Task 2

Tisza
“the Blonde River”

photo: Péter Vankó

11. 05. 2021.
Szeged
Hungary

Tisza, “the Blonde River”

The Tisza is the main river of Eastern Hungary, it flows from the Eastern Carpathians through the Great Hungarian Plain into the river Danube. Its total length was 1419 km but it has been shortened by regulation works in the 19th century to 962 km. The river drains 156 thousand km^2. Once, it was called “the most Hungarian river” because it flowed entirely within the historical Kingdom of Hungary. Today the Tisza begins in Ukraine, at the confluence of White and Black Tisza, collects water also from Slovakia, Romania, and Hungary, and it joins the Danube in Serbia.
At the Ukrainian-Hungarian border, the Tisza is a fast and green water river, which rolls pebbles in its bed. It becomes “blonde” 50 km down as the Szamos, a river originating in Romania mixes in its sandy brown water. As the Tisza flows towards Szeged it becomes slower and slower, and also its alluvium becomes finer and finer. If you walk along the river on the side opposite to the city centre of Szeged or if you make a paddle tour with a kayak or canoe you can find this very fine yellow sand.

The Tisza is the “life-giving” river of Szeged and Hungary. Besides its unique wildlife and ecosystem, it also plays an important role in field crop production. 90% of Hungary is endangered by drought, which could greatly determine the country’s agriculture. Plant biology research in Szeged goes back to more than 100 years, which focuses on how to resolve the disadvantages of drought and breeding of drought-resistant crops. In **Part A**, we will examine the effect of drought stress on plants, the understanding of which could help researchers to breed new drought-tolerant plant species.

In **Part B**, you will face some of the problems of our modern societies: environmental pollution. The *Tisza* is very susceptible to water contaminations, because it has a long, loopy pathway in Hungary, and it covers large areas of riverside locations, from which nearby industrial and agricultural sites may release pollutions into the water streams. Examples of polluting compounds are nitrite salts. Recently, significant levels of nitrite ion pollution have been discovered in samples collected along the Tisza, but the origin of the contamination is not known. Does the pollution come from a factory, built next to the banks of Tisza, or from an agricultural site next to one of the tributaries of Tisza? In this Part B, is your challenge to locate the sources of contamination!

In **Part C**, you will encounter the surprising behaviour of *granulated* materials. The most relevant material in our topic *Tisza* is of course the yellow sand but you will work with black volcanic sand, and poppy seeds, too. You will measure and investigate the angle of repose, density, patterns of spontaneous segregation, and jamming in real experiments in your lab. In addition, you will evaluate optical measurements made in Budapest about the sedimentation of your black sand sample.

Part A

Tisza is the 'life-giving' river of Szeged and Hungary. Besides its unique wildlife and ecosystem, it also plays an important role in field crop production. 90% of Hungary is endangered by drought, that could greatly determine the country's agriculture. Plant biology research in Szeged goes back to more than a 100 years, that focuses on how to resolve the disadvantages of drought and breeding of drought-resistant crops.

Prolonged drought affects most plants' susceptibility. The stress response depends on various factors, such as the intensity of the stress, its duration, the physiological and development status of a plant or its genotype. As a result of water deprivation, the homeostasis of plants gets disrupted, that leads to a decrease in shoot elongation, the size of leaf surface and photosynthetic activity and enhances the aging of leaves. As a result of prolonged drought, the yield and quality of crops also decreases. The stress response of plants shows a specific kinetics. As a result of drought, plants react with rapid stoma closure, with the purpose of reducing transpiration from leaves. In the long term, the photosynthetic activity decreases along with the amount of photosynthetic pigments, that reduces biomass production, and increases the accumulation of reactive oxygen species (ROS) thereby enhancing leaf senescence. In drought-tolerant plants, the accumulation of various compatible osmotic compounds (glycine betaine, proline, sugar alcohols) and antioxidants could aid successful acclimatization.

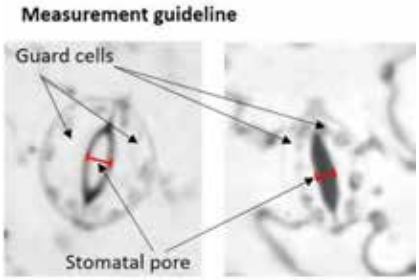
In this part, we will examine the effect of drought stress on plants, the understanding of which could help researchers to breed new drought-tolerant plant species.

A1. How can we detect the short-term effects of drought stress in plants? **A2.** How does prolonged drought stress affect plants? **A3.** How do plants defend themselves against drought stress?

Problem A1 *(32 points)*

We can monitor the rapid stress response of plants by measuring their stomatal closure.

Stomata are located in the epidermis of leaves and play an important role in the regulation of water balance and CO_2 uptake. A stoma consists of two guard cells that regulate the size of the stomatal pore in-between. The open and closed status of the stomata is regulated by various environmental factors (e.g. light/darkness, temperature, CO_2) and endogenous signals (e.g. abscisic acid, ROS). As a result of stress, stomata close rapidly, that is greatly determined by the hormone abscisic acid (ABA), the concentration of which also quickly increases in leaves during drought. The direct cause of stoma closure comes from a decrease in the guard cells' turgor pressure as a result of ABA-activated ion channels, through which K^+ and Cl^- efflux occurs from the guard cells, that is followed by water in a passive manner.



We can measure the open/closed status of the stomata by microscopy, using epidermis peels of leaves. In this experiment, the beginning of the artificial drought stress is 9:00 a.m. We prepare epidermis peels from well-watered plants and plants that underwent drought stress using forceps. Epidermal peels should be obtained from leaves with the same/similar development status for the sake of a fair comparison. The fresh samples are then immediately transferred to a buffer solution droplet on a glass slide that we then cover with a cover glass. The fresh dissections are then placed under a microscope and using the same magnification (e.g. 400x) we take images of the samples. We then measure the diameters of the stomatal pores on every image, calculate the average per image and represent the results on a diagram.

Devices and materials for this problem:

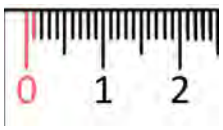
- Ruler
- Pen
- Calculator

A1.1 Measurement of the stomatal pore.

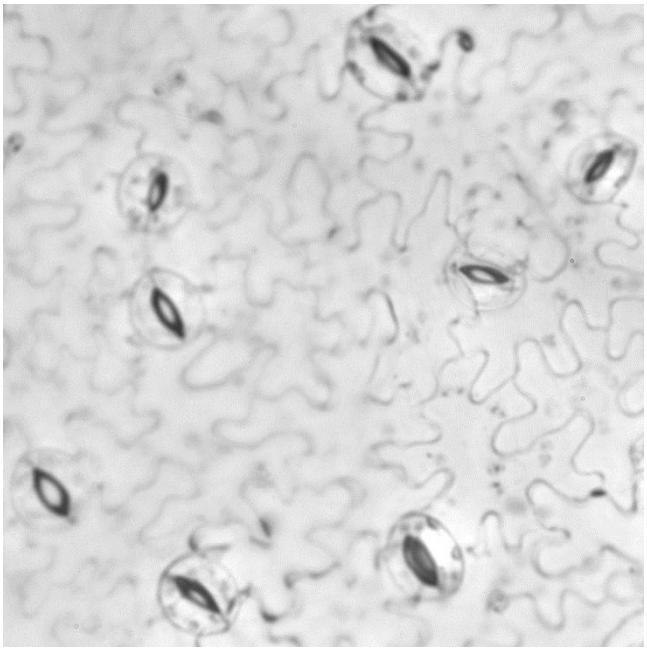
Using a ruler, we can measure the drought-induced stomatal pore diameter on the epidermis peels taken at different time points.

WARNING!

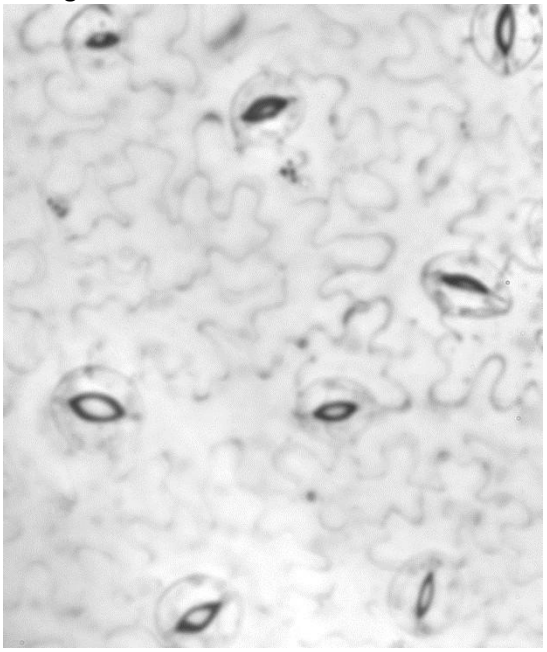
Please check the 'printed 2 cm' corresponds to 2 cm on the real ruler!



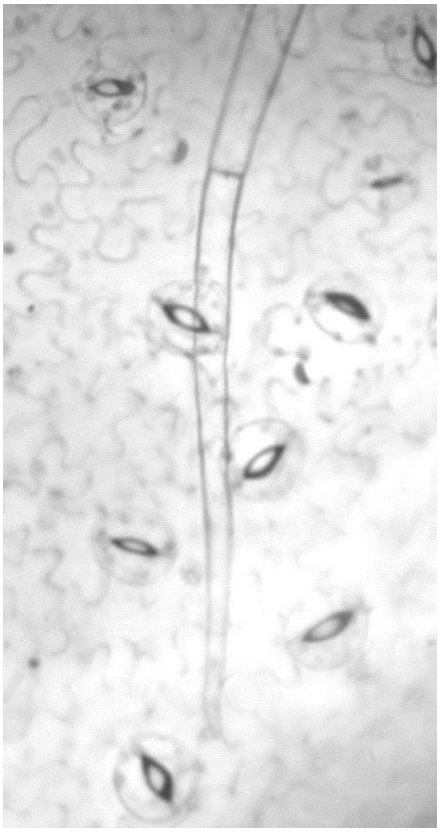
Control 9:00 a.m.



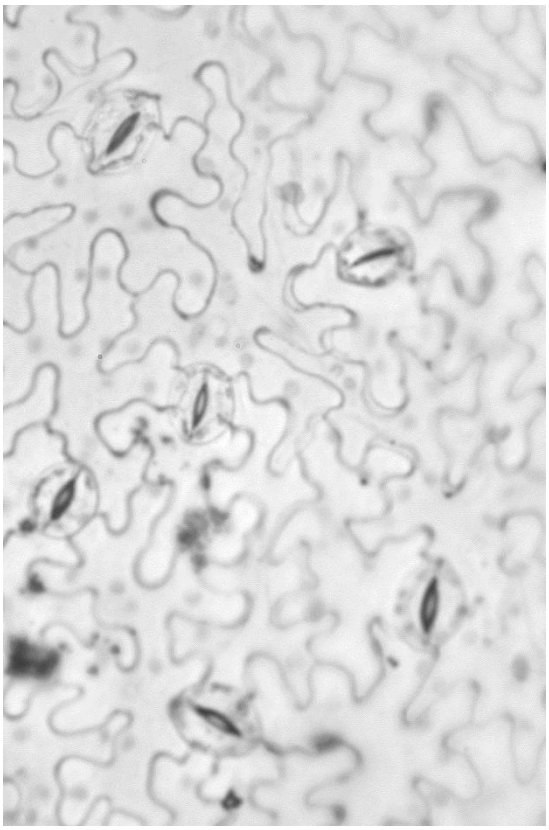
Drought stress 9:00 a.m.



