number of turns……(4)

3b. Shell is flat……..(8)

4a. Shell is sinistral……..(5)

4b. Shell is dextral……..(6)

5a. Shell is egg-shaped. Whorl height is less then aperture height. Yellow- brown or light brown……*Physa fontinalis.*

5b. Shell has spindle-like shape. Whorl height is twice the aperture height. Brown or dark brown……*Aplexa hypnorum.*

6a. Aperture height is significantly more than whorl height…(7)

6b. Whorl height is equal or slightly exceeds aperture height. Shell is up to 60 mm high……..*Lymnaea stagnalis.*

7a. Aperture height is approximately twice its width. Shell height is up to 25 mm, width – up to 15 mm……*Radix ovata*

7b. Aperture height and width are approximately equal. Shell height is up to 40 mm, width – up to 30 mm…….*Radix auricularia.*

8a. Aperture has bud-like shape, its height exceeds its width ……..*Planorbarius corneus.*

8b. Aperture has another shape, its width exceeds its height… (9)

9a. Diameter of the shell is over 8 mm, walls are thick, 5–6 turns, curved at top with flat bottom. Shell walls are opaque, dark-brown ……*Planorbis planorbis.*

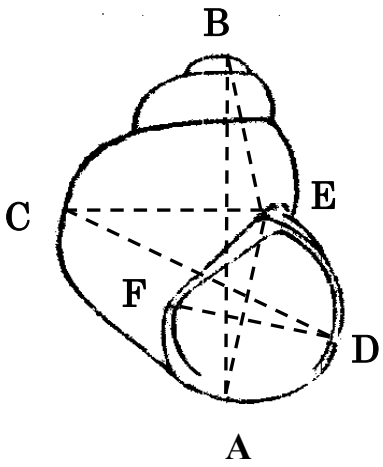
9b. Diameter of the shell is less than 8 mm, walls are thin, 4-5 turns, curved at top with plano-concave bottom. Shell walls are semi-transparent, light-brown. ……*Segmentina nitida.*

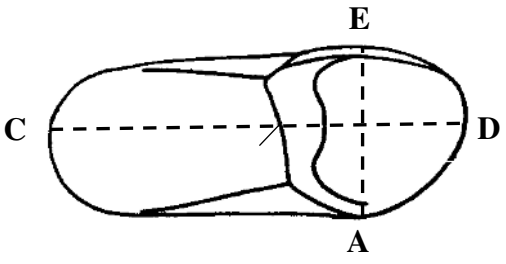




dextral shell

sinistral shell





Shell measurements of gastropod mollusks:

A–B — shell height,
C–D — shell width,
A–E — aperture height,
D–F — aperture width,
B–E — whorl height.

Should the mollusc shells become damaged, you can ask for a replacement.

Please do not forget to put zoological objects and instruments in their original positions when finished, as these will be used by the next group.

4.1.3 Laboratory «MICROBIOLOGY AND BIOTECHNOLOGY».

Dear Participants!

In the laboratory "**MICROBIOLOGY AND BIOTECHNOLOGY"** you will be given the following two tasks:

Task 1. Identification of microorganisms.

Task 2. Study of Bacterial cultures expressing different genes.

Duration of the lab work is 60 minutes.

Maximum number of points – **64** .

You MUST write down your results and answers on the **ANSWER SHEET** which will be collected by an assistant when the time elapses. It is not necessary to write anything in the task sheets.

Answer sheets taken away from the laboratory will not be accepted!

Please be careful when performing reactions and do not let the reagents and solutions come into contact with your skin and clothes!

PLEASE USE HAND DISINFECTION SOLUTIONS AFTER THIS PRACTICAL EXAMINATION

GOOD LUCK!

Country_____

First name_____ Family name _____

Code_____

Task 1. (46 points) Identification of microorganisms.

Materials and equipment

1. Bacterial strains in:

   - Three petri dishes with solid media (plate "GCO" – 1, plate “protease” – 1, plate “amylase” – 1);

   - tubes with solid medium (for “O/F-test”);

   - tubes with broth (for “H_2S-test” and “NR-test”).

2. Wooden toothpicks for transfer of bacterial biomass from solid medium onto glass slides.

3. Glass slides.

4. Pipettes.

5. KOH solution, 3 %.

6. H_2O_2 solution, 3 %.

7. Dimethylparaphenilendiamine (DMPA) solution, 1 % .

8. Lugol's solution (Lugol).

9. Griess solution, 1% (Griess).

Identification of bacteria is based on the study of certain biological properties, mostly morphological, physiological and biochemical characteristics. You have to identify five bacterial strains labelled № 1-5. For this you will have to perform five biochemical tests (1.1, 1.3, 1.4, 1.6 and 1.8). You will also use the results of the remaining tests given to you (tests 1.2, 1.5, and 1.7). Some tests are followed with additional questions on the corresponding topic that you have to answer.

