PHYSICS PRACTICAL EXAMINATION

GENERAL INFORMATION AND OPERATION MANUALS

P_Experiment A. Science and Measurements. Quality Assurance of Measurements

Science operates with reliable information. The data from measurements must be accurate and precise. The instruments must be checked and, if necessary, recalibrated using certified standard materials.

P_A1. Working with a Refractometer

General: A refractometer is an instrument measuring the refractive index or/and related parameters. Identify the instrument, it should be in the blue box. It has a prism at one end and an eyepiece (like a microscope) at the other.

You will use an instrument designed for honey quality control: the scale on the right side, "**WATER %**", is for the honey water content, the central scale, sucrose "**BRIX %**", is for the content of sweet substances (sucrose, sugars in general). You are not going to use the scale on the left side.

Total sucrose concentration is expressed in **Brix** (also **Bx**) percents, showing the mass of sucrose in grams present in 100 grams solution (w/w). A honey with 65 Bx% contains 65 g sucrose in 100 g honey. ***The sucrose Brix scale is a conventional scale for sugar content.***

How to measure:

Direct the prism to a light source and look through the eyepiece (don't use eyeglasses). Check the image clarity. If necessary, rotate the eyepiece until the image of the three scales becomes clear.

*Open the prism lid and use a stick to take 2-3 drops of the sample and apply it onto the prism surface. Gently press the lid to spread the fluid in a thin layer without air bubbles. Point the prism at an ambient light source and read on the central "**BRIX %**" scale a value at the edge of the blue zone. Similar method to read on the "**WATER %**" scale.*

After measurement clean the prism surface with wet wipes and finish with a soft cloth.

P_A2. Working with a conductometer

Your instrument is a combined „pH-meter /Conductometer".

Identify the instrument; it is labelled „pH-meter /Conductometer".

It has a protective cap at the bottom end. Remove the cap by pulling out: you will see exposed the cell/pH sensor (glass), the cell for conductometric measurements (a pair of metal electrodes), and a temperature sensor (black).

*Press the **on/off** button, marked with ⏻; it works only as **ON/OFF** button. You will see on display a symbol for battery capacity state, a label like „**pH**" or "**µS/cm**" (press **MODE** key to choose the convenient one), a numerical value, a temperature reading by default in ℃.*

*Keep the label "**µS/cm**" and the instrument works as conductometer.*

*The instrument switches off automatically after 3 minutes of inactivity to protect the battery. Simply press **ON** to reactivate it.*

*If you press the **HOLD** button, a small **H** letter appears in the left top corner and the instrument holds the last measured. Reactivate measuring by pressing again **HOLD**.*

*After each measurement remove the instrument from the solution, wash the sensors area using the wash bottle marked "**Deionized water**" and the "**Liquid waste**" tank. Use a dry tissue to remove excess water from sensor area.*

Conductivity is 1/resistivity, and conductance is 1/resistance. The conductance is measured in Siemens – symbol S (i.e., Ω^{-1}) – by applying an AC signal (low voltage, 25 – 1000 Hz) to avoid electrolysis. The specific conductivity of solutions is well related to the concentration of dissolved electrolytes.

How to measure:

Short press (and repeat if it is necessary) ***MODE/CAL*** *button to see the "**µS/cm**" label, the measuring unit for the specific conductivity. You are now in "**Conductivity**" mode of measurement.*

Turn off the instrument when not in use.

Calibration procedure: *calibrate for conductivity measurements (similar method) by immersing the instrument sensor area in a standard conductivity solution (a **1413 µS/cm conductivity standard solution** is available): press **MODE/CAL** for 6 s and then wait for a non-blinking reading.*

A3. Working with a pH-meter

General: a pH-meter is used to measure significant variations in the concentration of the H^+ ion (usually identified as hydronium ion H_3O^+).

$pH = -\log_{10}\ [H^+]$, i.e., $[H^+] = 10^{-pH}$, where [....] is concentration measured in $mol\ /\ dm^3$.

The pH-meter measuring cell of the pH-meter generates a voltage that is almost zero at *p*H = 7 and then varies by about 59 mV/*p*H unit (see Figure R1).

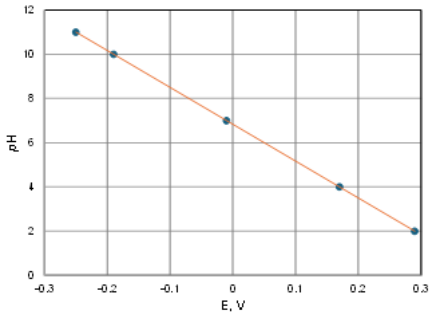


Figure R1. Variation of the electrochemical potential with *p*H

A pH-meter needs frequent checks and calibrations with calibration solutions having stable *p*H values (usually known as standard *p*H buffer solution). You have 3 standard *p*H buffer solutions for calibration and/or verification.

Your instrument is a combined „pH-meter /Conductometer".

How to measure:

*Press **MODE** key to choose the label „**pH**" and the instrument works as pH-meter.*

***Calibration procedure:** place the instrument in pH 4.01 standard buffer solution. Verify that the instrument is in the **pH** mode. Long press **MODE/CAL** button for 6 s and wait until the value 4.01 is not blinking anymore. The instrument recognizes automatically the buffer nominal value.*

Repeat the same sequence with the other two standard pH buffers (pH 6.86 and pH 9.18). Calibration is finished. Cap the standard pH buffers bottles with the corresponding lid (do not interchange) when not in use.

REMEMBER: after each change of measured solution, clean the sensor area with deionized water, and absorb the excess water with dry tissue.

P_A4. Working with a Digital Weighing Scale

Place the scale on a stable, horizontal surface. Press **ON/OFF** button ⏻ to turn on the scale. Wait 30 seconds before weighing, to allow equilibration.

Power will turn off *automatically* after one minute if not in use (in absence of any action); press and hold **T** button to see **A_OFF**, *to deactivate this function*.

If you don't see symbol **g** (for gram) in the right up corner, press repeatedly **MODE** button, to change accordingly.

Press **T** (Tare) button for a 0.00 reading (as initial value) or to subtract the mass of a weighing container.

Measuring procedure. Place the object on the scale (*in central position*) and read the value in grams.

Calibration procedure: *Press and hold (2-3 s)* ***MODE*** *button until you see 100.00 g (this is the weight you must use for calibration); the value can be changed by pressing* ***T****, don't change it). Short press again* ***MODE*** *and the value will flash. Place the calibration weight (labelled 100.00 g) on the scale and wait until* ***PASS*** *is displayed. Remove the weight. The scale is now calibrated.*

Close the instrument to protect the battery (you have disabled automatic turn off), when not in use.

Experiment B. Determination of sugar and water content of honey

Honey, syrups, juices, and sweet drinks have variable concentrations of dissolved solids, especially sucrose, and organic acids or salts. The water content of honey, % [g/100 g or w/w], is that quality criterion that determines the ability of honey to keep its composition and resist alteration by yeast fermentation. The higher the water content, the more likely it is to ferment during long storage periods.