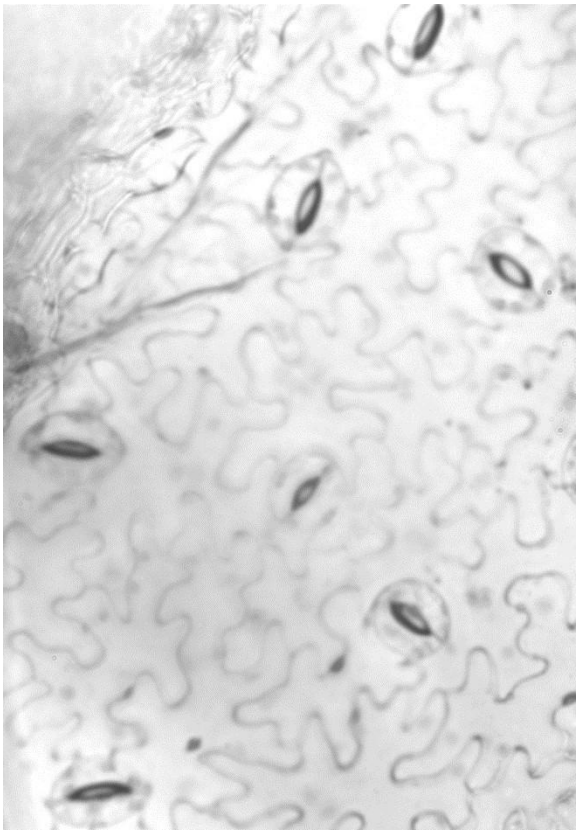
Control 10:00 a.m.



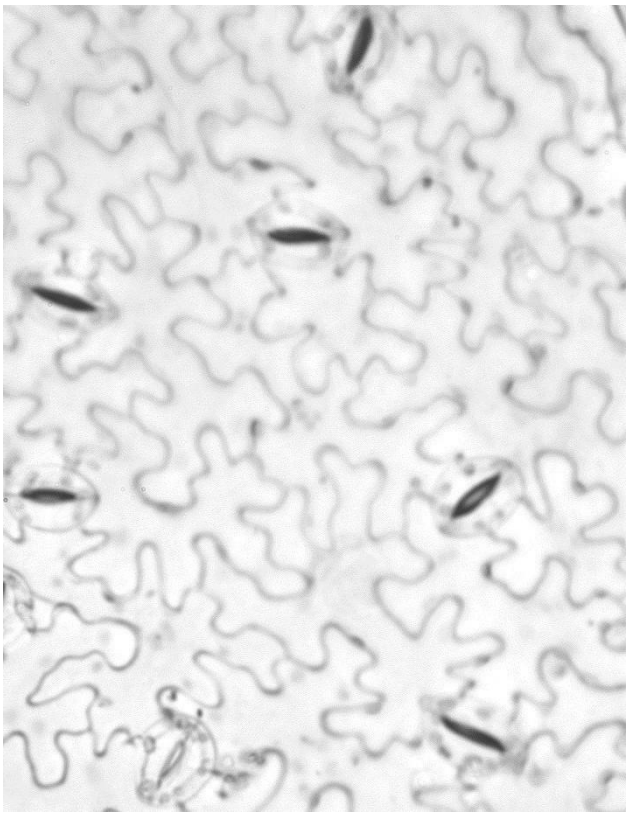
Drought stress 10:00 a.m.



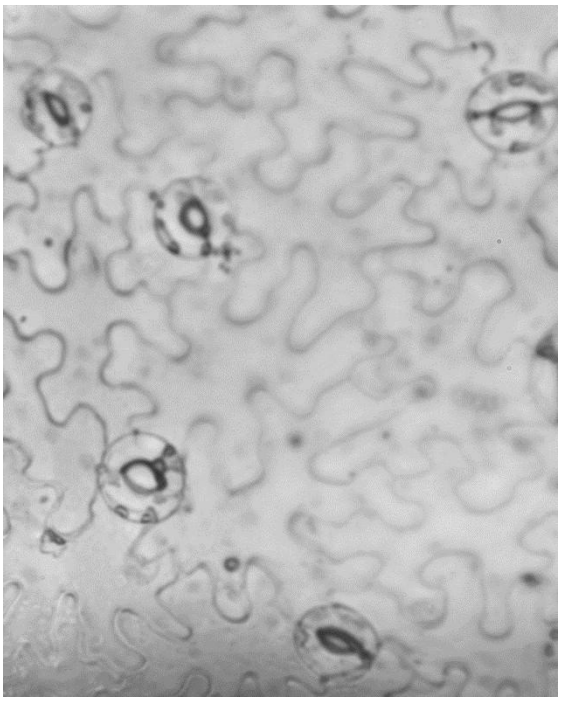
Control 12:00 a.m.



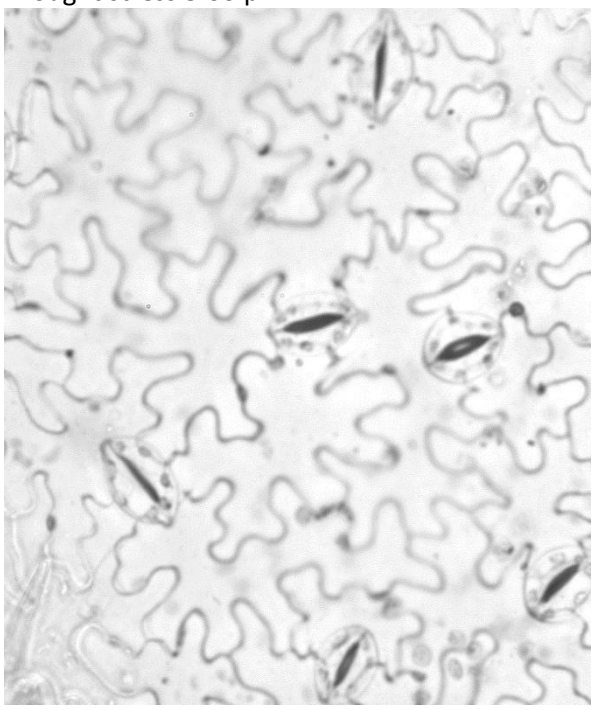
Drought stress 12:00 a.m.



Control 3:00 p.m.



Drought stress 3:00 p.m.



Measure the diameter of stomatal pores on the epidermis peels images at each time point in each condition! Measure all stoma on each photo! (Use the outer edge of the pore as the start/endpoint based on the figure in the introduction.)
Write your measurement data in the table below! (This table won't be evaluated.)

Treatments	Results (mm)
Control 9:00 a.m.	
Drought stress 9:00 a.m.	
Control 10:00 a.m.	
Drought stress 10:00 a.m.	
Control 12:00 a.m.	
Drought stress 12:00 a.m.	
Control 3:00 p.m.	
Drought stress 3:00 p.m.	

Question A1.1 Based on the measured data you collected in the table above, determine the closest average values at each time point in each condition! Write your answer in the field **A1.1** on the *Answer sheet*.

Treatments	Means (mm)
Control 9:00 a.m.	A) 2.91 B) 2.53 C) 3.01 D) 1.70
Drought stress 9:00 a.m.	A) 2.43 B) 1.40 C) 2.89 D) 3.31
Control 10:00 a.m.	A) 1.56 B) 2.81 C) 2.32 D) 3.07
Drought stress 10:00 a.m.	A) 1.60 B) 1.11 C) 1.93 D) 2.02
Control 12:00 a.m.	A) 2.67 B) 2.59 C) 1.66 D) 2.37
Drought stress 12:00 a.m.	A) 1.00 B) 1.82 C) 2.08 D) 2.11
Control 3:00 p.m.	A) 2.20 B) 3.13 C) 3.20 D) 2.96
Drought stress 3:00 p.m.	A) 0.90 B) 1.53 C) 1.29 D) 1.78

Question A1.2 Based on the average values, calculate the actual size (size in real life) of the stomatal pores in control and drought-stressed plants at 9:00 a.m. and 3:00 p.m.! Write your results in the field **A1.2** on the *Answer sheet*. **On the image 3cm = 100 µm.**

Question A1.3 Calculate how much did the stomatal pore size change under 6 hours as an effect of drought stress! Provide the answer in µm! Write your result in the field **A1.3** on the *Answer sheet*.

Question A1.4 Calculate the stomatal opening of drought-stressed plants at 3:00 p.m. to the control at 3:00 p.m.! Provide the answer in percentage (control plants are 100% open)! Write the correct answer in the field **A1.4** on the *Answer sheet*.

Question A1.5 Based on the experimental results, how is the physiological status in the leaves of drought stress-treated plants at 9:00 a.m. compared to the control? Write your answer in the field **A1.5** on the *Answer sheet*.

A) There are no significant differences in the transpiration
B) Water uptake is higher
C) The stomata closed
D) CO_2 uptake decreased

Question A1.6 Based on the experimental data, what physiological change could be observed in the leaves of drought stress-treated plants at 3:00 p.m. compared to the control? Write your answer in the field **A1.6** on the *Answer sheet*.

A) There is no significant difference in stomata movement
B) Water uptake is higher
C) Transpiration increases
D) CO_2 uptake decreases

Question A1.7 In terms of drought stress tolerance the breeding of what types of plant species could be an aim for scientists? Write the correct answer in the field **A1.7** on the *Answer sheet*.

A) Plants with low stomata amounts
B) Plants with large stomata sizes
C) Plants with large ABA concentrations
D) Plants with rapid ABA production

Problem A2 *(32 points)*

The effect of permanent drought on plants can be detected by changes in the amount of photosynthetic pigments in leaves.

A photosynthetic pigment's function lays in its ability to bind light energy in the chloroplasts of plant cells. Their characteristic includes the extended conjugated double bond system, through which they can be excited with photons of the visible spectrum (390 – 750 nm).

The energy of photons of the visible spectrum is enough to induce a photochemical reaction. Photosynthesizing organisms have various types of photosynthetic pigments, that differ in structure as well as in their function. Chlorophylls are the most important photosynthetic pigments in higher families of plants, that play a role in the binding of light energy as well as in conversion of light energy into chemical energy (charge separation). Chlorophylls contain four pyrrole rings as well as a cyclopentatonic ring that form a so-called chlorine structure. Magnezium ion is located in the center of a chlorine structure. To the chlorine structure, a 20 carbon atom long phytol chain is also attached.

In higher classes of plants, chlorophyll a (blueish-green color) and b (yellowish-green color) can also be found, that absorb at different wavelengths, and their amount as well as their ratio can also be altered upon stress. Accessory pigments can also be found in plants, those functions include the binding of light energy and its transfer (energy transfer) to the chlorophyll pigments. These pigments are not involved in charge separation, but play a role in the prevention of damages caused by excess light (e.g. antioxidant function). Such pigments include carotenoids (40-carbon atoms, dark yellow-orange color). Xanthophyll molecules (light yellow color) – that belong to one of the subgroups of carotenoids - play an important role in photoprotective processes, in the thermal dissipation of excessive energy.
During the experiment, photosynthetic pigments are extracted from the leaves of control plants and plants exposed to prolonged drought stress, then, after paper chromatographic separation, the amount and composition of pigments are compared.