Please fill your results in the table "Identification of bacteria" in the answer sheet using the following symbols: "+" for a positive reaction, "-" for lack of a reaction. A sample table is given on the next page. **Attention!** In the column "Gram reaction" you have to put "+" for Gram-positive bacteria and "-" for Gram-negative. In the column "O/F-test" put letter "F" for organisms with anaerobic respiration (fermentative metabolism) and letter "O" – for organisms with aerobic respiration (oxidative metabolism).

Fill all columns of the table except for the last one. Then identify your bacterium using identification table in the end of the task sheet and put the letter corresponding to the identified species into the column “ Result of identification”.

Identification of Bacteria (30 points)

Strain	Gram reaction	O\ F-test	The presence of:						Result of identification
			catalase	oxidase	protease	amylase	H_2S production	nitrate reductase	
1									
2									
3									
4									
5									

PLEASE BE CAREFUL WHEN PERFORMING REACTIONS AND DO NOT LET THE REAGENTS AND SOLUTIONS CONTACT YOUR SKIN AND CLOTHES!

PLEASE PUT USED PIPETTES, WOODEN TOOTHPICKS, GLASS SLIDES, FILTER PAPER, ETC. INTO A SPECIAL CONTAINER ON YOUR BENCH!

Test 1.1. Gram reaction

To perform this test you need:

1. Biomass of bacterial strains № 1-5 (from the GCO plate).

2. KOH solution (3 % KOH).

3. Five glass slides.

4. Wooden toothpicks.

Attention! You will need the «GCO» Petri dish later to perform tests 1.3 and 1.4. Please perform the tests in the suggested order: 1.1, 1.3, 1.4.

The method:

Using a dropping bottle, put a small drop of the 3 % KOH solution onto a glass slide. Using a toothpick, transfer some biomass (roughly 3-4 mm in diameter) of one strain to the KOH drop, trying not to transfer the agar. Mix the bacterial mass with the KOH solution thoroughly. If the mass sticks to the toothpick and moves behind it, the strain is Gram-negative, otherwise – Gram-positive. You may repeat the test if the results are not clear.

Using a new toothpick each time, repeat the test with the remaining strains. Put the results in the corresponding column of the "Identification of bacteria" table in the answer sheet using "+" for Gram-positive bacteria and "-" for Gram-negative.

Test 1.2. (O/F- test).

The O/F-test allows the determination of the ability of bacteria to utilise glucose in aerobic (oxidative metabolism) or anaerobic (fermentative metabolism) conditions. To determine the ability of your strains to utilise glucose aerobically and anaerobically, each strain was inoculated in advance into two tubes with agar medium containing the required mineral salts, glucose and a pH indicator ((water blue and rosolic acid) which is pink at neutral pH, blue at acidic pH and red at basic pH). To create anaerobic conditions, medium in the tubes labelled 1a – 5a was covered with vaseline oil immediately after inoculation, while the tubes 1b – 5b had no oil. The tubes were incubated in a thermostat for 24 hours.

Analyse the colour change in the tubes for each strain. Put the results in the column "O/F-test" in the table "Identification of bacteria" in the answer sheet. Use letter "F" for organisms with anaerobic respiration (fermentative metabolism) and letter "O" – for organisms with aerobic respiration (oxidative metabolism).

1.3. Catalase test.

To perform this test you need:

1. Biomass of bacterial strains № 1-5 (on the GCO plate).

2. Hydrogen peroxide solution (3 % H_2O_2).

3. Five glass slides.

4. Wooden toothpicks.

5. Pipettes.

The method:

Using a pipette, put a drop of hydrogen peroxide solution onto a glass slide. Using a toothpick, transfer some biomass of one strain from the GCO plate to the drop, trying not to transfer the agar. Mix the bacterial mass with the hydrogen peroxide solution thoroughly. Record the results while mixing the bacteria with the solution. Repeat the manipulation with the remaining four strains. Put the results in the corresponding column of the "Identification of bacteria" table in the answer sheet("+" if positive, "-" if negative).

Question 1.3.1. (2 points) Which reaction(s) is catalysed by catalase?

A. $3H_2O_2 + FADH_2 \rightarrow 3H_2O + O_2 + H_2 + FAD$
B. $2H_2O_2 \rightarrow 2H_2O + O_2$
C. $H_2O_2 \rightarrow 2HO^{\cdot}$
D. $H_2O_2 \rightarrow 2HO_2^{-} + H_2$
E. $2H_2O_2 + NADH + H^+ \rightarrow 2H_2O + NAD^+$

Put your answer code or codes into the line 1.3.1.

1.3.1. _____

Test 1.4. Cytochrome oxidase test.

To perform this test you need:

1. A Petri dish (GCO), with colonies of strains № 1-5.

1. 1 % solution of DMPA.

The method:

Using a dropping bottle, put a drop of DMPA onto each colony. 30-60 seconds later the colonies of oxidase-positive strains turn pink to dark red. Analyse the colony colour of each strain and fill the results in the corresponding column of the "Identification of bacteria" table in the answer sheet.

Question 1.4.1. (4 points) Which of the following statements are true for cytochrome oxidase positive bacteria?