The refractive index allows not only the evaluation of the water content, but also of the total level of sugars, if the available instrument presents multiple evaluation scales. Given the complex composition of many of these foods, their sucrose („sugar") content can be measured with an accuracy of about ± 0.5%. Total sucrose concentration is expressed in Brix % [w/w].

The main physico-chemical characteristics of Romanian honey are shown in **Table R1**.

Table R1. Main physico-chemical characteristics of Romanian honey

Honey type	Water content, % w/w	Sugar content, % w/w	*p*H	EC, mS/cm
Acacia	13.90 – 20.57	79.95 – 83.00	3.49 – 5.85	0.10 – 0.68
Linden	5.4 – 18.8	79.00 – 81.99	3.60 – 4.70	0.20 – 0.73
Polyfloral	4.8 – 19.6	79.96 – 82.38	3.20 – 4.60	0.23 – 0.83

P_Experiment C. Measuring of honey (electro)conductivity in a 20 % w/w aqueous solution

The electrical conductivity of honey is directly related to the concentration of mineral salts, organic acids, and proteins, varying significantly with the origin. Conductivity determination requires solutions of honey containing 20% (w/w) dry substance in double distilled water.

P_Experiment D. Measuring the *p*H of a honey solution (10% w/v in water)

Honey contains organic acids (0.6%) and amino acids (0.05%), so its *p*H can vary between 3.5 and 6. Gluconic acid is the most common, followed by acetic acid, butyric acid, citric, formic, lactin, malic, succonic and pyroglutamic. The most common of the 18 amino acids is proline. The **low *p*H value** makes honey compatible with foods with low acidity, inhibiting the development of microorganisms.

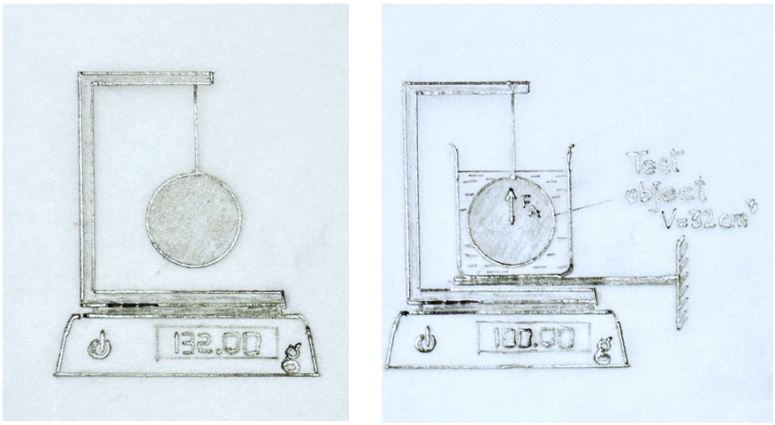
P_Experiment E. Archimedes' Principle and Application

Theory information

A body submerged (partially or completely) in a fluid is acted upon by a force equal in magnitude to the wheight of the displaced fluid (*Archimedes' principle*).

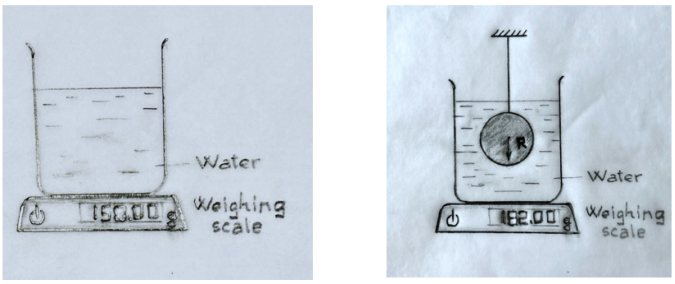
To every action, there is always opposed an equal reaction (*third law in Newtonian mechanics*), i.e. in this case the same body exerts a reaction force on the fluid (same magnitude, but opposite direction).

There are two schemes for the experimental setup, both including a weighting scale and a suspended ball: see Figure R2 for measuring the Archimedes' force and Figure R3 for measuring the reaction (force) to Archimedes' force.



(a) (b)

Figure R2. Experimental set-up for measuring the Archimedes' force.



(a) (b)

Figure R3. Experimental set-up for measuring the reaction (force) to Archimedes' force

Note: For your calculations use the following values:

Water density at room temperature is $1.000\ \mathrm{g/cm^3}$.

Gravitational acceleration, $g = 9.80\ \mathrm{m/s^2}$.

EXPERIMENTAL PART

Fill-in data only in the Answer Sheets, observing the task code.

A working time (**WT**) is recommended for each task.

***

P_Experiment A1: Verify the BRIX % scale (0.6 pt)

Use the fluid contained in the test tube labelled "**72.5% Brix**" as sample. Report the result in the **Answer Sheets**.

If the reading error is higher than ± 0.3 Bx % ask for assistance. The instrument can be then accurately calibrated. If the error is smaller, accept it, but correct subsequent readings with it.

WT: 5 min

Go to experiment B, task P_ B1.

Experiment A2. Working with a conductometer

Task P_A2.1: Calibrate the conductometer (1.0 pt)

After calibration measure:

- available **1413 µS/cm** standard conductivity solution.

and fill-in the first row of the ***Observation Table***.

WT: 10 min

Task P_A2.2: Measuring with a conductometer (0.3 pt)

After calibration measure:

- conductivity control samples CCS1 and CCS2.

and fill-in the ***Observation Table***.

WT: 5 min

Go to experiment C, tasks P_C1 and P_C2

P_Experiment A.3

Task P.A3.1: Verify the pH-meter (0.6 pt)

Fix the instrument in the clamp of the laboratory stand (with sensors exposed). Place the bottle with standard buffer of *p*H 4.01 under the instrument and adjust the height in the stand so that sensors are completely immersed in solution (approximately 1 cm depth). Gently stir the solution by circularly moving the bottle. When the displayed value is stable (after 10 – 20 s), read the pH value and record it in the ***Observation Table***.

WT: 10 min

Task P_A3.2: Calibrate the pH-meter (1.4 pt)

The instrument has new batteries. The specific calibration is lost when the batteries are replaced. Calibrate the pH-meter.

Measure again the 3 pH buffer solutions with the **calibrated** instrument. Fill-in the obtained values in the ***Observation Table***.

If all errors are less than ± 0.1 *p*H, the pH-meter is in a very good working state.

A good calibration will provide reading with errors below 0.2 *p*H units (errors within ±0.3 *p*H units are still acceptable for the aims here). If error exceeds ± 0.3 pH units, ask for help. Repeat the operations with the other two pH buffers.

WT: 15 min

Go to Experiment D, task P_D1

Experiment A4. Working with a Digital Weighing Scale

Task P_A4.1: Calibrate the scale (0.15 pt)

Your digital scale is not calibrated. Use the calibration weight to check. Calibrate the scale.

WT: 5 min

Task P.A4.2: Verify the mass of the calibration weight (0.05 pt)

After calibration verify the mass of the calibration weight in normal measuring mode.

WT: 3 min

Go to Experiment E.