Devices and materials for this problem:

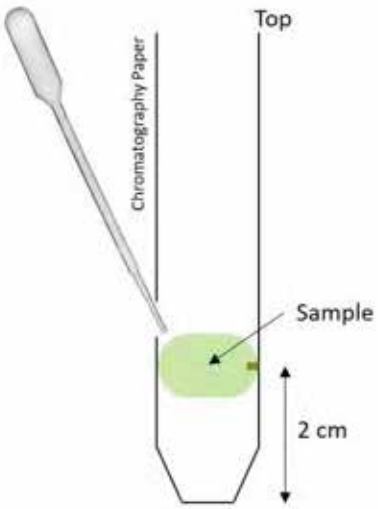
- Samples from control plants (**T2-A-2/1**) and plants exposed to prolonged drought (**T2-A-2/2**)
- Spatula
- 2x Weighing boat large
- 2x 15 mL Falcon-tubes
- 2x 50 mL Falcon-tubes
- 3x Plastic Pasteur pipettes
- 2x Chromatographic papers (**T2-A-2/3**)
- Glass beaker (250 mL)
- Acetone (10 mL)
- Acetone-petroleum ether 8:92 (10 mL) (**T2-A-2/4**)
- Plastic forceps
- Balance scale
- Ruler, Marker, Pencil, Pen
- Calculator
- Timer

SAFETY WARNINGS!
Keep acetone away from heat, hot surfaces, sparks, open flames and other ignition sources.
Do not breathe dust of acetone.
Protect your eyes from acetone.

A2.1 Detection of changes in photosynthetic pigments

Place a weighing boat on a balance, press the tare button (reset the displayed weight on the scale back to zero), and take 0.25 g from one of the plant samples using the spatula. Repeat the procedure with the other sample. Using a marker, label two 15 mL Falcon tubes according to the treatments. Add 5 mL of acetone in the 15 mL Falcon tubes and place the plant samples in the appropriate tubes. (Use the scales on the Falcon tubes to measure the amount!) Shake the samples for 5 minutes so that the acetone can extract the photosynthetic pigments from the samples. Place the Falcon tubes

in a glass beaker and wait a couple of minutes for the sediment to sink to the bottom of the Falcon tubes. In the meantime, aspirate 2 mL of eluent (acetone-petroleum ether 8:92) into each of the 50 mL Falcon tubes (two altogether) with a plastic Pasteur pipette. Label the two 50 mL Falcon tubes according to the plant samples. Put two paper chromatographs on the table horizontally. Measure 2 cm from the bottom of the paper chromatograph using a ruler and mark the distance at the edge of the paper with a pencil. Mark both of the paper chromatographs! Use a pencil to mark the paper, as the ink of a pen will be dissolved by the eluent. Use one paper chromatograph for one of the pigment extracts and the other chromatography paper for the other extract. Using a Pasteur pipette, aspirate 0.5 mL from the top half of one of the pigment extracts and carefully draw a line with it on one of the chromatography papers at a distance of 2 cm from the bottom. Do NOT push the entire liquid sample out of the pipette at once! By placing the tip of the pipette to the chromatography paper, it will slowly absorb some of the liquid. Repeat the 'drawing' five times in the same line with the remained pigment extract, exactly the same way as you did before. Try to make the strip as thin and as concentrated as possible! Wait until the pigment extracts dry on the chromatography papers between the 'drawing'. Perform this step also with the other pigment extract on the other chromatography paper. Wait until the pigment extracts dry on the chromatography paper. Once the papers dry, gently place them into the 50 mL Falcon tubes - containing acetone-petroleum ether 8:92 – with a forceps, so that the pigment extract strips are at the bottom of the paper, but just above the eluent. Close the 50 mL Falcon tubes to prevent the eluent from evaporating. Wait 10 minutes until the chromatograms occur. Using the forceps carefully remove the chromatograms from the Falcon tubes and place them onto the table. Wait until the chromatograms dry. Close the 50 mL Falcon tubes to prevent the eluent from evaporating.



Question A2.1 Identify the photosynthetic pigments in the control sample by numbering from the top of the chromatography paper! Write the letter of the appropriate pigment to the corresponding numbers in the field **A2.1** on the Answer sheet.

A) Chlorophyll *a*
B) Chlorophyll *b*
C) Carotene
D) Xanthophylls

Question A2.2 Measure the vertical width of each pigment streak with a ruler on the chromatographs and determine which pigments' amount altered due to the prolonged drought stress compared to the control! Write your answer in the field **A2.2** on the *Answer sheet*.

A) Increased
B) Decreased
C) Did not change

Findings
The amount of chlorophyll *b* in the drought stress-treated plants compared to the control:
The amount of chlorophyll *a* upon stress compared to the control:
The ratio of chlorophyll *a/b* as a result of prolonged drought stress:
The amount of carotene in the drought stress-treated plants compared to the control:
The amount of xanthophylls as a result of prolonged drought stress compared to the control:

Question A2.3 Based on the experimental results, what physiological alteration could be detected in the leaves of drought stress-treated plants at 3:00 p.m.? Write the correct answer in the field **A2.3** on the *Answer sheet*.

A) As a result of prolonged drought stress, photosynthesis efficiency increases due to the increased amounts of carotenoids.

B) As a result of drought stress, the efficiency of light absorption decreases, due to the increased amount of xanthophylls.

C) As a result of drought stress, biomass production decreases, because the altered chlorophyll *a/b* ratio reduces light absorption efficiency.

D) As a result of drought stress, photosynthesis efficiency decreases due to the increased amounts of chlorophyll *a*.

Question A2.4 In terms of drought stress tolerance the sublimation of what types of plant species could be an aim for breeders? Write the correct answer in the field **A2.4** on the Answer sheet.

A) Plants with low chlorophyll amounts
B) Plants with high chlorophyll amounts, but low carotenoids amounts
C) Plants that are not prone to loose high amounts of chlorophyll and carotenoids
D) Plants with elevated xanthophylls production only, because only these pigments play a role in photo-protective processes

Problem A3 *(36 points)*

The defense of plants against drought stress is monitored by the detection of changes in proline levels.

The water-soluble proline is an amino acid, whose concentration can increase almost up to a hundred-fold in plants during drought stress. Drought-stress induced elevated ABA concentrations play a key role in the rapid induction of proline synthesis. Proline is a compatible osmotic compound that provides membrane and protein protection during drought stress. Besides, it also plays a role in ROS scavenging.

During the experiment, first we will prepare a calibration dilution from a pre-determined stock solution of proline. Then, we will prepare extracts of leaves derived from both control plants and plants that underwent drought stress for several days. Droplets of both proline solutions and plant extracts will be placed on an isatin test paper and exposed to heat. Isatin reacts with proline in a specific manner. Based on the known concentrations of proline solutions, the proline content of plants can be determined.

Devices and materials for this problem:

- Control plant samples and samples from plants underwent drought stress
- Proline (0.3 g) in Eppendorf (**T2-A-3/1**)
- Spatula
- 2x Weighing boat large
- Weighing boat small
- 6x 15 mL Falcon tube
- 1x 50 mL Falcon tube
- 3x Plastic Pasteur-pipette
- 2x Isatin test paper covered with aluminum foil (**T2-A-3/2**)
- Glass beaker (250 mL)
- Ethanol (50 mL)
- Distilled water (50 mL)
- plastic forceps
- Balance scale
- Ruler
- Marker
- Pencil
- Pen
- Calculator
- Timer
- Hair dyer

SAFETY WARNINGS!

Keep ethanol away from heat, hot surfaces, sparks, open flames and other ignition sources.

Protect your eyes from ethanol.

A3.1 Examining plant defense against drought stress based on changes in proline amounts

Prepare 50 mL of 80% (V/V) ethanol solution from 100% ethanol, using distilled water.

> **Question A3.1** Calculate how much absolute ethanol (100%) and distilled water will you need for the solution! Write your answer in the field **A3.1** on the *Answer sheet*.

A) 30 mL ethanol and 20 mL distilled water
B) 40 mL ethanol and 10 mL distilled water
C) 45 mL ethanol and 5 mL distilled water
D) 48 mL ethanol and 2 mL distilled water

For preparing the solution, use a 50 mL Falcon tube. Use the scales on the Falcon tube to measure the amounts! Shake the solution profoundly!

Place a weighing boat on a balance scale, press the tare button, and take 0.1 g proline using the chemical spoon and pour it into a 15 mL Falcon tube, then add 10 mL 80% ethanol to the powder. Use the scales on the Falcon tube to measure the amounts! Shake the solution profoundly! Prepare the following dilutions from the proline stock solution using 80% ethanol: 2X, 5X, 50X, with a final volume of 10 mL for each.

> **Question A3.2** Calculate how much 80% ethanol and proline stock solution will you need! Write your answer in the field **A3.2** on the *Answer sheet*.

A) 2X: 8 mL proline + 2 mL 80% ethanol; 5X: 9 mL proline + 1 mL 80% ethanol; 50X: 5 mL from the 5X diluted proline + 5 mL 80% ethanol

B) 2X: 2 mL proline + 8 mL 80% ethanol; 5X: 5 mL proline + 5 mL 80% ethanol; 50X: 9 mLfrom the 5X diluted proline + 1 mL 80% ethanol

C) 2X: 5 mL proline + 5 mL 80% ethanol; 5X: 2 mL proline + 8 mL 80% ethanol; 50X: 1 mL from the 5X diluted proline + 9 mL 80% ethanol

D) 2X: 1 mL proline + 9 mL 80% ethanol; 5X: 5 mL proline + 5 mL 80% ethanol; 50X: 9 mL from the 5X diluted proline + 1 mL 80% ethanol

Label three 15 mL Falcon tubes according to the dilutions (2X, 5X, 50X). For the measurements use a plastic Pasteur pipette and the scaling on the Falcon tube! Shake the solutions profoundly!

Unwrap an isatin test paper from the aluminium foil and place it on the table using forceps. Divide the strip into four equal proportions with a ruler and a pencil. Carefully drop (c.a. 0.1 mL) from each proline solution (stock, 2X, 5X, 50X) to the isatin test paper using a Pasteur-pipette. Start from the most diluted solution and proceed onto the more concentrated solutions! Wait a few minutes, until the droplets dry. Dry the patches using hot air with the help of a hair dryer, until the color reaction occurs (c.a. 2 min)!

Question A3.3 Determine the color reaction of proline on the isatin test paper! Write the correct answer in the field **A3.3** on the *Answer sheet*.

A) Stock solution: dark blue; 2X: greenish-blue; 5X: blue; 50X: dark blue
B) Stock solution: pale blue; 2X: greenish-blue; 5X: blue; 50X: yellow
C) Stock solution: green; 2X: greenish-blue; 5X: yellow; 50X: yellow
D) Stock solution: dark blue; 2X: intense blue; 5X: blue; 50X: greenish-yellow

Question A3.4 Calculate the proline concentration of the dilution! Write the correct answer in the field **A3.4** on the *Answer sheet*. Use a calculator!

