European Union Science Olympiad

Jahresbericht 2018/19

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Regionales Netzwerk für Naturwissenschaften und Mathematik Kärnten

Vom

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European Union Science Olympiad

Die EUSO ist ein naturwissenschaftlicher Teamwettbewerb der Europäischen Union für Biologie, Chemie und Physik. Österreich war 2018 zum schon elften Mal mit zwei Teams bei der EUSO, die heuer in Ljubljana stattfand, vertreten.

Das Credo der EUSO

- begabten SchülerInnen die Möglichkeit geben, ihre Talente zu entfalten
- Das Interesse an Wissenschaft und des forschenden Lernens zu wecken bzw. zu fördern
- Durch die Eindrücke und Erfahrungen der EUSO auf eine mögliche Teilnahme an weiteren Internationalen Olympiadenvorzubereiten

Ziel des Wettbewerbs

- Öffentliche Interesse auf die naturwissenschaftliche Ausbildung lenken
- Ermittlung der besten SchülerInnen der Europäischen Union im naturwissenschaftlichen Bereich
- Wertschätzung der Wissenschaft in der Allgemeinheit
- Intensivierung der Zusammenarbeit zwischen europäischen Bildungssystemen
- Individuelle Ideen und Konzepte innerhalb der gesamten Europäischen Union zu verbreiten
- Vorbereitung europäischer SchülerInnen auf die Internationalen Facholympiaden

Mehr dazu unter: www.euso.eu und www.euso.at

Inhalt

1. Vorbereitungswoche an der BIKO mach MINT

33 SchülerInnen aus sechs Bundesländern, wurden, organisiert vom Regionalen Netzwerk Kärnten, vom 28. Jänner.-1. Februar. 2019 an der Bildungskooperation BIKO mach MINT am Educational Lab des Lakeside Parks Klagenfurt auf den Teamwettbewerb in Slowenien vorbereitet.

Bittner	Elena	BG/BRG Perchtoldsdorf
Bonfert	Fabian	BRG 18 Wien
Catharin	Amelie	Sir Karl Popper Schule
Dorfer	Paul	Gymnasium Tamsweg
Eminger	Simon	Sir Karl Popper Schule
Esterl	Johannes	BG/BRG Mössingerstraße Klagenfurt
Friedrich	Iris	Sir Karl Popper Schule
Friedrich	Isabelle-Catherine	Sir Karl Popper Schule
Girstmair	Birgit	Peraugymnasium Villach
Grosinger	Philipp	Sir Karl Popper Schule
Grubmüller	Lena	Sir Karl Popper Schule
Hofer	Moritz	BRG Zell am See
Högn	Tobias	BG/BRG Perchtoldsdorf
Hohl	Elias	Bischöfliches Gymnasium Graz
Kalla	Victoria	BG/BRG Mössingerstraße Klagenfurt
Klaus	Sophie	Europagymnasium Klagenfurt
Klimisch	Thomas	BG/BRG Carnerigasse Graz
Köhldorfer	Anna	Bischöfliches Gymnasium Graz
Kretz	Nicole	Peraugymnasium Villach
Lu	Jiwei Alexander	Polgargymnasium Wien
Miklautz	Anna	Europagymnasium Klagenfurt
Mirković	Elena Lucia	Peraugymnasium Villach
Neumeister	Kerstin	BORG Deutschlandsberg
Rainer	Eva Maria	BORG Wiener Neustadt
Rainer	Markus	Gymnasium Zell am See
Robič	Dalena	BRG Leibnitz
Rost	Lukas	Bundesgymnasium St. Pölten
Schrei	Anna Elena	BRG Leibnitz
Steiner	Amelie	BRG Leibnitz
Stepman	Helene	Gymnasium Schillerstraße Feldkirch
Taschler	Christopher	HTL1 Lastenstraße Klagenfurt
Vavra	Elisabeth	Sir Karl Popper Schule
Werner	Christopher	HTBLA Eisenstadt

2. Trainingstage an der BIKO mach MINT in Klagenfurt

Sechs Jugendliche schafften es in die Qualifikation und somit zum Intensivtraining, das heuer wieder in Kooperation mit dem deutschen EUSO-Nationalteam ebenfalls am Lakeside Park in Klagenfurt stattfand (8. - 12. April 2019). Diese Trainingstage mit den deutschen EUSO KandidatInnen waren sind inzwischen zur Tradition geworden und werden von der Teamleitung beider Länder als äußerst produktiv (Bericht des IPN Kiel)gewertet.