P_Experiment B

Task P_B1: Measure sugar content and water content (1.8 pt)

There are 5 samples (labelled as H1 to H5): three of them are honey from trusted sources, the other two are adulterated by addition of foreign substance or inferior honey. Measure the sugar content and the water content of each sample using the refractometer.

For each sample propose a diagnostic: **AUTHENTIC** or **ADULTERED**.

WT: 15 min

Go back to A.2

P_Experiment C.

Measuring of honey (electro)conductivity in a 20 % w/w aqueous solution

Task P_C1 (0.3 pt)

How much honey (having 20 % water content) should be weighed to prepare 100 g of 20% aqueous solution for the electroconductivity determination?

How much water should be added to the weighted honey?

WT: 5 min

Task P_C2 - Measuring of honey (electro)conductivity (1.5 pt)

There are 3 samples (labelled as EC 1 to EC 3) of different honey types, containing 20% dry honey substance. Measure their electroconductivities, in µS/cm, using the instrument in the "**Conductivity**" mode.

Record the conductivity and temperature values in the ***Observation Table*** when the reading is stable (after 10 – 20 s). Repeat the operations with the other samples.

Observe the main characteristics of honey of different origin in **Table R1** and propose a type (acacia, linden, polyfloral), if possible.

WT: 15 min

Go back to A3.

Experiment D. Measuring the pH of a honey solution (10% w/v in water)

Task P_D1: *p*H of honey samples (0.5 pt)

There is a 10% aqueous honey solution, labelled ACH1 or ACH2 or ACH3 (different honey types). A sample is **50 mL**.

Place the sample container on the working bench and adjust the pH-meter position (using the stand) so that the sensor area is immersed about 1 cm into the solution.

Read the solution **initial *p*H** and fill-in the ***Observation Table***.

WT: 5 min

Go back to A4.

Experiment E. Archimedes' Principle and Application

Use the appropiate experimental setup to accomplish the following tasks.

P_Task E.1 (1.8 pt)

Use the weighing scale, the laboratory stand, a beaker, and the small ball to mount the experimental setup in Figure R3.

Turn on the scale and use it after approximately 30 s.

Fill a transparent 150 mL beaker with 100 mL water, place it on the scale and press **T** button to read 0.00 g. Then completely submerge the ball in water (using the laboratory stand).

Read the value on the display and explain sign and value.

WT: 15 min

P_Task E.2 (0.5 pt)

Rearange the **Theory information** according to the pattern:

If ...(*stamenent 1*)... and if ...(*statement 2*)..., then ... (conclusion)...

Write the text in the **Answer Sheets**.

WT: 5 min

P_Task E.3 (0.1 pt)

Find the mass of the ball.

Describe the method.

WT: 3 min

P_Task E.4 (0.9 pt)

Experimentally find the ball volume. Describe the method used.

WT: 10 min

P_Task E.5 (0.1 pt)

Calculate the ball density.

WT: 3 min

P_Task E.6 (0.2 pt)

Calculate the Archimedes' force when the ball is completely submerged.

WT: 5 min

P_Task E.7 (1.8 pt)

A viscous fluid is provided as sample **F** for measuring its density.

Choose a convenient experimental setup.

Use the ball known parameters (from the previous tasks) to calculate the density of the viscous liquid.

WT: 15 min

P_Task F.8 (0.5 pt)

Propose and apply practically an alternative method to measure the density of the viscous liquid. Provide the result and compare accuracy, precision, and any difficulty.

WT: 10 min

Read the following instructions carefully

- Wear lab coat, safety goggles, and protective gloves when handling chemical compounds.
- Handle each substance with care.
- Do not try to taste or smell any chemical compound.
- Chemicals must be disposed properly in the beaker of 500 mL labelled "Waste".
- Ensure that you keep the answers sheet and question paper away from liquids.
- Immediately report all accidents, injuries to the supervisors present, however minor they may be.
- Eating any kind of food is strictly forbidden during the experimental task.
- Keep your voice low when you discuss with your teammates during the experimental task.
- Keep the work environment clean.
- Do not leave the examination hall until you have permission to do so. You may start working only when the start signal is given.
- You have 3 hours to carry out the experiments, to record the results on the answer sheets.
- **Only the answer sheets will be evaluated. All results must be written in the designated boxes on the answer sheets. Data written elsewhere will not be graded.**

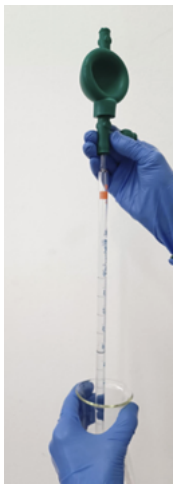
Pipette operation

There are three letters on the pipette filler: “A (air), S (suction) and E (empty).

- Insert the top of the pipette in the bottom of the pipette filler (if is not already assembled).
- Release air from the pipette filler by pressing “A” valve on the top of the pipette filler while simultaneously squeezing the bulb.
- Insert the tip of the pipette into the solution P1 for liquid aspiration by pressing the “S” valve. Adjust the liquid level to the mark using “S” and “E” valves.
- For liquid aspiration, be careful to keep the tip of the pipette in the liquid and not to let the liquid go up into the pipette filler.
- Using “E” valve, release the liquid from the pipette up to the desired volume into the conical flask.
- Recover the solution left in the pipette in the small Berzelius beaker, in the same way.







C_I. Identification of the compounds in provided solutions (6.75 points)

Materials provided:

Bottles labelled from 1 to 6 containing the aqueous solution of the inorganic compounds that must be identified
Test tubes and test tubes rack
7 plastic Pasteur pipettes
Tray
Permanent marker and paper towel
Wash bottle with distilled water
Large Berzelius beaker for residual solutions and washing waters

In the 6 bottles, labelled from 1 to 6, there are aqueous solutions of the following reagents: **barium nitrate, potassium iodide, sodium carbonate, lead nitrate, zinc chloride and sodium sulphite** (not in this order).

Check for each solution possible chemical reactions (forming a precipitate) with the other solutions and write the observations in Table 1 (from the Answer sheet) as follows: in the appropriate cell in **Table 1**, if a precipitate forms, write the colour and "pp.", otherwise mark with "X".

Based on the experimental observations, fill the **Table 2** from Answer sheet:

C_I.1 Put "X" sign in the appropriate cell if no precipitate forms
C_I.2 Identify the chemical compound from each bottle and write the chemical formula of the compounds in the last row of Table 2.
C_I.3 In the appropriate cell of Table 2, write the chemical formula of the resulting precipitate (marked with "↓") and indicate its colour.

Hint: The chemical reactions will be performed in test tubes using small volumes of substances (several drops of each reagent) sampling with plastic Pasteur pipettes. Observe the resulting precipitates after several minutes.

Consider all substances used in the experiments as toxic, harmful if swallowed, irritant for eye and skin

C_II. Titrimetic determination of KH_2PO_4 (6.25 points)

Materials provided

Burette
Stand and burette clamp
Pipette and pipette filler
Thymolphthalein indicator solution in a bottle with dropper. Its transition range is: **pH 8.8 (colorless) - pH 10.5 (blue)**.
2 conical flasks (Erlenmeyer) of 250 mL
Volumetric flask with cap of 50 mL containing the sample
2 small Berzelius beakers (one for filling the burette and one for sample solution)
Bottle of 250 mL with the KOH solution
Permanent marker
Paper towel
Wash bottle with distilled water
1 large Berzelius beaker for residual solutions and washing waters.