A) 2X: 50 mg/mL; 5X: 20 mg/mL; 50X: 2 mg/mL
B) 2X: 50 mg/mL; 5X: 20 mg/mL; 50X: 1 mg/mL
C) 2X: 5 mg/mL; 5X: 2 mg/mL; 50X: 0.2 mg/mL
D) 2X: 5 mg/mL; 5X: 2 mg/mL; 50X: 1 mg/mL

Place a weighing boat on a balance scale, press the tare button, and take 0.25 g from one of the plant samples using the spatula. Repeat the procedure with the other sample. Using a marker, label two 15 mL Falcon tubes according to the treatments. Place the plant samples in the appropriate 15 mL Falcon tubes and add 5 mL 80% ethanol to each. (Use the scales on the Falcon tubes to measure the amount!) Shake the samples for 5 minutes so that the 80% ethanol can extract the proline from the samples. Place the Falcon tubes in a glass beaker and wait a couple of minutes for the sediment to sink to the bottom of the Falcon tubes. In the meantime, place another isatin paper on the table with forceps. Divide it into two and label them with a pencil according to the treatments. Carefully drop (c.a. 0.1 mL) form each plant extract to the isatin paper using a plastic Pasteur-pipette. Wait a few minutes, until the droplets dry. Dry the patches using hot air at close distance with the help of a hair dryer, until the color reaction occurs (c.a. 2 min)!

Question A3.5 Determine the color reaction of the extracts of plant samples on the isatin test paper!
Write the correct answer in the field **A3.5** on the Answer sheet.

A) Control: dark blue; drought stress treated: yellow
B) Control: yellow; drought stress treated: blue
C) Control: yellow; drought stress treated: green
D) Control: dark blue; drought stress treated: greenish-blue

Question A3.6 Calculate the proline concentration in the plant samples! Use the previous calibration (A3.4. and A3.5.)! Write the correct answer in the field **A3.6** on the *Answer sheet*.

A) drought stress treated: 50 mg/mL
B) drought stress treated: 2 mg/mL
C) drought stress treated: 1 g/mL
D) drought stress treated: 0.2 mg/mL

Question A3.7 What could be the purpose of proline detection? Write the correct answer in the field **A3.7** on the *Answer sheet*.

A) It directly indicates ROS amounts during drought stress
B) It indicates changes in amino acid composition during drought stress
C) It correlates with the amount of ABA
D) It can be used as a marker of drought stress

Part B
Detective story – spotting contamination sources along the paths of Tisza and its tributaries

Improper industrial or municipal wastewater treatment and disposal are one of the most frequent environmentally relevant problems all over the world. Polluted waters entering streams and rivers can then have adverse effects on various time and volume scales. They may cause only local damages to aquatic living systems, but in extreme cases, they can generate severe regional conflicts.

Although effluent streams from agricultural sites and bioprocessing plants generally do not contain high levels of hazardous components, large volumes of aqueous solutions containing organic and inorganic nutrients are potentially toxic in aquatic environments. Industrial plants, but even smaller factories may cause even greater levels of contamination.

Nitrite anions are not among the most abundant contaminants, but may originate from various sources ranging from agriculture to heavy industry.

Problem B (*100 points)*

The abnormal change of some riverside plant species at certain areas along the Tisza aroused strong suspicion that **Tisza and some of its side rivers** (tributaries) have recently been **polluted by nitrite anion**s. As a quick measure, the Environmental Authorities already collected several samples from the inland waters, starting from the incoming point of Tisza near Ukraine, until the exit point at the Serbian border. Meanwhile, authorities of the neighbouring countries also collected samples at close-border checkpoints. However, at present, no one knows exactly what is (or what are) the source(s) of the nitrite contamination. Does it come naturally, just it hasn't been spotted yet? Is there a single source, or are there multiple sources? Does anyone accidentally, or worse, neglectfully, "poison" effluent waters? In this problem, **envision yourself as the chief chemist, who is commissioned as the responsible to locate unambiguously the contaminating site(s).**

Meanwhile, you received help: the Environmental Engineer Team searched for potential contaminating sites along the whole Eastern Hungary. These are indicated on the map found in the section **B.3** (note: the same map is found separately as an A4 sheet). This map (**Figure 2**) also indicates the 18 sampling sites from which water samples were collected.

SAFETY WARNINGS!

1) Mouth pipetting is not allowed!

2) Insert the top of the pipette in the bottom of the pipetting ball. Be careful, don't break the glass pipette!

3) Watch out! Do not draw liquid into the ball!

4) Wear protective gloves and lab-coat during the whole set of titration experiments.

Equipment and materials for this problem:

EQUIPMENT:

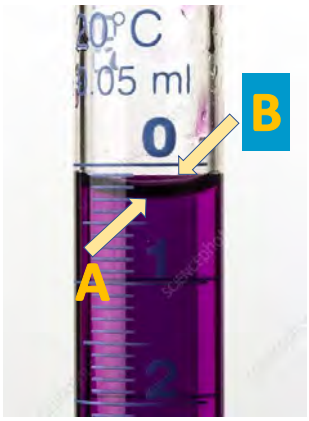
- Volumetric flask, 500 mL volume
- Volumetric glass pipette, 10.00 mL volume (non-graduated) – for nitrite salt solutions
- Volumetric glass pipette, 10.00 mL volume (non-graduated) – for oxalate salt solution
- Pipetting ball
- Burette, 10.00 mL volume
- Clamp and stand for burette
- Beaker, 50 mL volume – for filling up the burette with the titrant solution
- Beaker, 50 mL volume – for pipetting of oxalate salt "auxiliary reagent"
- Beaker, 250 mL volume – for dissolution of solid oxalate salt
- Beaker, 250 mL volume – for disposal of waste solutions (titrants, etc.)
- Erlenmeyer flask with standard ground joints, 250 mL volume (with caps) – for titration
- Pasteur pipette, graduated – for addition of sulphuric acid solution
- Funnel
- Water Flask
- 1 blank A4 sheet (for visibility of titration endpoint, optional)
- Balance
- Electric heater

MATERIALS/SAMPLES:

- titrant solution – c ≈ 0.02 mol/dm^3 $KMnO_4$ solution – in 0.5 L dark glass, noted as "T2-B/1"
- 4 × dilute sulphuric acid solution – c ≈ 0.9 mol/dm^3 – in 40-mL vials, noted as "T2-B/2"
- 1 × solid, anhydrous Na-oxalate ($Na_2(COO)_2$) – in 40-mL vial, noted as "T2-B/3"
- 3 × Tisza river sample – checkpoint #°5, collected @ Tiszalök – in 40-mL vials: "T2-B/#5"
- 3 × Körös river sample – checkpoint #°11, collected @ Öcsöd – in 40-mL vials: "T2-B/#11"
- Deionized (DI) water

Please pay attention to the following:

- Due to the shortage of time, plan to make only two parallel titrations for each sample. We suggest a third parallel titration only if the difference between the End-point volumes is unexpectedly large, e.g. above 3 %. It is more useful to leave enough time (ideally more than 1.5 hour) for the calculation.
- Aqueous solutions of nitrite ions are well preserved in the leak-tight sealable plastic containers you have received. The quantitative analysis of NO_2^- can be precisely performed when these solutions contact with air upon opening the vials, making samples, titration, etc. However, unnecessary opening of the vials and leaving these exposed to air for long time may result in atmospheric oxidation of a small fraction of nitrite ions and thus erroneous measurement results.
- In permanganometry, the correct reading of the liquid level should be done using the upper, liquid level (horizontal rim), and not the lower, curved meniscus (**Figure 1**). This is because the permanganate solution is usually deep purple and not dilute enough to allow correct visualization of the lower curvature.



- Do not use any periodic tables in the room/laboratory you are staying in. **Use the following relative atomic masses** for your calculations:
$A_r(H) = 1.01$; $A_r(C) = 12.01$; $A_r(O) = 16.00$; $A_r(Na) = 22.99$
- It is strongly advised to rinse the pipettes with a few millilitres of the respective solution before use.
- IMPORTANT: when you finished adding the stoichiometric numbers on the "**B-1**" and "**B-3**" pages (**Questions B.1.1. & B.2.1**) on the *Answer* sheet, give these pages to the laboratory assistant, who will provide you with a sheet with the two, correctly balanced equations. Once you received these balanced equations, you cannot receive that part of the answer sheet back!

Figure 1. Liquid meniscus of a less concentrated $KMnO_4$ solution that allows distinction between lower meniscus and the upper rim. Arrow A: curved meniscus level (0.3 mL). Arrow B: rim level (0.1 mL).

Experiment

The (unknown) nitrite salt content of the aqueous samples can be determined by permanganometry, which is one specific branch of titrimetric methods based on redox reactions. In redox titrations, the ions/molecules to be investigated will react with a chemical agent, termed as "titrant". In the present case, the latter is potassium permanganate ($KMnO_4$) that is added to the solution of the investigated material as an aqueous solution of precisely known concentration. Manganese can exist in various oxidation states and chemical forms depending on the redox conditions in the solution. Under fairly strongly acidic conditions, permanganate ions are strong oxidants and they undergo reduction to divalent manganese ions by the following ionic equation:

$$MnO_4^- + 8\ H^+ + 5\ e^- = Mn^{2+} + 4\ H_2O$$

Under normal laboratory conditions, $KMnO_4$ titrant solutions can only be stored very shortly after preparation without any decomposition. This is because trace organic and inorganic contaminations may react with $KMnO_4$ and reduce a part of it to manganese dioxide. In practice, permanganate titrant solutions are not used when freshly prepared: they are left standing for more than a week, filtered from the precipitated manganese-containing compounds, and stored in dark containers. You receive this solution after the same, thorough preparation procedure. Your first task is to determine the exact, analytical (molar) concentration of the $KMnO_4$ titrant.

B.1 Standardization of potassium permanganate (titrant) solution

The exact concentration of the potassium permanganate solution is determined by titrating sodium oxalate (here abbreviated as "OX") solution of exactly known concentration. This reaction proceeds unambiguously, resulting in the formation of carbon dioxide and manganese(II) ions.

Question B.1.1 What is the correct form of the stoichiometric equation related to the redox reaction between MnO_4^- and $(COO)_2^{2-}$ ions? Supplement the equation at **B.1.1** on the *Answer sheet* with the missing stoichiometric numbers!

Perform the standardization as follows:

A) preparation of OX stock solution

Prepare a stock solution of sodium oxalate (OX). See Question **B.1.2** to assess the relative molecular mass of this compound, which you receive in an anhydrous form (free of crystal water). Calculate the mass of $Na_2(COO)_2$ needed to prepare 500.0 mL stock solution with c = 0.0480 mol/L concentration. Fill in the table **B.1.3** on the answer sheet.

Take one of the 250 mL beakers and weigh in the calculated mass of OX. NOTE: you do not need to measure exactly the calculated mass. If you want to save time, it is enough if you slightly over- or undermeasure the calculated mass (stay within ± 1%) and use the exact mass for the calculation of the concentration of OX (c_{OX}).

Dissolve the solid OX powder with deionised water and transfer it quantitatively into the 500 mL volumetric flask. After filling the liquid level of the flask (V = 500.0 mL) to the line by deionised water, mix the solution well.