A. Capable of using O_2 as terminal electron acceptor in the respiratory chain.

B. All are unable to undertake anaerobic respiration.

C. All are strict aerobes (obligate aerobes).

D. All are strict anaerobes (obligate anaerobes).

E. All are facultative anaerobes.

F. Cytochrome oxidase takes part in chemosynthesis in some strains.

Put your answer code or codes into the line 1.4.1.

1.4.1. _____

1.5. Proteolytic activity test.

For determination of proteolytic activity you must analyse a Petri dish with media containing casein, inoculated in advance with strains № 1-5. This plate is labelled "protease". Record the results in the table in the answer sheet.

Test 1.6. Amylase test.

The plate labelled "amylase" contains rich solid medium supplemented with 0.2% of starch and has been inoculated with strains № 1-5 in advance. Cover the surface of this plate with Lugol's solution (Lugol) and determine which bacteria have the amylolytic activity. Record the reaction results into the corresponding column of the "Identification of bacteria" table in the answer sheet.

1.7. Test for hydrogen sulphide generation (H_2S-test).

Here you must analyse five tubes prepared previously. The tubes contain meat broth that was inoculated with test strains some time before. The tubes also contain pieces of white indicator paper saturated with the solution of lead acetate. Record the results in the table in the answer sheet.

Record in the answer sheet the single letter code for the correct answer for each of the two questions below:

Question 1.7.1. (4 points) When bacteria which are capable of producing H_2S grow on meat broth medium, H_2S is generated from:

A. RNA.

B. DNA.

C. Arginine.

D. Methionine.

E. Serine.

F. Glycine.

G. Thiamine.

H. Biotin.

I. Taurine.

J. Cysteine.

Put your answer code or codes into the line 1.7.1.

1.7.1.: _____

Question 1.7.2. (2 points) Which reaction(s) is/are responsible for the colour change of the indicator paper?

A. $2CH_3COOH + H_2S = (CH_3CO)_2S + 2H_2O$

B. $Pb^{2+} + S^{2-} = PbS$
C. $(CH_3COO)_2Pb + H_2S = 2CH_3COOH + Pb + S$
D. $2CH_3COOH + H_2S = CHSCOOH + 2H_2$
E. $2CH_3COOH + Pb + 2H_2S = 2C_2H_6 + PbSO_4 + S$

Write your answer code or codes down in the line 1.7.2. of the answer sheet

1.7.2.:_____

1.8. Nitrate reductase test (NR-test).

For this reaction you need:

1.Tubes with suspensions of cells of strains № 1-5 marked as "NR".

2. Griess reagent, 1 % (Griess).

3.Pipettes.

Add 1 ml of the 1% Griess (Griess) reagent to the suspension of bacteria. The presence of nitrate reductase activity results in the appearance of red colour within 1 minute. Record the results in the table in the answer sheet.

Question 1.8.1. (4 points) **The presence of nitrate reductase allows:**

A. The use of nitrate as an electron acceptor in the electron transport chain during chemosynthesis.

B. The use of nitrate as an electron donor in the electron transport chain during respiration.

C. The use of nitrate as an electron donor in the electron transport chain during chemosynthesis.

D. The use of nitrate as an electron acceptor in the electron transport chain during respiration.

E. The use of nitrites as nitrogen source.

Write your answer code or codes down in the line 1.8.1. of the answer sheet.

1.8.1.:_____

Use your results and the identification table to identify the species of your strains.
Fill the results in the table in the answer list.

Identification table

	Genus, species	Gram reaction	O\ F-test	The presence of:					
				catalase	oxidase	protease	amylase	H_2S production	nitrate reductase
A	Escherichia coli	–	F	+	–	+	–	+	+
B	Xanthomonas campestris	–	O	+	–	+	–	+	–
C	Lactobacillus delbrueckii	+	F	–	–	+	–	+	–
D	Erwinia herbicola	–	F	+	–	–	–	+	+
E	Clavibacter michiganensis	+	O	+	–	–	+	+	–
F	Staphylococcus saprophyticus	+	F	+	–	–	–	–	–
G	Pseudomonas mendocina	–	O	–	+	–	–	–	+
H	Pseudomonas putida	–	O	+	+	+	–	–	–
I	Sarcina lutea	+	F	+	–	+	–	–	–
J	Streptobacillus moniliformes	–	F	–	–	–	–	–	–
K	Agrobacterium tumefaciens	–	O	+	+	–	–	+	+
L	Pseudomonas fluorescens	–	O	+	+	+	–	–	+
M	Bacillus subtilis	+	F	+	–	+	+	+	–
N	Streptococcus lactis	+	F	–	–	+	–	+	+

Task 2. (18 points) Study of Bacterial cultures expressing different genes.