Potassium dihydrogen phosphate monohydrate ($KH_2PO_4 \cdot H_2O$) is commonly used as a fertilizer, food additive, and buffering agent in various industrial applications. It is also known as MKP. In agriculture, MKP is used as a source of phosphorus and potassium, which are essential nutrients for plant growth.

For quantitative determination of KH_2PO_4 from the volumetric flask carry out the following steps:

1. Carefully fill the clean burette with 0.0973 mol/L of KOH aqueous solution using one of the small Berzelius beaker (label it "KOH").
2. Bring the sample solution from volumetric flask to the final marked volume by topping up with distilled water, resulting the **solution P1**. Carefully pour about 30 mL of the solution P1 from the volumetric flask in the other small Berzelius beaker (label it "P1").
3. Using the pipette, place 8.0 mL of the solution P1 into the 250 mL conical flask (Erlenmeyer). Add about 25 mL distilled water into the same conical flask and 10 drops of thymolphthalein indicator.
4. Titrate the resulting solution (from 3) to the endpoint with KOH solution (blue colour).
5. Repeat the titration (operations from 3 and 4) three times.

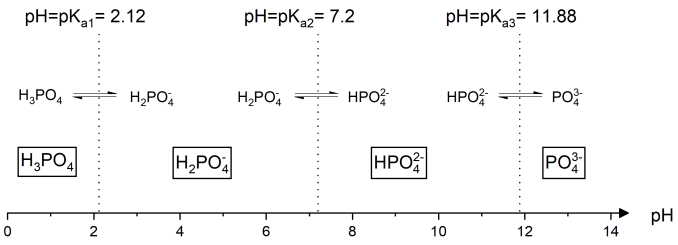
C_II.1 Record the used volume of KOH for each titration in **Table 3** (from the answer sheet) and calculate the average volume of KOH solution.

C_II.2 Write and balance the chemical reaction of titration.

C_II.3 Calculate the mass in grams (4-digits) of KH_2PO_4 (as anhydrous salt) in 8.0 mL of P1 solution.

C_II.4 Establish the KH_2PO_4 concentration (mol/L; 4-digits) of the P1 solution.

Molecular weigh of KH_2PO_4: 136 g/mol



Scheme 1. Simplified diagram for the dominant H_3PO_4 species versus pH in aqueous solution.

DO NOT PROVIDE YOUR FINAL ANSWERS HERE, USE THE ANSWER SHEET.

THE EXPERIMENTAL TEST

BIOLOGY

ORPHAN BEARS GET ANOTHER CHANCE

THE STORY

Near the Ursus Valley, Romania, there is a large brown bear population consisting of several families, with a total of 10 cubs.

A physical-spatial location analysis of the bear families demonstrated that three of the families, which together have 5 cubs (marked as ”cub 1”, ”cub 2”, ”cub 5”, ”cub 7” and ”cub 10”) have their dens in the North West (NW) part of the village , two families are located in the South East (SE) part and 3 cubs were identified (”cub 3”, “cub 4” and “cub 9”), and the rest of the bears are located in the Far East part area of the village, where 2 cubs were also identified (“cub 6” and “cub 8”).

Most of the time the bears feed on what they find in the forest, especially different types of berries, but sometimes they can also descend into human settlements to look for food. Residents of Ursus Valley reported to the authorities that a bear cub often visits their village and scares domestic animals. That's why the authorities decided to move this cub, together with its family.

Therefore, the cub needs to be identified, so they enlisted the help of forensic researchers. They collected different types of samples, both from the locality and from the surrounding areas where the different families of bears are located. These samples were represented by scats, hairs, leaves of most prevalent coniferous tree species. Different coniferous species were present in the NW area, SE area and Far East area.

To ensure that only the bear that visits Ursus Valley will be relocated, the authorities requested the help of forensic geneticists and biologists. They collected biological samples (hair, scats, etc.) from the location visited by the animal and hair samples from all the 10 bear individuals that make up the population from the forests near settlement Ursus Valley.

Your role, as investigators, is to identify, based on the data obtained from the analysis of the samples, which bear took the habit of visiting the village and therefore could potentially represent a danger for the local inhabitants. You have to undertake different approaches to accomplish this task.

Glossary of terms used in the practical test of the Biology exam.

STR = microsatellite = a set of short repeated DNA sequences at a particular locus on a chromosome, which vary in number in different individuals and so can be used for genetic fingerprinting.

Amplicon = DNA products of a polymerase chain reaction (PCR)

Haplotype = a group of alleles in an organism that are inherited together from a single parent.

DNA profiling (also called **DNA fingerprinting** and **genetic fingerprinting)** = the process of determining an individual's deoxyribonucleic acid (DNA) characteristics.

Hypodermis: A layer or layers of cells located just below the epidermis, often serving as a protective or supportive tissue.

Resin duct: Tube-like structures in some plants (like pines and spruces) that transport and store resin, a sticky substance produced to protect the plant from injury or infection.

Septate chlorenchyma: Refers to the presence of internal walls (septa) towards the inside of the cells that divide cells.

Palisade chlorenchyma: A type of chlorophyll-rich tissue (chlorenchyma) consisting of elongated, tightly packed cells, usually found just below the upper epidermis of leaves and involved in photosynthesis.

Spongy chlorenchyma: Loosely arranged, irregularly shaped cells with air spaces between them, aiding in gas exchange and photosynthesis.

Vascular bundle sheath: A layer of cells surrounding a vascular bundle (xylem and phloem) in plants, providing structural support and regulating the flow of substances.

Sclerenchyma: A type of plant tissue composed of thick-walled, dead cells that provide mechanical support and strength to the plant.

Collenchyma: A type of flexible plant tissue made up of living cells with unevenly thickened walls, providing structural support while allowing growth.

Vascular bundle: A strand of tissue in plants containing xylem (which transports water) and phloem (which transports nutrients), responsible for internal transport.

Trichome: Hair-like structures on the surface of plant epidermis.

Cuticle: A waxy, protective layer covering the epidermis of leaves, stems, and other plant parts, helping to reduce water loss.

Epidermis: The outermost layer of cells in a plant, serving as a protective barrier against environmental factors like pathogens and water loss.

Aquifer tissue refers to specialized plant tissue involved in the storage or movement of water.

Aerenchyma tissue contains large air-filled spaces or cavities between the cells.

EXPERIMENT 1 - FORENSIC GENETICS

WHICH BEAR?

One important method is based on forensic analyses. Forensic analyses utilize various scientific methods such as DNA profiling, fingerprinting, and dental records comparison to establish distinct individual characteristics within a population, aiding in identification processes.

Therefore, investigators extracted DNA from biological hair samples and performed molecular fingerprinting analysis, using a set of 10 STR type markers, polymorphic DNA regions with 2-6 bp long repeat units. The number of repeats in STR markers is highly variable among individuals, making these markers effective for use in forensic applications. Amplicons for each marker and each individual were separated using a genetic analyser equipment and analysed with the appropriate software, using standard procedures.