Insgesamt beteiligte TrainerInnen in Klagenfurt

TrainerIn	Stamminstitution	Fach
Mag Brachtl	Karl	RN Kärnten
Mag. Holub	Peter	RN Kärnten
Mag. Holub	Sigrid	RN Kärnten
Dr. Morgenstern	Christina	PH Kärnten, Bundeslehrerin
Mag. Winkler	Dieter	Bischöfliches Gymnasium Graz, Bundeslehrer
Lobnig	Stefan	NMS St Ursula, Landeslehrer
Dr. Willitsch	Peter	Physiklehrer im Ruhestand

3. EUSO 2019 in Portugal

Die Organisation in Almada war ausgezeichnet. Das Freizeitprogramm war sehr spannend, die Aufgabenstellungen fordernd, Fächer übergreifend und gut vorbereitet, allerdings extrem schwierig. Vor allem, weil sie in der Zeit kaum bewältigbar waren. Das kam unserem erfahrenen A-Team entgegen, das mit diesem Setting hervorragend zurechtkam.

4. Team AUSTRIA 2019

Delegationsleitung: Mag. Peter Holub

Mentorin Biologie: Dr. Christina Morgenstern

Mentor Chemie: Mag. Karl Brachtl

Mentor Physik: Mag. Dieter Winkler

Team A: Elias Hohl, Victoria Kalla, Sophie Klaus, *Goldmedaille*

Team B: Moritz Hofer, Lukas Rost, Johannes Esterl, *Bronzemedaille*

5. Erstes Gold seit 2015, sowie einmal Bronze in Almada!

Anlässlich der diesjährigen Europäischen Science Olympiade in Portugal kann Österreich einen großen Erfolg vorweisen. Unser A-Team errang den hervorragenden 5. Platz bei 50 teilnehmenden Teams und damit eine der begehrten sechs Goldmedaillen! Renommierte Nationen wie Ungarn und die Tschechische Republik rangieren dahinter. Auch unser junges B-Team schlug sich ausgezeichnet, verpasste Silber nur um 0,5 Punkte (maximale Punktezahl: 720).

Goldmedaillen

Germany B	(1)		1 Nantje Nageler 2 Damian Groß 3 Franz Loose
Latvia A	(2)		1 Rolans Lopatko 2 Ilmars Stolcers 3 Viesturs Spulis
Ireland A	(3)		1 Oscar Despard 2 Oisín Ó Feinneadha 3 Tommy Connolly
Estonia A	(4)		1 Andreas Simson 2 Martin Rahe 3 Hendrik Vilja
Austria A	(5)		1 **Victoria Kalla** 2 **Elias Hohl** 3 **Sophie Klaus**
Germany A	(6)		1 Maximilian Mittl 2 Fabian Kutz 3 Tobias Messer

Die österreichische Delegation in Portugal 2019

Von links nach rechts hinten: Dr. Christina Morgenstern, RECC für Naturwissenschaften der Pädagogischen Hochschule Kärnten, Mentorin Biologie, Lukas Rost, BG/BRG St. Pölten, Team B, Victoria Kalla, BG/BRG Mössingerstraße, Team A, Tomás Chora, guide, Mag. Dieter Winkler, Bischöfliches Gymnasium Graz, Mentor Physik, Moritz Hofer, BRG Zell am See, Team B, Mag. Karl Brachtl, RN Kärnten, Mentor Chemie, Mag. Sigrid Holub, NAWImix, Trainerin Biologie, Mag. Peter Holub, RN Kärnten und BIKO mach MINT, Bundeskoordinator, **vorne:** Sophie Klaus, Europagymnasium Klagenfurt, Team A. Elias Hohl, Bischöfliches Gymnasium Graz, Team A, Johannes Esterl, BG/BRG Mössingerstraße Klagenfurt, Team B.

Sowohl die Trainingswoche im März als auch die Trainingstage mit den deutschen Nationalteams im April wurden vom Regionalen Netzwerk für Naturwissenschaften und Mathematik Kärnten koordiniert und fanden in den brandneuen Experimentierräumen der Bildungskooperation BIKO mach MINT am Educational Lab, Lakeside Park Klagenfurt statt.
Drei der sechs Goldmedaillen entsprangen dieser Trainingsgemeinschaft, in der als TrainerInnen auch Magdalena Lederbauer, Schülerin der Sir Karl Popper Schule, Dr. Peter Willitsch, NAWImix und Stefan Lobnig, BIKO mach MINT, einen wesentlichen Teil zum Erfolg geleistet haben.