Materials and equipment

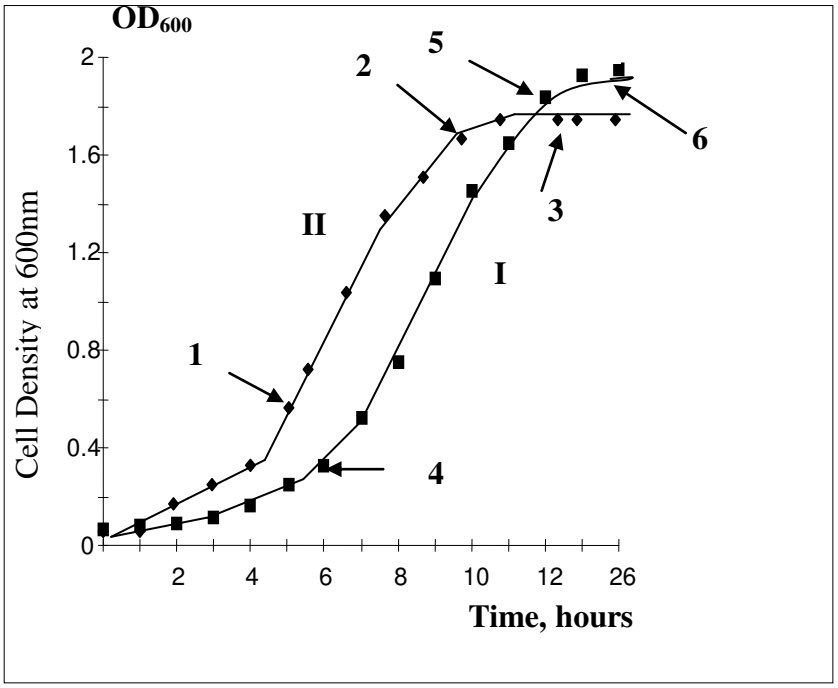
1.	**Six tubes with cells taken from cultures at different stages of growth.**	**6**
2.	**Distilled water (flask A).**	**1**
3.	**Dropping bottle with 0.5 M catechol solution (flask B).**	**1**
4.	**Pipette.**	**1**

The *xylE* gene coding the enzyme catechol-2,3-dioxygenase is often used as a reporter to study the expression of various genes. This enzyme catalyses the conversion of colourless catechol into a yellow coloured product called - hydroxymuconic semialdehyde. Fusing the promoterless *xylE* sequence to the promoter of gene of interest allows the expression of this gene to be analyzed according to the appearance and intensity of the yellow colour of reaction products.

Two strains of *Escherichia coli* have been constructed experimentally in which the *xylE* gene was fused to promoters of two different genes, gene C and gene D. Figure 1 shows growth curves for these bacteria, labelled I and II (I - *E. coli* with *xylE* fused to gene C promoter, II - *E. coli* with *xylE* fused to gene D promoter). The arrows in Figure 1 show when the cell samples were taken from the cultures. The number on the tube corresponds to the number of the arrow in Figure 1.

Determine the phases of culture growth in which genes C and D are expressed.

Figure 1



To do this you need to perform the following actions:

1) Fill the pipette to the mark using water (from flask A). Pipette this volume to each tube.

2) using the dropping bottle (flask B), add one drop of catechol solution to each tube and mix the contents of the tube by shaking,

3) leave the tubes at room temperature for 3 to 5 minutes,

4) examine the appearance of yellow colour in each tube.

Determine in which growth phases genes C and D are expressed and fill the table in the answer sheet, putting the "+" sign in the corresponding column.

Strain	Gene	The gene is expressed in		
		early log phase	late log phase	stationary phase
I	**C**			
II	**D**			

4.1.4 Laboratory «GENETICS».

Dear Participants!

In the laboratory **"GENETICS"** you will be given the following two tasks:

Task 1. Genetic analysis of inheritance of seed coat colour in *Phaseolus vulgaris L.*

Task 2. Identification of the *trp* mutations in the yeast *Saccharomyces cerevisiae.*

Duration of the lab work is **60 minutes.**

Maximum number of points – **61**.

You have to write down your results and answers on the **ANSWER SHEET** which will be collected by an assistant when the time elapses. It is not necessary to write anything on the task sheets.

Good luck!

Country_____

First name_____ **Family name** _____

Code_____

<u>Task 1. (30.5 points)</u> Genetic analysis of inheritance of seed coat colour in *Phaseolus vulgaris L.*