You must keep in mind that for a precise identification of the individual, there must be at least a 99.99% match between the DNA fingerprint of the unknown sample and the DNA fingerprint of the individual.

Using the genotype information presented in **Table I-1,** answer the following questions.

Table I-1. Individual wild bear genotypes and the genotype of the unknown sample for each of the 10 STR markers. UA are the name of STR markers. ID1, ID2....ID10 are the identification number of bear individuals from the wild population

STR loci	Unknown sample	Wild bear individual ID									
		ID_1	ID_2	ID_3	ID_4	ID_5	ID_6	ID_7	ID_8	ID_9	ID_{10}
UA03	15, 16	16, 15	16, 16	17, 15	16, 15	16, 15	16, 18	20, 15	15, 15	18, 15	16, 15
UA06	10, 11	11, 10	11, 10	11, 10	11, 10	11, 10	11, 10	11, 10	11, 10	11, 10	11, 11
UA14	8, 10	11, 10	7, 10	10, 10	11, 10	8, 10	8, 12	8, 8	8, 10	8, 10	8, 10
UA17	27, 24	24, 27	24, 27	24, 27	24, 27	24, 27	24, 27	24, 27	24, 27	27, 27	24, 27
UA25	13, 14	13, 14	13, 14	12, 14	13, 14	13, 14	15, 14	13, 14	14, 14	13, 14	13, 14
UA51	12, 14	14, 12	14, 12	14, 12	14, 12	14, 12	14, 12	14, 12	14, 12	14, 12	14, 12
UA57	17, 17	17, 17	17, 17	17, 17	17, 17	17, 17	17, 17	17, 17	17, 17	17, 17	17, 17
UA63	8, 12	12, 9	12, 7	10, 8	12, 8	12, 8	11, 8	12, 8	12, 8	14, 8	13, 8
UA68	14, 19	19, 14	19, 20	19, 14	17, 14	19, 14	15, 14	19, 14	19, 14	19, 13	19, 14
UA16	22, 28	22, 28	22, 28	22, 28	22, 28	22, 28	28, 22	22, 28	22, 28	22, 28	23, 28	22, 28

UA03, UA06....UA16 – name of STR markers

ID_1, ID_2....ID_{10} – identification number of bear individual from the wild population

QUESTIONS:

DO NOT PROVIDE YOUR FINAL ANSWERS HERE FOR ANY OF THE QUESTIONS.

USE THE ANSWER SHEET!

B_I.1 [1.5 points, 0.15 per statement]

Using the information provided in Table I-1, write in the table in the ANSWER SHEET the ID number of each individual bear that has the same genetic profile as the unknown sample, for each marker (found in the STR loci column).

STR loci	Bear individuals with the same genotype as the unknown sample
UA03	
UA06	
UA14	
UA17	
UA25	
UA51	
UA57	
UA63	
UA68	
UA16	

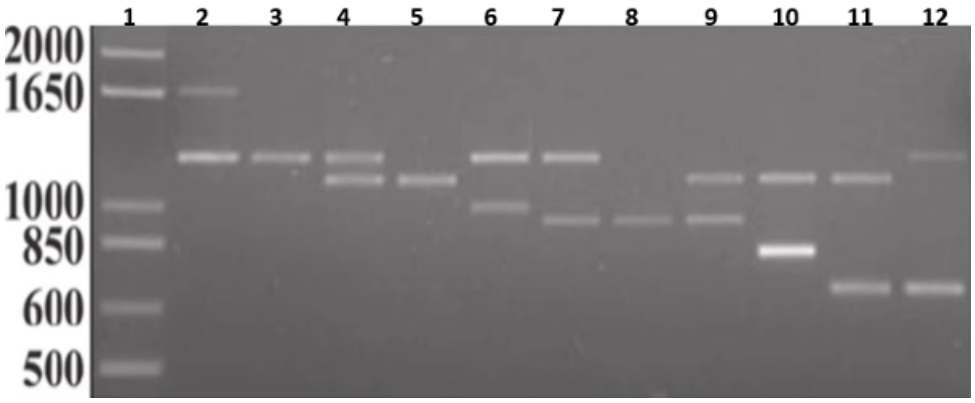
B_I.2 [0.25 points]

Based on your responses in the table, in the ANSWER SHEET, identify the ID number of the bear cub that visited the village. Write your answer in the answer sheet.

The conclusion of your investigation
The ID number of the bear cub that has the habit of visiting the village is:

B_I.3 [1.25 points, 0.25 per statement]

The figure below shows an image of a DNA fingerprint for one STR marker across 11 different individuals (lanes 2 to 12). A DNA ladder was loaded in lane 1, with the length of each fragment labelled beside the image. Using your knowledge of DNA markers, indicate whether each of the following statements is true or false by marking an "X" on your ANSWER SHEET.



Statement	True	False
The individual from Lane 10 has a heterozygous genotype for this marker and has the same alleles as the individual in Lane 7.		
Individual from Lane 8 is homozygous for an allele that is approximately 1000 bp.		
The allele that individuals from Lane 6 and 11 have in common is approximately 850 bp long.		
All 11 individuals from this analysis are heterozygous for this marker.		
Individual from Lane 12 is heterozygous and one of the allele has approximatively 600 pb.		

EXPERIMENT II – "*WHITE EAR*" OR NO "*WHITE EAR*"?

LINKAGE ANALYSIS ON A BEAR PEDIGREE

In the same population of bears, was identified a family, consisting of a mother bear, a father bear and two cubs, a family in which the mother bear and the cubs had an interesting characteristic: a "**white ear".**

The rangers noticed that the mother bear was pregnant again and they wondered if the unborn cub would also have that "**white ear"**, so they, again, asked for the help of the geneticists.

Using autosomal STR markers and biological samples from the mother bear, father bear and the two already born cubs, they performed linkage analyses and found that there is positive linkage between this phenotype and a maternal region spanning 0.699 cM from chromosome 4. You have to keep in mind that STR marker alleles are inherited in a Mendelian manner and that linkage analysis encompasses a group of methods that examine the inheritance pattern of DNA markers within families. It is used to determine if there is a relationship between a particular region of the genome and a phenotype of interest.

Using the same markers, they also genotyped a DNA sample from the unborn bear cub. Each individual genotype is presented in Table II-1.

Your role is to determine, based on the individual genotypes for each locus, which haplotypes the bear cubs have inherited from each parent and whether it is possible for the unborn bear cub to have the "white ear" phenotype, similar to his mother and siblings.

Table II-1. With determined genotypes for each STR locus and each individual from the bear family. Note that this time the allele "name" is given in CAPITAL LETTERS.