Question B.1.2 Calculate the relative molecular mass of OX. Write your result in the left box of **B.1.2** on the *Answer sheet*. Calculate the mass of OX, which needs to be dissolved for the preparation of 500.0 mL 0.0480 mol/L OX stock solution, by the precision of three decimals. Note it in the box, at the right hand side.

Question B.1.3 Note the mass value that you measured by balance (left box). Use this value to calculate the concentration of OX solution and write it into the right box of **B.1.3** on the *Answer sheet* with 4 decimals precision.

B) titration of OX solutions by permanganate solution

Pour some OX solution into one of the 50 mL beakers. Draw 10.00 mL of the already prepared ~0.0480 mol/L OX solution by a pipette and transfer it to one of the Erlenmeyer flasks. Add ca. 10 mL of 0.9 mol/L H_2SO_4 solution by the graduated Pasteur pipette, rinse the wall of the flask by a few drops of DI water and put the flask on the heater to reach ca. 60-80 °C (it is enough if the flask is "very hot" but do not let the liquid boil). Meanwhile, take the other, small beaker of 50 mL and rinse it with a few mL of the $KMnO_4$ titrant solution. Pour the content into the "waste" beaker, that is the leftover beaker of 250 mL. Pour some of the titrant into the 50-mL beaker and fill the burette with the $KMnO_4$ solution to the level of 0.00 mL. NOTE: Before filling the burette, it is highly recommended to rinse it (1-2 times) with a few mL (4-5 mL) of the titrant.

Titrate the warm (60-80°C) OX solution by $KMnO_4$ solution. In the beginning, add the titrant portions slowly and wait until its colour disappears. After this induction period, the titration can proceed more swiftly. Take care to keep the temperature of the liquid in the Erlenmeyer flask around 60-80°C throughout the titration until reaching the End-point. The titration is

considered to be completed when a very light purple colour of the solution remains for ca. 1 minute. Read your burette and note the End-point titrant volume to the field **B.1.4** on the *Answer sheet*.

Wash the flask with DI water or take a new one. Fill up the burette to 0.00-mL. You should repeat the whole titration procedure of the 10.00 mL OX solution at least one more time. Note every End-point titrant volumes to the field **B.1.4** on the *Answer sheet*.

Question B.1.4 What is c_{KMnO4} ? Write down your titration results in the field **B1.4** on the *Answer sheet* and calculate the exact concentration of the $KMnO_4$ solution (5 decimals) using the stoichiometry consideration in **B.1.1**.!

B.2 Determination of the exact concentration of nitrite ions in the water samples

The value of c_{KMnO4} tells you the "oxidizing" capacity of your titrant solution. Now, you have all tools to figure out the level of the nitrite ion content in the water samples. Given your short time, the Technician Team helped you with the titration of the collected water samples – except two samples, which you need to analyse yourself: one of these was collected along the Tisza river (**@ checkpoint 5**, **Tiszalök**) and the other one was collected along the Körös river (**@ checkpoint 11, Öcsöd**).

IMPORTANT: all solutions, provided here or titrated by the Technician Team were pre-concentrated (10 × concentration) from the initial ones to fit the titratable concentration range! **You do not need to calculate with this pre-concentration factor** but be not surprised if you find unrealistically high nitrite ion concentrations! Also, the Technician Team found that the amount of oxidizable contaminations (other than nitrite) in the water samples is negligible. Therefore, you can be sure that the consumption of permanganate is only related to the reaction with the nitrite content.

Determination of nitrite ions by permanganometry

Nitrite ions (NO_2^-) can be oxidized rapidly by permanganate ions to nitrate ions according to the reaction equation found in the *Answer sheet*.

Question B.2.1 What is the correct form of the stoichiometric equation related to the redox reaction between MnO_4^- and NO_2^- ions? Supplement the equation at **B.2.1** on the *Answer sheet* with the missing stoichiometric numbers!

In acidic media, nitrite ions are protonated and transform to nitrous acid (HNO_2). The latter compound, however, is decomposing to nitrogen oxides, a part of which can escape from the titrating vessel that would cause an error in the analysis. Therefore, the determination of nitrite ions can be performed via a so-called double back-titration procedure. First, we add the permanganate titrant solution to the nitrite solution in an excess amount and then acidify the solution. Since oxidation of the nitrite ions proceeds significantly faster than the decomposition of nitrous acid, the nitrite ions can be quantitatively transformed to nitrate ions. Now, the unreacted $KMnO_4$ could be backtitrated by the OX solution that you have already used for point **B.1**. The "beauty-spot" is the following: direct titration of permanganate ions by oxalate ions is not providing precise results because permanganate

ions may noticeably react with the solvent at elevated temperature. For this reason, the sodium oxalate solution is also added in an excess relative to the unreacted permanganate fraction. Then, the excess of the added OX solution is measured (titrated) by the $KMnO_4$ titrant by the already known reaction, specified in the **Question B.1.1** of the *Answer sheet*.

Perform the titration as follows:

> **Titration of the NO_2^- solutions by the permanganate titrant**
>
> Draw 10.00 mL of one of the nitrite ion containing river water samples (e.g. from checkpoint #5) by a pipette and transfer it to one of the Erlenmeyer flasks. You may use any of the flasks after rinsing with DI water, even if droplets of water remain inside.
>
> Add exactly 10.00 mL of titrant solution from the burette into the Erlenmeyer flask containing already the previously pipetted water sample. Rinse the wall of the Erlenmeyer flask with a small amount of DI water, add 10 mL 0.9 mol/L H_2SO_4 solution by the graduated Pasteur pipette and then close the flask immediately with the glass cap. Next, shake the flask rigorously, but aim towards horizontal motion, to reduce chance of liquid leakage via an improperly attached cap! Leave the sample standing for 15 minutes and shake it periodically, in ca. every two-three minutes). After 15 minutes, pipette 10.00 mL of the OX solution into the sample, which should result in the disappearance of the colour. Place the flask on the heater (without cap) to reach ca. 60-80 °C, then the excess of oxalate ions can be titrated by the permanganate titrant. Note the End-point titrant volume to the field **B.2.2** on the *Answer sheet*.
>
> Wash the flask with DI water or take a new one. Repeat the whole titration of the 10.00-mL sample at least one more time.
>
> Finally, titrate the *other water sample* also twice, according to the procedure detailed above. Note all of the End-point titrant volumes also to the field **B.2.2** on the *Answer sheet*.

> **Question B.2.2** Write down your titration results in the field **B2.2** on the *Answer sheet* and calculate the NO_2^- concentration (with 5 decimals precision) of the water samples collected at checkpoints **#5** and **#11**!

B.3 Location of contamination sites

Figure 2 shows the long, curly pathway of Tisza river with its numerous tributaries. Those **rivers entering into the country were not found to contain any nitrite salts** as contaminations. However, various agricultural and industrial sites may secretly or accidentally **release nitrite ions** into the adjacent water streams **steadily,** thereby causing a permanent concentration level of NO_2^-. Your colleagues listed 14 potential contamination sites, indicated as small icons and a capital letter on the map (A-N). These sources can be identified by considering the NO_2^- ion concentrations measured in water samples at the 18 checkpoints indicated with numbers (1-18) on the map.

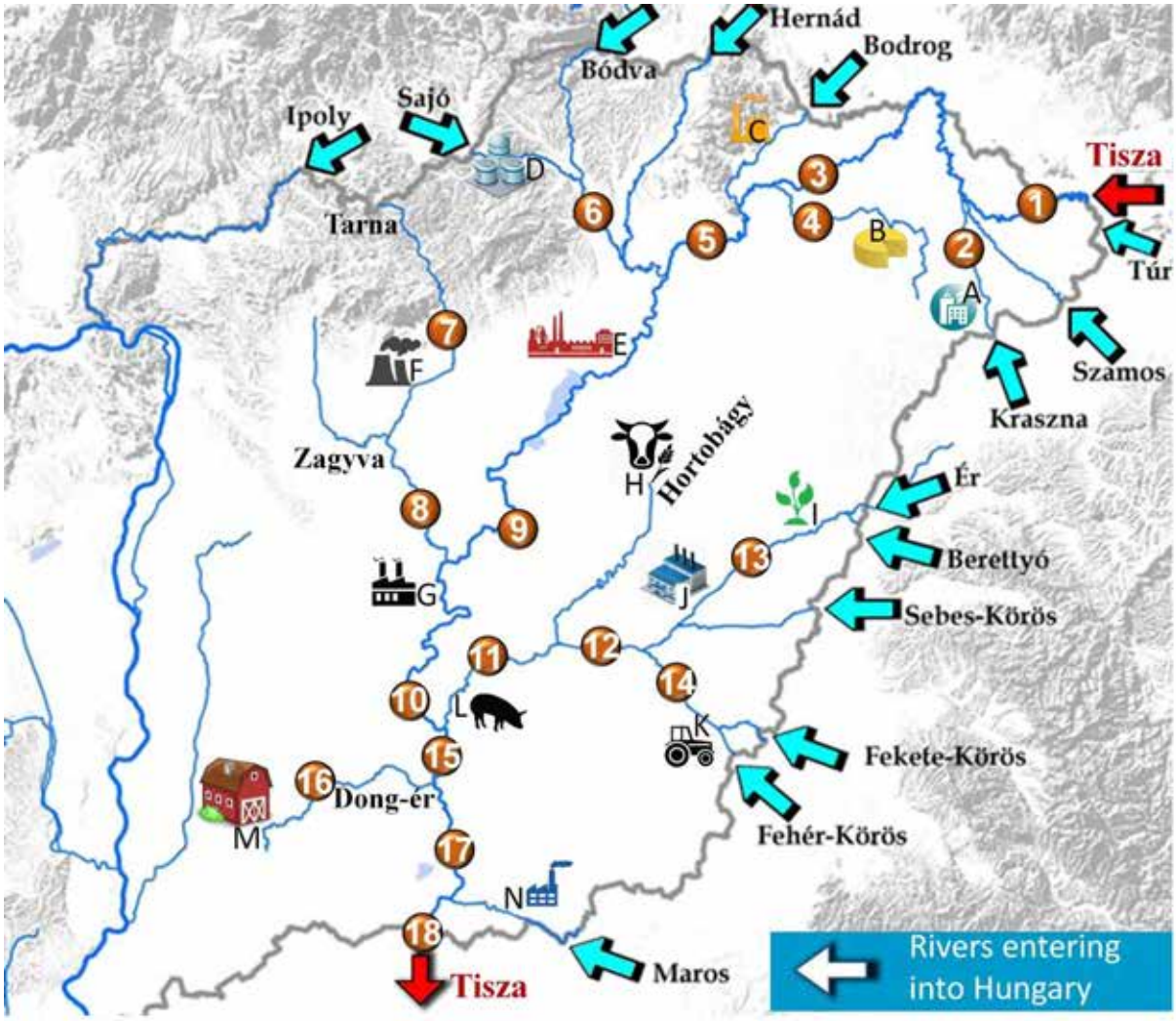


Figure 2. Map of Eastern Hungary with Tisza river (entrance point 1; exit: 18) and its tributaries. Numbers in red circles indicate the checkpoints where water samples were drawn. Small icons and associated capital letters indicate the potential contamination sites. This map is reproduced as a separate A4 sheet in larger size, for your convenience, but these two figures are otherwise identical.