Time for carrying out this task must not exceed 25 minutes

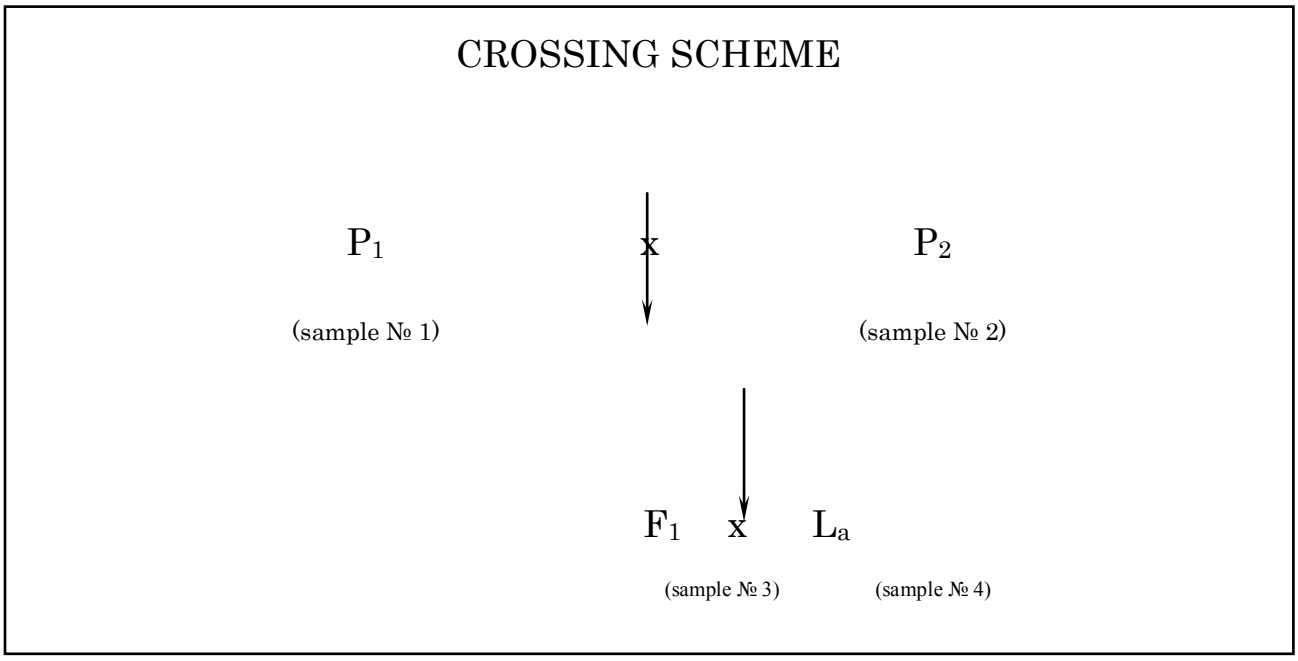
Materials and equipment

1.	Parental sample seeds (P_1).	sample № 1
2.	Parental sample seeds (P_2).	sample № 2
3.	Hybrid seeds (F_1).	sample № 3
4.	Test cross line seeds (L_a).	sample № 4
5.	Seeds of F_a generation.	sample № 5
6.	Petri dishes for seeds.	2
7.	Sheet of white paper.	1

The seed-coat colour of common beans (*Phaseolus vulgaris L.*) is controlled by a number of genes, which are responsible for the synthesis of pigments and distribution of the seed coat colour, as well as modifying genes, that can enhance, attenuate or change colour in another way. In the preliminary experiments breeding of two types of common beans (P_1 and P_2)differing in seed-coat colour was conducted. Seeds of F_1 plants were cultured. Plants (F_1) gave seeds of F_1 phenotype.

On the next stage of the experiment test-crossing of F_1 plants with testcross line plants (L_a) was conducted. Grown hybrids (F_a) gave seeds of F_a phenotype. For the next analysis, one seed from each F_a plant was taken.

Scheme of the experiment.



Stages of the work:

You are given parental sample seeds P_1 (sample № 1) and P_2 (sample № 2), hybrid seeds F_1 (sample № 3), testcross line seeds L_a (sample № 4) and seeds of F_a generation (sample № 5).Differences between parental samples are determined by different combinations of two pairs of non-allelic genes A and B (different gene loci). Gene A controls synthesis of pigment ("A" = dominant allele -pigment is present, "a" = recessive gene -pigment is absent). Gene B is a modifying gene, that influences colour intensity (B = dominant allele – modification is present, and b = recessive allele – modification is absent). Different combinations of two pairs of non-allelic genes A and B cause the development of three types of seed-coat colour (Table 1).

Table 1

Kind of seeds	Seed-coat colour	Code of the colour
	White	**w**
	Yellow-brown	**y**
	Black	**b**

You should accomplish the next problems:

Determine if parental samples P_1 and P_2 are pure-breeding lines (homozygous at each gene locus).

- Determine the type of inheritance of seed-coat colour in common beans (presence of interaction of non-allelic genes A and B).
- Determine the genotypes of the parental forms of P_1 and P_2, hybrid seeds F_1, seeds of F_a generation and testcross line seeds L_a
- Determine if the investigated non-allelic genes are linked.

Attention! The differences in viability of zygotes or gametes of different types of analyzed common bean (***Phaseolus vulgaris L.***) samples were not detected. Genes A and B are localized in the nucleus.

Problem 1.1. Determine if the parental samples P_1 and P_2 are pure-breeding lines (homozygous by every pair of non-allelic genes) by seed coat colour ? To answer this question you must analyze F_1 seeds.