Name of STR loci	Papa-bear genotype	Mama-bear genotype	Cub-("daughter") genotype	Cub ("son") genotype	Unborn cub genotype
UA03	H, A	D, B	A, D	D, H	B, H
UA06	C, L	B, I	I, C	I, L	B, L
UA14	L, I	C, A	L, C	C, I	A, I
UA17	C, C	D, D	C, D	C, D	C, D
UA25	B, D	A, D	B, D	D, D	A, D
UA51	D, B	A, B	A, D	A, B	B, B

QUESTIONS:

DO NOT PROVIDE YOUR FINAL ANSWERS HERE FOR ANY OF THE QUESTIONS.

USE THE ANSWER SHEET!

B_II.1 [1 point]

Draw within the rectangular space provided in the ANSWER SHEET the pedigree representing the bear family. Use the appropriate symbols for male, female and un-born cub.

B_II.2 [2 points]

Use the information in Table II-1 regarding the bear genotype for each of the markers and write in the table in the ANSWER SHEET the haplotypes of each member of the bear family.

Individual haplotypes of the bears within the family

Name of STR loci	Papa-bear genotype		Daughter genotype		Son genotype		Unborn cub genotype		Mama-bear genotype	
	H_P1	H_P2	H	H	H	H	H	H	H_M1	H_M2
UA03										
UA06										
UA14										
UA17										
UA25										
UA51										

H_P1 = one of the paternal haplotype, H_P2 = the other paternal haplotype, H_M1 = one of the maternal haplotype, H_M2 = the other maternal haplotype, H = haplotype.

B_II.3 [0.35 points]

Identify the maternal (H_M) and, respectively paternal (H_P) haplotypes of the unborn bear cub. Write the sequence of CAPITAL LETTERS (*e.g. ABCDE*) that represents each haplotype, as you determined in Table II-1.

		Haplotype
Unborn bear cub	**Maternal haplotype**	
	Paternal haplotype	

B_II.4 [0.25 point]

Identify the maternal (H_M) haplotype associated with the *"white-ear"* phenotype. Write the sequence of CAPITAL LETTERS (*e.g. ABCDE*) that represents the haplotype.

Maternal haplotype associated with "white ear":

B_II.5 [0.25 point]

Considering the haplotype inherited by the unborn cub from its mother, is it possible for the cub to exhibit the "White-ear" phenotype? Mark with an" X" your answer in the table in the ANSWER SHEET.

	ANSWER
YES	
NO	

EXPERIMENT III

PLANT LEAVES SPECIMENS

In forensic investigations, plant anatomy can play a crucial role in identifying the origin and movement of animals, like bear cubs, by analyzing plant fragments present in collected samples. Coniferous tree species in particular have distinctive anatomical features — such as leaf structure, resin ducts, and stomatal patterns — that can vary significantly between species and even across geographic regions. When these plant parts are found, they provide indirect evidence of an animal's location or habitat.

In this case, the anatomical differences in the leaves of coniferous species from the NW, SE, and Far East regions could serve as geographic markers. By comparing the coniferous leaf anatomy found in the samples to reference samples from these areas, researchers can narrow down where the cub might have been, as the conifer species' specific traits could indicate its environment. This use of plant anatomical analysis helps forensic researchers trace the cub's potential movements and locate its origins.

Materials:

1. 3 envelopes with leaves of three coniferous species (labelled "A", "B" and "C")
2. 1 dark bottle with colorant – labelled "DYE"
3. 1 transparent bottle with DI H_2O (distilled water) – labelled "H_2O"
4. 12 pieces of microscope slides placed in one Petri dish
5. 1 box of coverslips
6. 1 scalpel
7. 1 plastic needle
8. 1 tweezer
9. 2 pencils
10. 2 red-capped plastic recipients labelled "WASTE" for waste
11. 1 pack of tissue paper (for use in all experiments)
12. 3 pairs of gloves - 3 each of sizes S, M, and L (for use in all experiments)
13. 1 compound light microscope

The DYE is specific to cell walls. It stains cellulose walls red and lignified cell walls yellow.

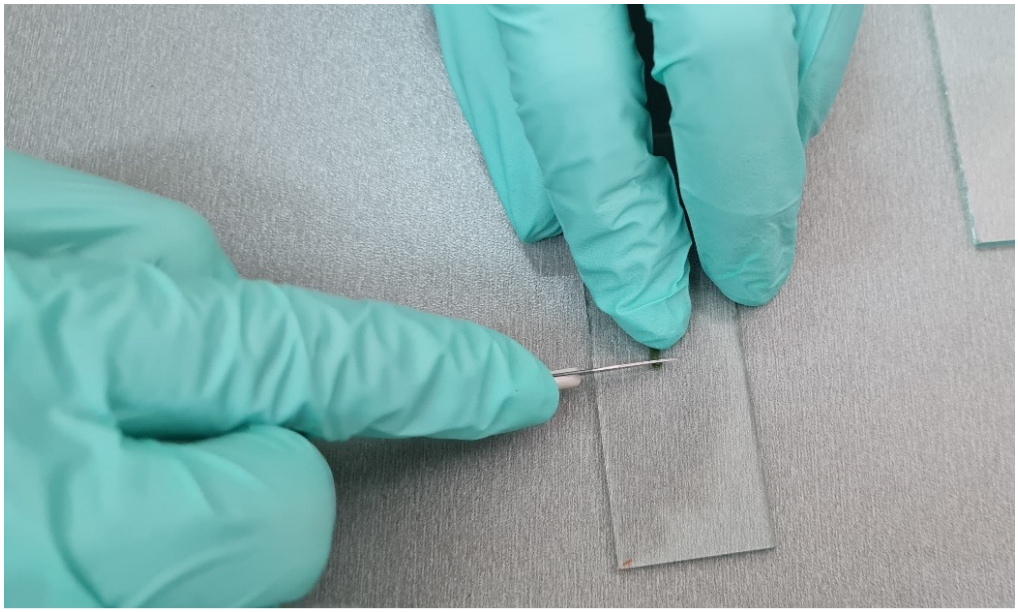
Instructions

Prepare microscope slides from each of the 3 plant leaf specimens, by transverse section and study their anatomies under a microscope. Instructions are as follows.

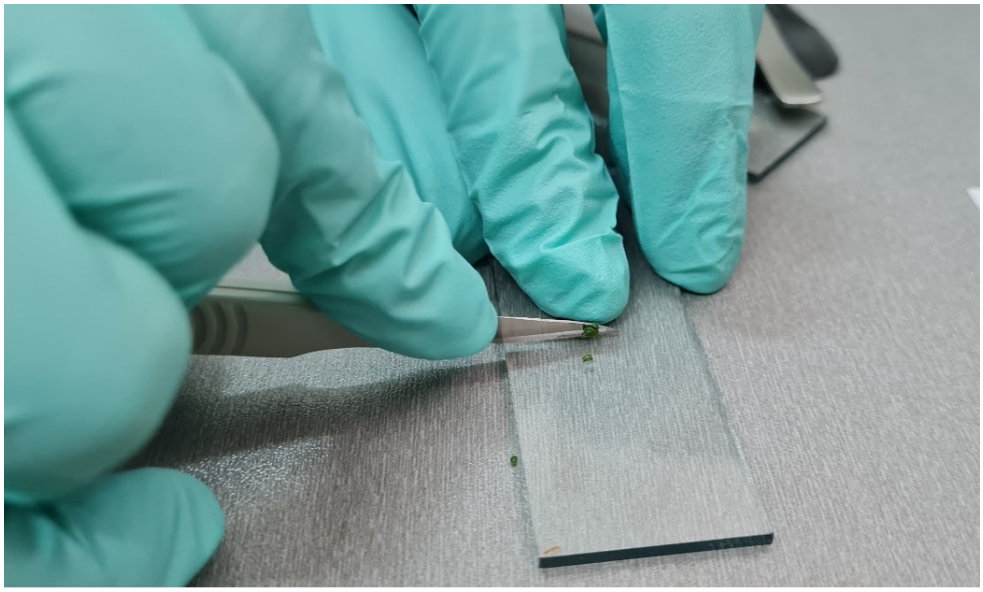
1. Transverse section of specimens: Use the provided scalpel to cut thin cross-sections of each of the plant leaf specimens as follows:

Note: Repeat the following steps for each of the plant leaf specimens provided, independently, so that you do not mix the species specimens.