Nitrite ion concentrations need to be determined for all checkpoints, not only for the two you already have. For your support, however, your Technician Team were of help and all the other checkpoint concentrations are now obtained as summarized by the table below. Do not fill the two missing values in this table but use it to identify checkpoints and their nitrite ion concentrations and to know their **volumetric flow rate (in m^3/s) of the water stream** (the flow rate, marked as "w", is the total volume of water that passes through the whole cross section of the river within 1 second). These flow rates are important to be known, because the confluence of a polluted river with a clean one will cause dilution, that is, the decrease of the nitrite ion concentration. When water streams meet and flow together, their **flow rates can be simply added together** to know the flow rate of the unified river. For simplicity, we assume that the flow rates of the rivers are constant starting from their sources (or their Hungarian entry points) until they reach their confluence point (uniting with another river).

Checkpoint number #	Checkpoint name	River	Flow rate, w (m^3/s)	c/ NO_2^- (mol/L)
1	Szatmárcseke	Tisza	240	0
2	Mátészalka	Kraszna	3	0.02
3	Dombrád	Tisza	364	0.000165
4	Nyírbogdány-Kónyatelep	Lónyay-főcsatorna	1	0
5	Tiszalök	Tisza	480	?
6	Miskolc	Sajó	38	0.03145
7	Verpelét	Tarna	3	0
8	Zagyvarékas	Zagyva	16	0
9	Tiszabő	Tisza	550	0.0301
10	Lakitelek	Tisza	566	0.02925
11	Öcsöd	Körös	102	?
12	Gyomaendrőd	Körös	100	0.0045
13	Bakonszeg	Berettyó	15	0.018
14	Köröstarcsa	Körös	70	0
15	Szentes	Tisza	668	0.03125
16	Kiskunhalas	Dong-ér	1	0.045
17	Hódmezővásárhely	Tisza	670	0.03125
18	Szeged	Tisza	820	0.02554

For some of your calculations, you will also need to know the flow rates of those rivers at which no checkpoints were established. These are found in the table below: NOTE: You **may not need to use all** of these data in your calculations!

River	Flow rate of the river (m^3/s)
Szamos	121
Bodrog	115
Hernád	32
Bódva	9
Sebes-Körös	30
Hortobágy	2
Maros	150

In the final step of the task, you will need to locate the contamination sites. In the table below, we also helped you to calculate the flow rates at the polluting sites. Knowing the concentration and flow rate at the *checkpoints*, you can calculate the **concentration of nitrite ions in the river section just before reaching each site** using the material balance equation, which reflects that the nitrite ions are never lost at confluences:

$$c_X w_X = c_Y w_Y + c_Z w_Z + \ldots$$

where c_X is the nitrite ion concentration of the unified river, c_Y, c_Z,… are the concentrations of the main river and its tributary(ies) before their confluence, w_X is the flow rate of unified river, and w_Y, w_Z,… are the flow rates of the main river and tributaries before their confluence. By the same way of thinking (at some places it is so simple that you can just use common sense), you can also calculate the nitrite

ion **concentration in the river section just after it passed each site.** From these two data (concentration before and after the site) you can simply calculate the nitrite ion concentration difference (Δc) by which the potential polluting site contributes to the nitrite ion level. If Δc > 0, there is a clear indication that the respective site discharged nitrite contamination to the river.

Furthermore, for the characterization of the contamination level of the spotted site (company), the emitted rate of nitrite ions (P) are more feasible than simply the concentration difference, Δc. The emitted rate of pollution (P) equals to Δc×w. Does this value indicate a minor or a major contamination level? You can decide by comparing it to the limit values in the field **B.3.1** of the *Answer sheet.*

> **Question B.3.1** Calculate the nitrite ion concentration difference (Δc) by which the potential polluting site contributes to the nitrite ion level in the water. This difference is the circumstantial evidence that the company is releasing nitrite compounds into the environment! Which are these contamination sites? Check the field **B.3.1** in the *Answer sheet* and supplement it with data. Locate the sites! Calculate also Calculate also the emitted rate of pollution (in mol/s) and determine their pollution level based on the ranges scpecified in the *Answer sheet*!

Notation on map	Company name	Flow rate, w (m^3/s)	Polluting level
A	Fábiánháza Brewery	3	?
B	Hungry Cat Cheese Factory	1	?
C	Zemplén Tool Factory	115	?
D	BorsodChem	29	?
E	Tiszanewcity Chemicals	550	?
F	Mátra Power Plant	3	?
G	Szolnok Rail Cargo	566	?
H	Wild West Cowboys Dairy Farm	2	?
I	Bihari Tobacco Fields	15	?
J	Szeghalom Thermoelectrics Ltd	15	?
K	Mezőberény Croplands	70	?
L	Kunszentmárton Pig Farm	102	?
M	Treeclimbing Goat Pastures	1	?
N	Makó Rubber Factory	150	?

Could you capture some of the polluting sites? Well done!
Could you identify all of the polluting sites in due time? Congratulations! You completed a “Mission Impossible”!!

Aftermath:

Thanks to your and your Team’s precision and diligence, the Environmental Authorities were able to impose important measures on the polluting Companies, which changed their production practices. Now, the aquatic wildlife along the Tisza river is about to be recovered from the damage.

Part C

In this part, there are two problems. In the first one, you will perform real experiments and measurements with different granulated materials. In the second problem of this part, you will study and evaluate optical measurements with sand sedimentation in water.

Problem C1 *(50 points)*

Devices and materials for this problem:

- plastic kitchen tray, Bunsen stand with test tube clamps and cross clamps
- kitchen measuring cup
- jeweller's scale with transparent plastic tray
- yellow sand in a plastic bag (ca. 120 g)
- black sand in a plastic bag (ca. 80 g)
- poppy seeds in a plastic bag (ca. 20 mL)
- flat foamboard frame with transparent covers, 2 small foamboard pieces
- transparent plexiglass tube (11 cm long)
- plastic sample holder (ca. 20 mL, 2 pieces)
- mini funnel, small plastic spoon
- plastic coffee cup (2 pieces)
- rubber "fingers" and bands (2 pieces)
- tesa film, paper towels, permanent marker, calculator, pens, pencil, rulers, ...

SAFETY WARNINGS!

Work carefully with granulated materials, work above the kitchen tray. Keep the scale clean and dry. Wipe the table and devices with a paper towel if necessary.

Introduction:

Granulated (or granular) materials have surprising behaviours, which are in the centre of scientific interest for decades, but today only a small part of the experimental results can be explained fully by theoretical models. These materials occur in nature, but also in industrial and everyday practice. They range in size from rocks of several metres in scree slopes to micron-sized dye powders.

Everybody has played with sand. Dry sand can be poured similarly to liquids, but the poured sand does not spread out completely but forms a mound. Its slope is characteristic for the material, depends on many different factors like form, size distribution, coefficient of friction, etc.

More surprising phenomena are spontaneous segregation when a mixture separates into its components due to shaking or pouring; jamming, when the material changes from fluid-like behaviour to solid-like; and pattern formation, as dunes and ripples of sand.

In this problem you will measure the slope of two different sand samples, you will investigate the segregation of a mixture of yellow sand and poppy seeds due to pouring, and the jamming of sand samples in a tube, and at last you will measure the mass density of the sand samples.

Measuring the slope of sand samples and investigating spontaneous segregation.

Mount the flat foamboard frame with transparent covers with a test tube clamp of the Bunsen stand. Use the two small foamboard pieces for better fixing. Place the mini funnel into the hole of the frame. Check the verticality of the frame.
Pour ca. 40 mL from the *yellow sand sample* in a plastic coffee cup. Measure the volume with a plastic sample holder. Its volume is ca. 20 mL.
Use the small plastic spoon and very carefully, very slow, in small amounts (like salting a meal) pour the sand into the funnel.
If all material is poured, carefully lay a ruler on the frame parallel to the slope, and mark the intersection points of the line at the horizontal and vertical walls of the frame. **WARNING! Be careful, do not shake the frame, you could change the slope.**

Question C1.1a Read the x and y distances of the markings from the bottom corner of the frame, calculate the angle α of the slope to the horizontal, and write the values in the first line of table **C1.1** on the *Answer sheet*.

Demount the frame, pour out the yellow sand into its original plastic bag.
Mount again the frame in the same way and repeat the whole process described above with the *black sand sample*.

Question C1.1b Write your readings and the calculated value in the second line of table **C1.1** on the *Answer sheet*.

Demount the frame, pour out the black sand into its original plastic bag.
Mount again the frame in the same way for the next experiment.
Mix ca. 20 mL of poppy seeds and 20 mL of yellow sand in a plastic coffee cup. For measuring use a plastic sample holder. **WARNING! Shaking does not help. Use the small plastic spoon.**
Use the small plastic spoon, and very carefully, very slow, in small amounts (like salting a meal) pour the mixture into the funnel. Try to take equally from the bottom and the top of the mixture. During the pouring you can observe the interesting behaviour of the mixed material: sometimes a layer of one of the components rolls down, and in this way, segregated layers of poppy seeds and yellow sand are formed.
If all material is poured, count the number n of layers formed from poppy seeds, and measure the thickness d of the layers. (d_i is the thickness of the i^{th} layer, $1 \leq i \leq n$.) **WARNING! Be careful, do not shake the frame, you could destroy the layers.**
Calculate the average thickness d_{avr} of the layers.

Question C1.2a Write your results in the first line of table **C1.2** on the *Answer sheet*.

Demount the frame, pour out the mixture back to the coffee cup.

Mount again the frame in the same way. Repeat the experiment two more times.

Question C1.2b Write your results in the second and third lines of table **C1.2** on the *Answer sheet*.

Demount the frame, pour out the mixture back to the coffee cup (and leave it there).

Jamming in a tube.

Between the grains of the granulated material and the wall of the vessel, and between the grains of the granulated material, there is a frictional force also at rest – unlike liquids, where there is internal friction only in motion. This friction can cause the jamming of the granulated materials inside a vessel, and therefore unexpected forces can also occur. In this part of the problem, you will compare the behaviour of yellow sand and water in a transparent vertical tube.

Prepare yellow sand into the other plastic coffee cup. Place the jeweller's scale on the base of the Bunsen stand. Place the square-shaped transparent plastic tray on the scale.

With a permanent marker make 10 markings on the transparent plexiglass tube 1 cm apart. The first marking should be also 1 cm from one end of the tube.

Mount the tube above the center of the scale by two test tube clamps on the Bunsen stand. The tube should be as close to the Bunsen stand as possible. Check that all components of the stand fit tightly and without play. **WARNING! The stiffness of the stand is very important for successful measurements.** Adjust the tube vertically.

Switch on the scale, press the 'T' (Tare) button and place a piece of paper on the tray on the scale. Lower the tube so that it gently touches the paper. Check that the rim of the tube fits well to the plane of the tray. Pull out the paper carefully. The scale must show zero (or a very small weight) – if not, repeat the process.

Place the small funnel in the top hole of the tube. Use the small plastic spoon and very carefully, very slowly, in small amounts (like salting a meal) pour sand into the funnel until the sand level reaches the first marking at $h = 1$ cm. Read the value m shown on the scale (in grams) – this value is proportional to the force exerted by the sand on the balance.