1.1.1. (1.5 points) Look over samples № 1 and № 2. Specify the seed phenotypes of parental forms and F_1 using the symbols from Table 1 (Page 4). Fill in the table in the answer sheet:

Plant seeds	Sample	Seed phenotype
P_1	№ 1	
P_2	№ 2	
F_1	№ 3	

1.1.2. (2 points) Analyse the seed-coat phenotypes of parental samples and F_1 hybrids. Select the correct answer. On the answer sheet record in the symbols of correct answers:

A. Both parental plants are homozygous.
B. Both parental plants are heterozygous.
C. Plant P_1 is homozygous, plant P_2 is heterozygous.
D. Plant P_2 is homozygous, plant P_1 is heterozygous.
E. Using the data presented it is impossible to determine, if the parental genotypes are pure-breeding lines.

1.1.2.:_____

Problem 1.2. Determine the type of inheritance of seed-coat colour in common beans. You need to analyze the seeds of F_a plants, which were received after breeding of F_1 plants with L_a plants.

1.2.1. (1 point) Carefully place the seeds from sample № 5 (F_a plant seeds) on to the sheet of white paper. Identify the quantity of the phenotypic classes of F_a by seed-coat colour. Group the seeds of F_a by phenotypic classes by putting them into Petri dishes for seeds. Using the codes from Table 1 specify the phenotypes of F_a. Record in the table in the answer sheet.

№ of class	Seed phenotype
Total number of classes	

1.2.2. (3 points) **Using your findings about the quantity of F_a classes, choose the type of interaction of non-allelic genes A and B, which control seed-coat colour in common beans. Record the symbols of correct answers on the answer sheet.**

A. There is no interaction of non-allelic genes in the experiment conducted.
B. Incomplete dominance.
C. Duplicate genes
D. Epistasis
E. Codominance.
F. Pleiotropic gene action.

1.2.2:_____

Problem 1.3. Determine the genotypes of the parental samples P_1 and P_2, hybrid seeds F_1, seeds of F_a generation and testcross line seeds (L_a)

1.3.1. (4 points) Specify all of the possible genotypes of P_1, P_2, F_1, F_a, and L_a plants using symbols "*A*" and "*B*" to mark the dominant alleles, symbols "*a*" and "*b*" to mark the recessive alleles of the investigated genes in the boxes of the table below. Fill in the table in the answer sheet.

Plants	Seed phenotype		
	Black	**Yellow-brown**	**White**
P_1			
P_2			
F_1			
L_a			
F_a			

Problem 4. Determine if the investigated non-allelic genes A and B are linked.

1.4.1. (1 point) Determine frequency of phenotypic classes in F_a by seed colour. To answer this question calculate the number of seeds in each class. Use the codes from Table 1. Fill in the table in the answer sheet.

№ of class	Seed phenotype	Number of seeds
Total number of seeds		

1.4.2. (3 points) Determine the ratio of the different phenotype classes by the colour of the seeds in F_a. Fill in the answer sheet using the code of the correct answer:

Code	White	Yellow-brown	Black
A.	**0.50**	**0.25**	**0.25**
B.	**0.50**	**0.19**	**0.31**
C.	**0.56**	**0.16**	**0.28**
D.	**0.42**	**0.14**	**0.44**
E.	**0.44**	**0.15**	**0.41**
F.	**0.50**	**0.14**	**0.36**

1.4.2.:_____

1.4.3. (3 points) **To determine whether there is linkage between the genes being investigated you must specify the** expected ratio **in F_a in the case of** no **linkage. You an receive the points for this task only if your answer for 1.2.2. is correct. Record in the table in the answer sheet:**

Phenotypic class	Ratio (%)
White seeds	
Yellow-brown seeds	
Black seeds	

1.4.4. (3 points) **Specify the** expected ratio **by seed colour in F_a if the investigated genes *A* and *B* are linked completely. You can receive the points for this task only if your answer for 1.2.2. is correct. Record in the table in the answer sheet:**

Phenotypic class	Ratio (%)
White seeds	
Yellow-brown seeds	
Black seeds	

1.4.5. (3 points) Using χ^2 method, determine whether to reject or not-reject (accept) your hypothesis

Calculate the χ^2 value for H_0 (null hypothesis)being "No linkage" using the formula below:

$$\chi^2 = \Sigma((E_i - O_i)^2 / E_i),$$

where E_i is the expected frequency of the phenotype class i. O_i is the practically observed frequency of the same class. Use two decimal places during your calculations. Record in the answer sheet by the χ^2 value (with two decimal places).

1.4.5. _____

1.4.6. (3 points) Use the table of χ^2 distribution to determine what is the maximum probability (p) of your H_0 (null hypothesis)not being rejected (being accepted). Write the codes of the answers on your answer sheet.