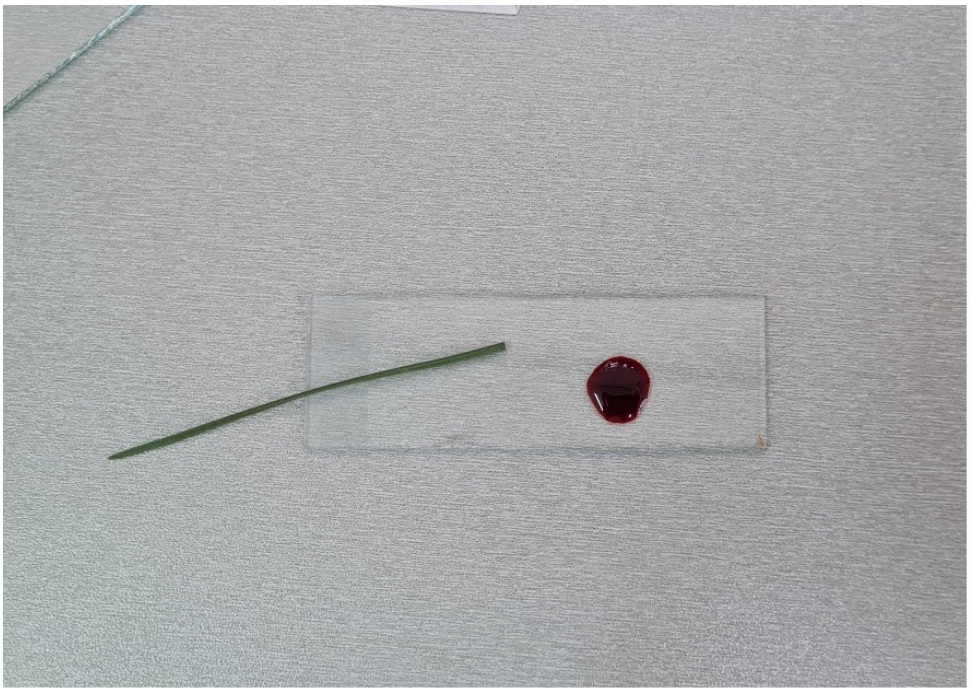
1.1 Hold the specimen down directly on a microscope slide with one hand, as shown in the figure below:



1.2 With the other hand, use the scalpel to cut the specimen vertically to obtain a thin cross-section slice, as shown in the figure below. Repeat this step several times to obtain multiple slices of each of the plant leaf specimens.

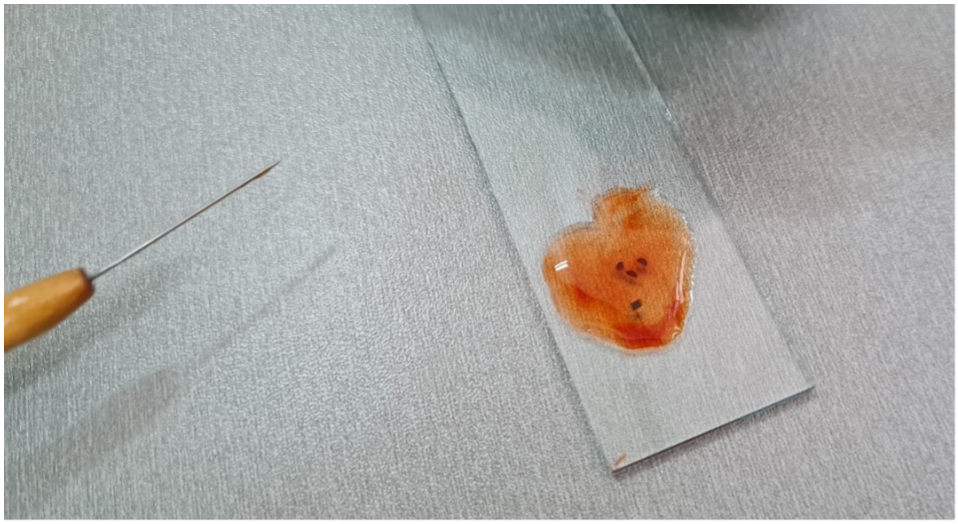


2. Choose as many slices as you like (which you consider the thinnest and whole) of each of the plant leaf specimens and transfer them on a microscope slide. Add a drop of colorant from the bottle labelled ”DYE” on top of the slices from the microscope slide and leave the colorant for approximately 1 minute.

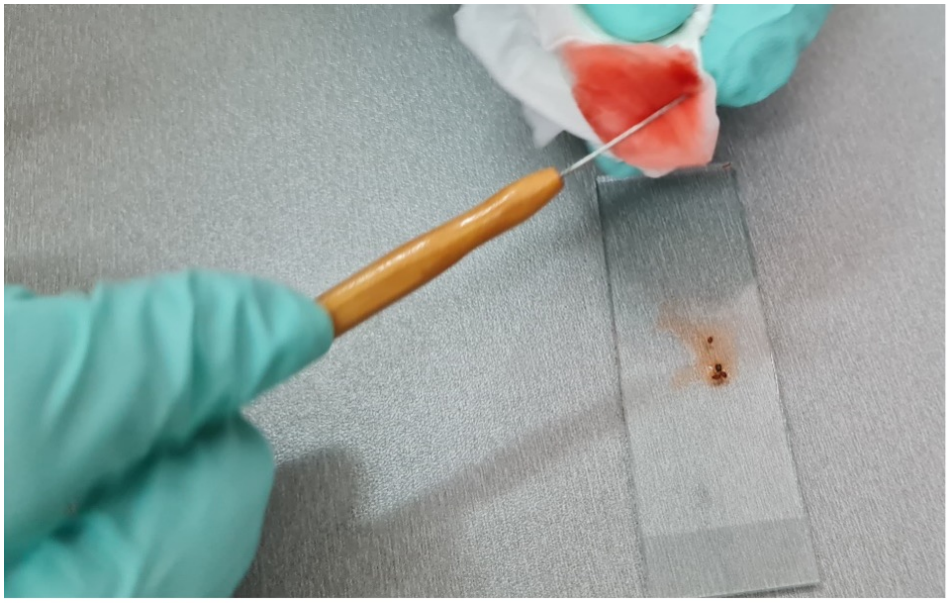


3. After about 1 minute, carefully remove the colorant by draining it with a thin tissue paper. You should be careful not to remove the specimen slices. Use the plastic needle to carefully move the specimen slices on a side, while removing the colorant.