Continue pouring the sand in the same way (very carefully, very slowly, in small amounts), and read the values shown on the scale at every marking. Stop the process at the last marking below the top end of the tube.

Question C1.3a Write your readings in the first 10 boxes (1 – 10) in the first empty line of table **C1.3** on the *Answer sheet*.

The test tube clamps and all parts of the Bunsen stand are not a fully rigid structure. They bend slightly due to the weight of the sand.

Try to lift carefully the tube with your hands to "help" the Bunsen stand carrying the weight. **WARNING! Do not loosen the screws of the stand.** The value shown on the scale becomes lower, and it reaches a minimum value.

Question C1.3b Write the minimum value shown on the scale in the column marked by ↑ in the first empty line of table **C1.3** on the *Answer sheet*.

Release the lifting. Surprisingly the value shown on the scale becomes higher than before the lifting.

Question C1.3c Write the increased value shown on the scale in the column marked by ↓ in the first empty line of table **C1.3** on the *Answer sheet*.

Release the screws of the cross clamps, and lift the tube higher by a few cm. The sand runs out onto the tray on the scale. The value shown on the scale corresponds to the weight of the sand.

Question C1.3d Write the value shown on the scale in the column marked by W in the first empty line of table **C1.3** on the *Answer sheet*.

Carefully pour back the sand into the plastic coffee cup.
Repeat the whole process **C1.3a-d** two more times with the same material.

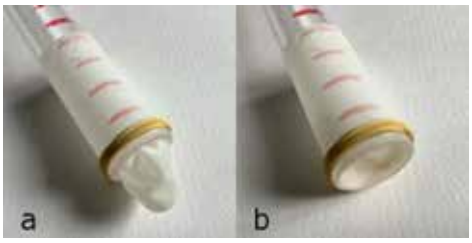
Question C1.3e Write your readings in the appropriate columns of the second and third empty lines in table **C1.3** on the *Answer sheet*.

After you have finished the last experiment pour back the sand into its original plastic bag.

Question C1.3f Calculate the avergae values in every column and write your results in the appropriate boxes of the last line in table **C1.3** on the *Answer sheet*.

For comparison, examine how a liquid behaves in the tube. The tube can not fit waterproof on the scale, you have to make a waterproof closure that exerts only a very small force.

Pull a rubber "finger" loosely over an end of the tube (see *figure part a*). **WARNING! It is important to keep it loose.** Fix it at the rim of the tube with a rubber band, then push the loose small "bag" into the tube with your finger (see *figure part b*).



Mount the tube above the center of the scale by two test tube clamps on the Bunsen stand, as before. Switch on the scale, and press the 'T' (Tare) button. Lower the tube so that it is very close to the tray but does not touch it. The scale must show zero.

Take some tap water into the kitchen measuring cup. Place the small funnel in the top hole of the tube. Use a plastic sample holder and/or the small plastic spoon, and very carefully, very slowly pour water into the funnel until the water level reaches the first marking at $h = 1$ cm. Read the value m shown on the scale (in grams) – this value is proportional to the force exerted by the water on the balance.
Continue pouring water in the same way, and read the values shown on the scale at every marking. Stop the process at the last marking below the top end of the tube.

Question C1.4a Write your readings in the first 10 empty boxes (1 – 10) of table **C1.4** on the *Answer sheet*.

Demount the tube from the stand and pour the water from the tube into the tray to measure its weight. (If the scale has been switched off, first switch it on again, and press button "T".)

Question C1.4b Write the value shown on the scale in the box marked by W in table **C1.4** on the *Answer sheet*.

Question C1.5 Plot on a *graph paper* labeled as *'graph C1.5'*, the calculated average values m_{avr} for the yellow sand (the first 10 boxes of the last line in table **C1.3a-e**) and the measured values m_{w} for water (the first 10 data in table **C1.4a-b**) in the function of the material level h. **Plot both functions in the same graph and use different markers for the two materials, respectively.**
Draw a horizontal line in both cases at the value corresponding to the total weight of the material in the tube (column 'W').
For yellow sand, mark both the minimum and maximum average values (columns '↑' and '↓' of the last line in table **C1.3a-e**) at $h = 10$ cm, too.
Actually, we measure forces all the time, but for simplicity use the gram unit shown on the scale everywhere.
Do not forget to attach *'graph C1.5'* to the answer sheet!

Interpret the observed phenomena! Different forces are acting on the materials (yellow sand or water) in the tube. The gravitational force F_{g} (acting at the center of mass), the normal force exerted by the tray on the scale F_{s}, and the normal and frictional forces exerted by the wall of the tube F_{n} and F_{f}. (The effect of air pressure is not taken into account, its resultant is negligible.)

Question C1.6 Draw the forces acting on the materials in sketches **C1.6** on the *Answer sheet*. In case **(a)** for sand at the moment when the value, shown on the scale was minimal (**C1.3b** '↑'), in case **(b)** also for sand but at the moment when the value was maximal (**C1.3c** '↓'), and finally in case **(c)** for water when the water level was $h = 10$ cm (**C1.4a** '10'). Use notations F_{g}, F_{s}, F_{n}, and F_{f}. Mark the direction of the force, and try to express its relative magnitude by the length of the arrow.

Measuring the density of sand samples.

For granulated materials, we can distinguish two different densities: one of them is the average density $_{avr}$ of the granulate, taking into account the volume of the cavities between the grains, the other one is the real density of the material of the grains. In this last, short part of the problem you will measure the densities of the sand samples. To do it follow the next procedure.

WARNING! After this measurement you can not reuse the devices and materials, therefore make this measurement only if you are ready with all other measurements in Problem 1.

Wipe dry both plastic sample holders. Switch on the scale (the transparent plastic tray must be on it), and press button "T". Place an empty plastic sample holder on the scale.

Question C1.7a Read the weight m_e of the empty sample holder and write the value in the proper box of table **C1.7** on the *Answer sheet*.

Take off the sample holder from the scale. Fill it completely with tap water. Wipe carefully dry the outside of the sample holder if necessary. Place back it on the scale. (If the scale has been switched off, first switch it on again, and press button "T".)

WARNING! Never fill any materials in the sample holder above the scale.

Question C1.7b Read the weight m_w of the sample holder full with water and write the value in the proper box of table **C1.7** on the *Answer sheet*.

Take off the sample holder from the scale, pour out the water, and wipe it dry. Fill it completely with yellow sand. Use the mini funnel and the small plastic spoon. **WARNING! Pour the sand carefully. Do not compress the material.** Place back it on the scale.

Question C1.7c Read the weight m_{ds} of the sample holder full with dry sand and write the value in the proper box of table **C1.7** on the *Answer sheet*.

Take off the sample holder from the scale. Add very carefully and slow water to the sand with the small plastic spoon. Give time to the water to sink, and to the air bubbles to rise. Continue adding water till the surface remains wet and the water would flow over. Wipe carefully dry the outside of the sample holder if necessary. Place back it on the scale.

Question C1.7d Read the weight m_{ws} of the sample holder full with wet sand and write the value in the proper box of table **C1.7** on the *Answer sheet*.

Take the other sample holder and repeat steps **C1.7c** and **C1.7d** with your black sand sample.

Question C1.7e Write your readings in table **C1.7** on the *Answer sheet*.

Question C1.7f Derive expressions for calculating the average density $_{\text{avr}}$ of the granulate and the density of the material of the grains. Use **only** the measured quantities m_{e}, m_{w}, m_{ds}, m_{ws}, and the known density $_{\text{w}}$ of water. Write the steps of the derivation and the derived expressions in box **C1.7f** on the Answer sheet.

Question C1.7g Calculate the numerical values of average density $_{\text{avr}}$ and density for both yellow and black sand samples. Use $_{\text{w}} = 1\ \text{g/cm}^3$ for the density of water. Write the results in the appropriate boxes of table **C1.7a-e** on the Answer sheet. You can use the empty columns for calculations.

Problem C2

In this problem, a sedimentation method is used to analyse the particle size statistics of a given sand sample. The sand is put into a vessel filled with water. The greater is the sand particle, the faster it sinks. Stokes law will be used to model the velocity of the sinking. The sand particle concentration of the water is changing as a function of time depending on the particle size distribution.
The particle concentration of the water is determined by an optical transmission measurement. A horizontal laser beam is transmitted through the vessel, and the transmitted light power is registered by a photodetector as a function of time. Our goal is to evaluate the particle size statistics from this power - time function.

C2.1 Particle concentration

Optical setup

In this section, a simple measurement setup is introduced which is applicable to measure the sand concentration of the water in an optical way. The setup is shown in Fig.1.

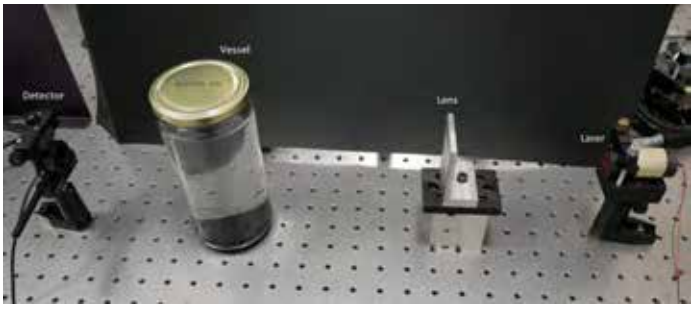


Fig.1 Measurement setup

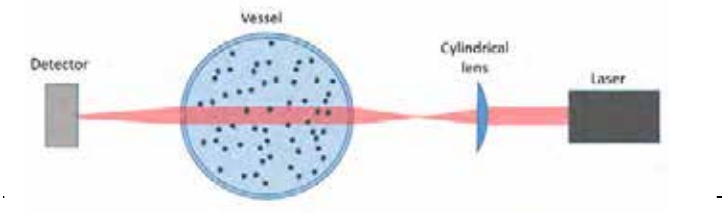


Fig.2 Horizontal plane cross section of the beam propagation.

The light source is a $620\ \text{nm}$ wavelength, $1.8\ \text{mW}$ diode laser, that produces a collimated beam with a $5\ \text{mm}$ diameter. This beam is transmitted through the sedimentation vessel. It is a conventional cylindrical glass jar filled with water. The outside diameter of the jar is $80\ \text{mm}$, the height is $180\ \text{mm}$. We have to recognise that the cylindrical vessel focuses the beam in the horizontal plane. Thus the beam would converge in the water even if the original incident beam contains only parallel rays. We would like to realize a collimated beam in the vessel, so correction optics should be applied. A cylindrical lens is inserted between the light source and the vessel as it is shown

on the horizontal plane cross-section of the setup in Fig.2. This cylindrical lens is focusing the beam only in the horizontal plane. The lens is set so that the rays in the vessel propagate parallel.
The light transmitting through the jar is detected by a light power meter. This instrument has a calibrated silicon photodetector, and it displays the light power in mW. The device can be connected to a computer by a USB port. Using software we can register the power data as a function of time with $0.2\ \mathrm{s}$ sample interval. We measure the transmitted light power in case the water is clear. This light power is referred as $I_0 = 1.67\ \mathrm{mW}$.

> Before further work, **watch the video** *'sedimentation.mp4'* on the tablet/notebook provided.