Table of χ^2 distribution

df	Value (p) of a significance level χ^2									
	0.99	**0.95**	**0.90**	**0.75**	**0.50**	**0.25**	**0.10**	**0.05**	**0.025**	**0.01**
1	-	-	0.02	0.10	0.45	1.32	2.71	3.84	5.02	6.63
2	0.02	0.10	0.21	0.58	1.39	2.77	4.61	5.99	7.38	9.21
3	0.11	0.35	0.58	1.21	2.37	4.11	6.25	7.81	9.35	11.34
4	0.30	0.71	1.06	1.92	3.36	5.39	7.78	9.49	11.14	13.28
5	0.55	1.15	1.61	2.67	4.35	6.63	9.24	11.07	12.83	15.09
6	0.87	1.64	2.20	3.45	5.35	7.84	10.64	12.59	14.45	16.81
7	1.24	2.17	2.83	4.25	6.35	9.04	12.02	14.07	16.01	18.48
8	1.65	2.73	3.49	5.07	7.34	10.22	13.36	15.51	17.53	20.09
9	2.09	3.33	4.17	5.90	8.34	11.39	14.68	16.92	19.02	21.67
10	2.56	3.94	4.87	6.74	9.34	12.55	15.99	18.31	20.48	23.21

A. < 0.01
B. > 0.01
C. < 0.05
D. > 0.05
E. 0.01
F. 0.05

1.4.6._____

1.4.7. (3 points) **Using your value of p, determine if genes A and B are linked. Calculate the distance between genes A and B (in cM) if they linked. Record in the answer sheet the code of correct answer.**

A. There is complete linkage between genes A and B. The distance between the genes is 6.94 cM.

B. There is complete linkage between genes A and B. The distance between the genes is 12.36 cM.

C. There is complete linkage between genes A and B. The distance between the genes is 27.78 cM.

D. There is incomplete linkage between genes A and B. The distance between the genes is 6.94 cM.

E. There is incomplete linkage between genes A and B. The distance between the genes is 12.36 cM.

F. There is incomplete linkage between genes A and B. The distance between the genes is 27.78 cM.

G. Genes A and B are not linked. The distance between the genes is 6.94 cM.

H. Genes A and B are not linked. The distance between the genes is 12.,36 cM.

I. Genes A and B are not linked. The distance between the genes is 27.78 cM

J. Genes A and B are not linked

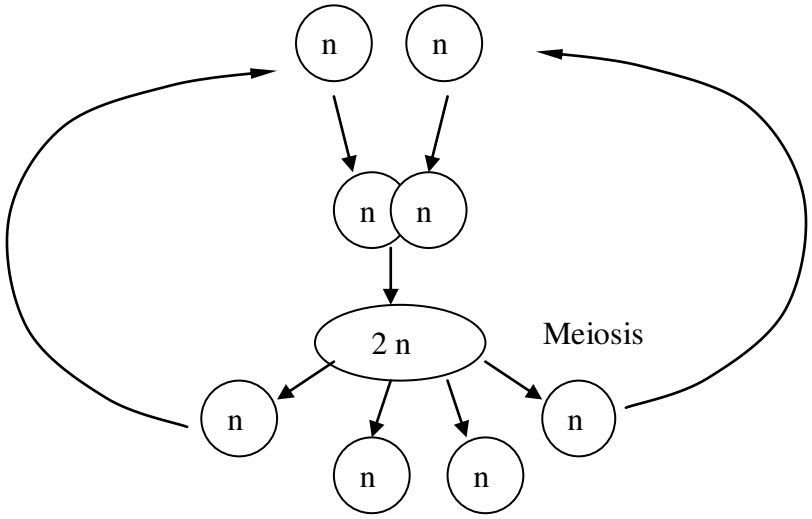
1.4.7:_____

Task 2: (30.5 points) Identification of *trp* mutations in yeast *Saccharomyces cerevisiae*

Materials and equipment

1.	Tubes with culture liquid.	12
2.	A plate with 12 wells.	1
3.	A tube with Erlich reagent.	1
4.	A tube with indole solution.	1
5.	A tube with anthranilate solution.	1
6.	A tube with water.	1
7.	1 ml pipette.	13
8.	A sheet of white paper.	1
9.	A container for used pipettes.	1
10.	Paper towels.	1

You are given the yeast *Saccharomyces cerevisiae* as an experimental organism. The scheme of life cycle of this organism is presented below.



These yeasts have alternating haploid and diploid phases during their life cycle. The fusion of haploid cells gives rise to a diploid cell which through meiosis can produce four haploid cells with different genotypes.

The scheme below shows the pathway of tryptophan biosynthesis in the yeast *Saccharomyces cerevisiae.* The scheme shows some intermediate products and genes responsible for the synthesis of enzymes of this pathway.

chorismate ⟶ anthranilate ⟶ ⟶ ⟶ indole ⟶ ⟶ tryptophan

trp2 gene *trp4* gene *trp5* gene

Mutations in the *trp* genes lead to the accumulation of the intermediates in the culture liquid. Two intermediates of this biosynthetic pathway, anthranilate and indole, can be detected in the culture liquid of the corresponding mutants through colour reactions with the Erlich reagent.

2.1. (1.5 points) Using a special pipette, add 0.5 ml of Erlich reagent to the control tubes with standard solutions of anthranilate, indole and to the tube with water (with no anthranilate and indole). Observe the colour change and record it in the table in the answer sheet using single letter colour code.