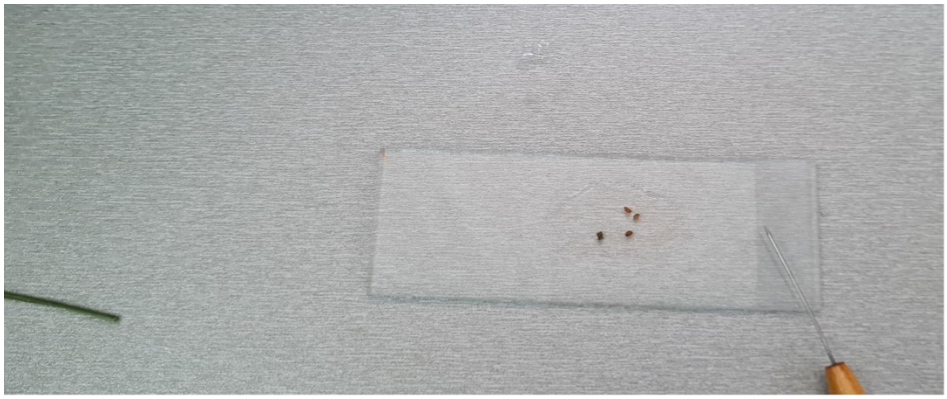
4. Add 1-2 drops of distilled water from the bottle labelled "H_2O" and using the microscope needle, gently move the specimen slices around so that the excess of the colorant is washed out.



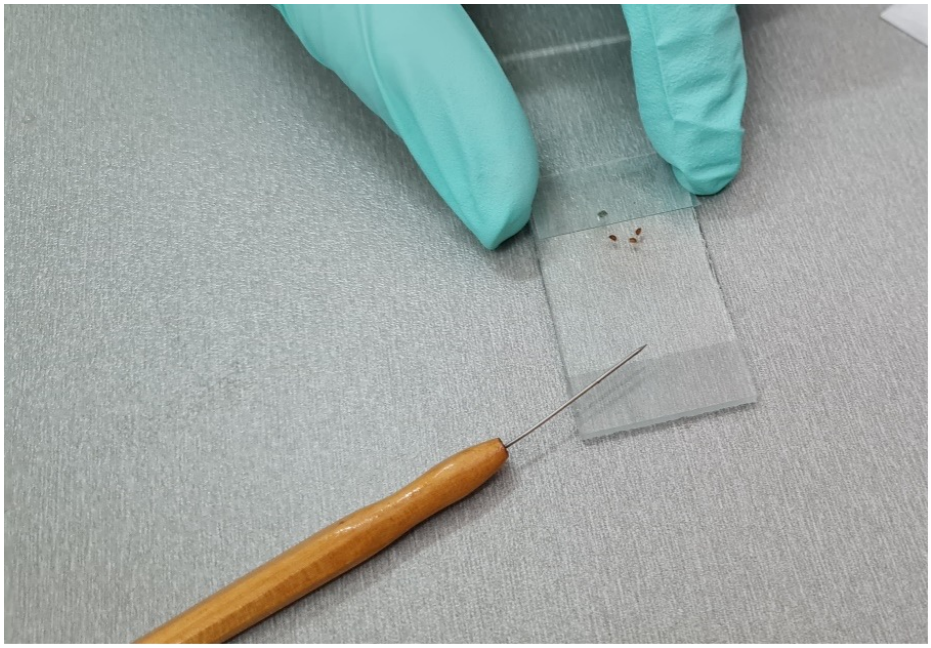
5. Carefully remove the water by draining it with a tissue paper, but take care not to remove also your specimen slices.



6. Add another 1-2 drops of water from the bottle labelled "H_2O". If the water becomes red, drain it again, and repeat the procedure until the added water remains colourless. Then proceed to the next step.



7. Cover the specimen slices with a coverslip.



8. Repeat steps 1-7 for each plant specimen.

9. Observe the prepared samples for each of the plant specimens under the provided microscope.

10. Continue to the questions.

QUESTIONS:

DO NOT PROVIDE YOUR FINAL ANSWERS HERE.

USE THE ANSWER SHEET!

B_III.1 [1.4 points]

Identify the anatomical characteristics of each leaf specimen. In Table III-1, mark an "X" in the corresponding box to indicate the presence of each identified characteristic.

Table III-1

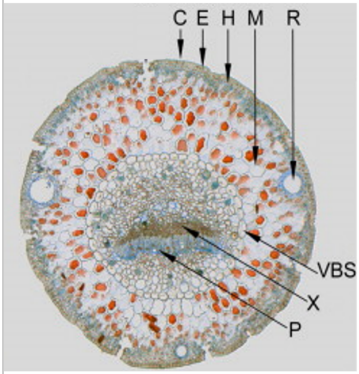
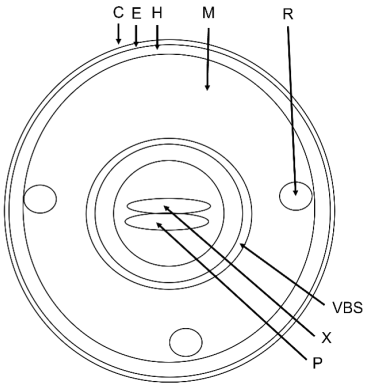
	Specimen A	**Specimen B**	**Specimen C**
Cuticle			
Trichomes			
Spongy chlorenchyma			
Palisade chlorenchyma			
Septate chlorenchyma			
1 vascular bundle			
2 vascular bundles			
More than 2 vascular bundles			
1-2 resin ducts			
More than 2 resin ducts			
Hypodermis – as Sclerenchyma			
Hypodermis – as Collenchyma			
Hypodermis – as Aquifer tissue			
Aerenchyma			

B_III.2 [4.5 points, 1.5 points for each leaf specimen]

Draw a schematic representation of each specimen's leaf in the rectangular space provided. Orient the leaf drawing with the upper epidermis facing upward on the page. Use circles to represent resin ducts (if present) and ovals to represent vascular bundles.

Label with: C – cuticle, E – epidermis, H – hypodermis, M – mesophyll, R – resin duct, VBS – vascular bundle sheath, X – xylem, P – phloem.

Note: an example for schematic representation is given bellow:

Example of a schematic representation – real cross section left, schematic representation right
 

Specimen A – schematic representation

Specimen B – schematic representation

Specimen C – schematic representation

B_III.3 [0.25 points]

Identify which plant specimen (A, B, or C) corresponds to the leaf of the plant species dominant in the North West (NW) area of the village. Note that this species has a leaf shape closest to a rectangle and is singly attached. Write ”X” in the corresponding box in the table.

	Specimen A	Specimen B	Specimen C
The plant species in the NW are of the village			