Transmittance

The laser beam has an $L = 76\ \mathrm{mm}$ path length in the water with a beam diameter of $d = 5\ \mathrm{mm}$. Thus we have a cylinder shape illuminated volume with approximately homogeneous intensity distribution in case of clear water. In this case, the transmitted power is I_0.
Let us suppose that a small sphere shape particle appears in the illuminated volume. The radius of the particle is R. It reduces the transmitted power by ΔI_1.

> **Question C2.1a** Supposing that the particle absolutely shadows the rays illuminate its cross-section, express the ΔI_1 power by the given parameters. write the expression on the *Answer sheet*.

If more particles appear in the illuminated volume, the light power loss becomes higher. We suppose that if N pieces of the same size particle are in the illuminated volume, the transmitted power decrease is $\Delta I = N\Delta I_1$ In this model we suppose that the particles are not shadowing each other. It is approximately true in the case of low particle concentrations.
In our experiments, we use the *black sand* which has been analysed in *Problem C1*. There you have determined the mass density ρ_S of the material of the sand in *Question C1.7g*. Let us suppose that we measure ΔI power loss in the transmitted light related to the clear water. We suppose that all the particles have the same radius R and the same density ρ_S. Knowing these parameters we can calculate the mass concentration of the particles in the water suspension. The mass concentration of the particles is defined by the next expression: $C_R = \frac{m_R}{V}$ where m_R is the sum of mass of the particles have radius R in the illuminated volume, and V is the volume of the illuminated region.

> **Question C2.1b** Express the C_R as a function of ΔI and R using the given parameters. Write the expression on the *Answer sheet*.

C2.2 Sedimentation

The theory of sedimentation is well known from the honey viscosity measurement of *Task 1.* We suppose that the sinking sand particles have a characteristic velocity determined by the equilibrium of the gravitation force, the buoyancy, and the drag force comes from the viscosity of the water. We suppose that the particles have spherical-shape with radius R. In this case, the drag force can be described by Stokes's law: $F_\mathrm{d} = 6\pi\eta R v$, where η is the viscosity of the medium, and v is the velocity of the particle. In *Task 1* you have measured the *unknown* viscosity of honey. In this experiment we

know the viscosity of water, it is $\eta = 10^{-3}$ Pas at room temperature. We also know the density of water, it is $\rho_w = 1000\ \mathrm{kg/m^3}$. For the density of the *black sand* particles use your result in Question **C1.7** (in this measurement the same black sand was used).The gravity of the Earth is $g = 9{,}81\ \mathrm{m/s^2}$.

> **Question C2.2a** Write the equation of the equilibrium of the forces acting on a sinking particle. (Similarly as in Task 1.) Derive a formula to determine the velocity as the function of the radius R using the given parameters. Write it on the *Answer sheet*.

Let imagine that we put some sand into the vessel filled with water, and it is shaken up to disperse the particles. We suppose that at the $t = 0$ moment the distribution of the particles is uniform in the volume of the vessel. We also suppose that all the particles have the same radius R. In this ideal case, all the particles have the same sinking velocity. It implies that a sinking plane can be defined. Under this plane, the concentration of the particles is the same as the initial one, but over this plane theoretically, we could not find any particle. As it is visualized in Fig.3. We call this plane the "ceiling", this ceiling sinks with uniform velocity.

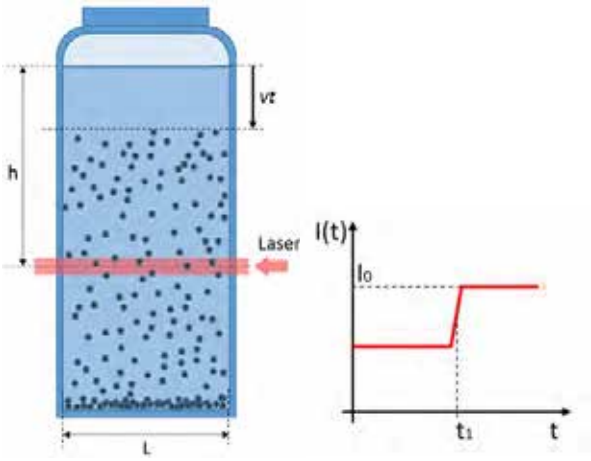


Fig.3 Schematics of the sedimentation and the changing of the transmittance in case all the particles have the same radius.

If the vessel is illuminated by a horizontal laser beam at the depth h, then the transmitted power $I(t)$ changes as a function of time as it is plotted on the schematics graph in Fig. 3. The critical time t_1 depends on the sinking velocity and the h parameter.

Let us suppose that the sand is a mixture of spherical particles that have two different radius R_1 and R_2. In the initial moment, the particles have a uniform distribution in the volume, but later we have two ceilings sinking with different velocities correspond to the two particle sizes. The changes of light power $I(t)$ in the function of time are demonstrated in Fig.4.

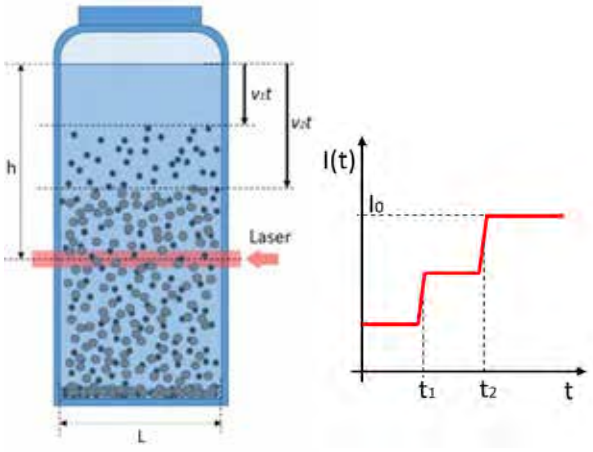


Fig.4 Schematics of the sedimentation and the changing of the transmittance in case the sand is a mixture particles have two different radius.

In a realistic sand sample, there are a lot of particles of different sizes. Thus in a real experiment, we cannot recognise steps on the $I(t)$ function, rather we can measure a continually grown graph. However, this realistic sedimentation also can be successfully modeled by the "infinite number of sinking ceilings" that have different velocities. In this measurement, you have to analyse the $I(t)$ function of realistic sedimentation experiments.

Measurement

In the following measurements, we use the setup shown in Fig.1. We put 3 g of the *black sand* into the jar filled with water. The distance between the laser beam and the water surface is $h = 53$ mm. The covered vessel is shaken up, and then we replaced it to its original position. The data collection of the light power meter was started at this moment. The software registered the light power with 0.2 s time resolution.

After that, we set the distance between the laser beam and the water surface to $h = 102$ mm and we repeat the experiment. The result is plotted in Fig.5. The time axis has a logarithmic scale.

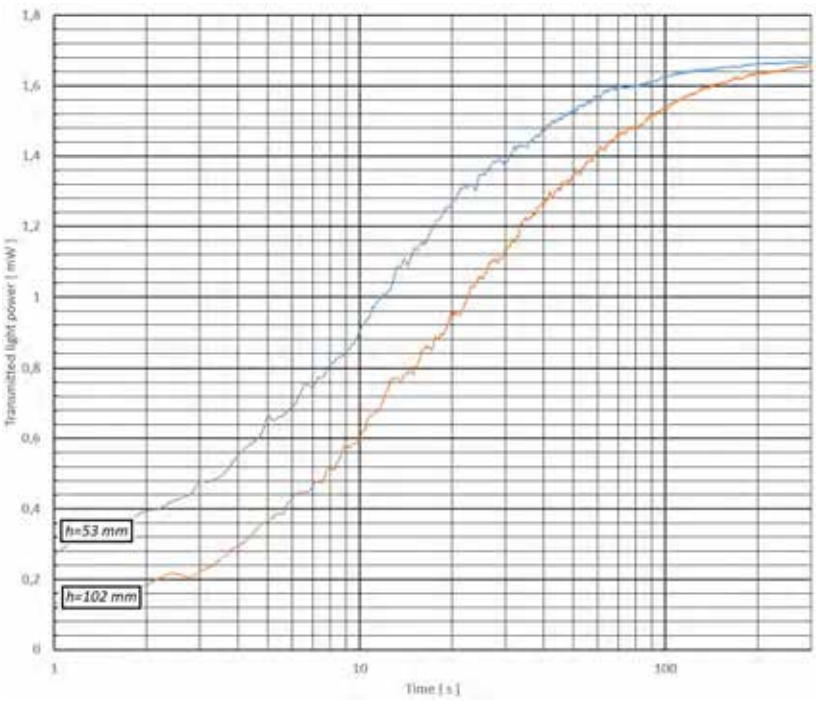


Fig.5 Transmitted light power versus time at two cases of h parameter

In next, you have to analyse this curve to evaluate the particle size statistics of the sand sample. Some characteristic particle radii have to be prescribed to set the size ranges that are analysed. These characteristic radii are the follows:

$R_1 = 10$ µm $\quad R_2 = 15$ µm $\quad R_3 = 20$ µm
$R_4 = 25$ µm $\quad R_5 = 30$ µm $\quad R_6 = 35$ µm
$R_7 = 40$ µm $\quad R_8 = 45$ µm $\quad R_9 = 50$ µm

At first, we have to know the time points when the sinking ceiling corresponding to the given radii reaches the level of the laser beam. After these time points, there are not any particles larger than the given radii in the vessel volume over the laser beam.

Question C2.2b Evaluate numerically the time points corresponding to the given particle sizes R_i. Use the formulas derived in question **C2.2a**. Evaluate them in the case of $h = 53$ mm and $h = 102$ mm. Fill the table with the time results on the *Answer sheet*.

Question C2.2c Use the diagram of Fig.5. Read the transmitted light power values I_i.corresponding to the determined time data of **C2.2b**. Write these power data into the table on the *Answer sheet*.

In the following, we divide the particle radii into ranges. The i^{th} range is defined by the next: $R_i < R < R_{i+1}$, where $i = 1 \ldots 8$. Our goal is to evaluate the mass concentration of the particles that have a radius in the given range. We know that the mass concentration of particles that have a given radius can be determined by the transmitted light power changing ΔI. Thus we have to evaluate the power enhancements ΔI_i corresponding to the defined ranges by the following difference: $\Delta I_i = I_i - I_{i+1}$

> **Question C2.2d** Calculate the transmitted light power enhancements and fill the table on the *Answer sheet*.

Knowing the transmission changes, we can calculate the mass concentration C_i of the particles in the given radius range. Let us use the expression derived in **C2.1b**. We have to note that the cited equation contains an exact radius parameter R. However, we are going to evaluate the concentration corresponding to a range of radius. To eliminate this problem, let us substitute to the expression the average radius R_a of the given range defined by the follows: $R_\text{a} = \frac{R_i + R_{i+1}}{2}$

> **Question C2.2e** Calculate the mass concentrations C_i corresponding to the given radius ranges. Fill the table on the *Answer sheet*.

> **Question C2.2f** Make bar histograms to summarize the evaluated size distribution of the suspension. Use a graph paper and label it '*graph C2.2*'. Plot simultaneously both data derived from the measurements at laser position $h = 53$ mm and $h = 102$ mm. Indicate on the graph which column belongs to which measurement.
> **Do not forget to attach '*graph C2.2*' to the answer sheet!**