Compound	Colour after Erlich reagent addition
Water	
Anthranilate	
Indole	
Colour code:	У – yellow
	R – red
	N – no colour change

2.2. (1.5 points) Which compounds will accumulate in the culture liquid if the mutants are grown in the rich medium? Fill in the table below in the answer sheet using one letter code.

Mutant	Accumulated intermediate
trp 2 $^-$	
trp 4 $^-$	
trp 5 $^-$	
Code:	A – anthranilate
	I – indole
	O – neither anthranilate nor indole

2.3. (6 points) **Three classes of double mutants have been constructed in haploid *S. cerevisiae* named as *trpX*$^-$ *trpY*$^-$ *trpZ*$^+$; *trpX*$^-$ *trpY*$^+$ *trpZ*$^-$; *trpX*$^+$ *trpY*$^-$ *trpZ*$^-$(sign «– » denotes mutant genes, sign « + » denotes wild type genes; all *trp* genes are located on different chromosomes).**

Three matings between these mutants have been performed as shown in the table below. Each mating has generated all possible types of haploid progeny. Please write down in the answer sheet the genotypes of all possible progeny from each cross.

№	**Mating**	**Possible progeny genotypes**
I	***trpX*** $^-$ ***trpY*** $^-$ ***trpZ*** $^+$ × ***trpX*** $^-$ ***trpY*** $^+$ ***trpZ*** $^-$	
II	***trpX*** $^-$ ***trpY*** $^-$ ***trpZ*** $^+$ × ***trpX*** $^+$ ***trpY*** $^-$ ***trpZ*** $^-$	
III	*trpX*$^-$ *trpY*$^+$ *trpZ*$^-$ × *trpX*$^+$ *trpY*$^-$ *trpZ*$^-$	

2.4. (12 points) **Clones produced by these matings have then been grown in liquid medium, cells removed by centrifugation and supernatant collected for analysis. You now need to identify these clones.**

Please test each of the 12 culture liquid samples for the presence of the tryptophan metabolic intermediates and use these data for the identification of the *trpX*$^-$, *trpY*$^-$ and *trpZ*$^-$ mutations. You are given tubes with supernatants from 12 cultures of *S. cerevisiae.* The tubes are labelled according to the mating (I, II and III) and clone number (1-4).

To test the accumulation of particular compounds, transfer 1 ml of liquid from each tube to the wells of the 12-well plate. Use a new pipette for each transfer!

Add 0.5 ml of the Erlich reagent (using a special pipette) to each well containing the 1 ml of supernatant. Record the colour changes (using a single letter code) in the table in the answer sheet.

Determine which compound has accumulated in each culture and record this in the same table in the answer sheet using a single letter code.

№	Mating	Tube №	Colour after Erlich reagent addition	Accumulated intermediate
I	$trpX^- trpY^- trpZ^+$ × $trpX^- trpY^+ trpZ^-$	I.1		
		I.2		
		I.3		
		I.4		
II	$trpX^- trpY^- trpZ^+$ × $trpX^+ trpY^- trpZ^-$	II.1		
		II.2		
		II.3		
		II.4		
III	$trpX^- trpY^+ trpZ^-$ × $trpX^+ trpY^- trpZ^-$	III.1		
		III.2		
		III.3		
		III.4		
Code:			У – yellow	A – anthranilate
			R – red	I – indole
			N – no colour change	O – neither anthranilate nor indole

2.5. (3 points) **Identify the *trpX*⁻, *trpY*⁻ and *trpZ*⁻ mutations. Write down names of the genes in which the *trpX*⁻, *trpY*⁻ and *trpZ*⁻ mutations are located in the table in the answer sheet.**

Gene	Mutation
trp 2	
trp 4	
trp 5	

2.6. (3 points) **How would the experimental results change if the *trpX*⁻ and *trpY*⁻ genes were completely linked? Record in the answer sheet the letter corresponding to the correct answer:**

A. The number of different progeny genotypes would be reduced.
B. The results would not be changed.
C. Phenotypically wild type yeast may be produced.
D. The number of single and triple mutants would increase.

2.6.:_____

2.7. (1.5 points) **How many genotype classes would be obtained if the three genes were located on the same chromosome and were 100 per cent linked? Write the number for each mating in the answer sheet.**

2.7.: I_____

II_____

III_____

2.8. (0.5 points) **Which mating will give the single mutant accumulating anthranilate? Write the mating number (I, II or III) in the answer sheet.**

2.8.:_____

2.9. (0.5 point) **Write the genotype of this mutant in the answer sheet using the actual gene names (*trp 2*, *trp 4* or *trp 5*).**

2.9.:_____

2.10. (1 point) **Which of the double mutants has to be mated with this anthranilate-accumulating single mutant to get progeny with wild type genotype? Write the genotype of this double mutant in the answer sheet using the actual gene names (*trp 2*, *trp 4* or *trp 5*).**

2.10.:_____