6. Unterstützung durch

Land Kärnten

Klagenfurt am Wörthersee

Industriellenvereinigung Kärnten

Stadt Villach

Regionales Netzwerk für Naturwissenschaften und Mathematik Kärnten

IMST- Innovationen machen Schulen Top

7. Anhang – Aufgabenstellungen 2019

TASK 1 CORK

INTRODUCTION

Cork is a plant tissue formed by cells filled with a gas mixture similar to air and bound by natural polymers, the main components being suberin (45%), lignin (27%) and polysaccharides (12%). This extraordinary product of nature is nothing more, nothing less than the bark of the cork oak, the only tree with an auto renewable bark.

Portugal accounts for almost 50% of the global cork oak area and is the largest cork producer in the world, contributing with 55% of the annual world production.

Cork removal is performed only by specialized professionals who manage to extract the bark without damaging the tree so that the oak can be stripped every nine years. However, a cork oak needs to be 25 years old before it can be stripped for the first time. This first cork can be used as raw material for thermal and acoustic insulation products. Cork is widely exploited due to its unique properties and today its industrial application goes far beyond cork stoppers and cork flooring. For example the Vega rocket from the European Space Agency's (ESA) was launched into space in 2012 and, in order to prevent the rocket from overheating, cork was placed in the nose cone and other areas sensitive to temperature. In a Sustainable Development context, cork can play a relevant role, because it is a natural, renewable, recyclable and non-toxic resource, with exceptionally good environmental qualities, and a high potential of innovative technological characteristics.

On a trip to the province of Alentejo, two friends, Vasco and Isabel, were very surprised when they saw large beautiful trees which bark had just been removed.

They approached the workers and asked if that process might harm the trees. The workers smiled at them and explained that this particular bark they were extracting was simply cork, the material that is used as bottle stoppers.

Isabel then remembered of pictures from her parents when they visited the Portugal Pavilion at Expo 2000 in Hannover (Figure A), whose walls were made of cork! They were really curious and decided they wanted to learn more about this fantastic oak tree, the cork that it produces and its possible applications.

Figure A – Portugal Pavilion at Expo 2000 Hannover.
Álvaro Siza Vieira and Eduardo Souto Moura, Pritzker Prize winners, Portuguese architects, used pure expanded cork boards and high density cork boards as external coating on some facades of the building.

In this task you will get to know a little more about cork and, hopefully, you will understand why it is considered an extraordinary natural product.

With this aim, you will show Vasco and Isabel how to identify the best cork-producing tree that will allow making premium wine bottle stoppers. You will also show them how to evaluate the quality of a cork stopper and will demonstrate that cork can really be an efficient thermal insulator.

This assignment includes 4 individual tasks which consist on:

Task 1 - 1 – Identification of Key Characteristics of Cork and of the Cork Producing Tree 60 Marks

Task 1 - 2 – Selection of the Perfect Cork Plank to make Premium Wine Bottle Stoppers 60 Marks

Task 1 - 3 – Determination of Phenolic Content and Evaluation of Cork Quality. 120 Marks

Task 1 - 4 – Cork as Thermal Insulator 120 Marks

TASK 1 - 1.: IDENTIFICATION OF KEY CHARACTERISTICS OF CORK AND OF THE CORK PRODUCING TREE

Introduction

There are approximately 200 species listed under the genus *Quercus*, but only one species has the ability to continuously produce a structure with technological characteristics that make it highly valuable. This structure is commonly called cork and it is a protective structure named phellem by plant anatomists.

Cork is evaluated according to its quality, with two main physical-chemical factors being evaluated: plank thickness and plank porosity. Plank thickness relates with cork annual growth, i.e. the number of cells produced per year. Currently, the cork production cycle takes 9 years, the annual rings being visible in the planks (Figure 1 - 1.1). Cork planks are classified according to its thickness and are normalised into calliper classes. However, there is not a direct relationship between thickness and quality. Very thin planks are not suitable for industry purposes while thick planks have too much gas permeability (negative trait for cork quality). If cork is used for stopper production and there are too much permeability, it will allow too much air to diffuse it may damage the wine. Additionally, if the plank is too thick, too much unused cork is waste in the production line. When having a suitable plank thickness, the plank is evaluated for its porosity.

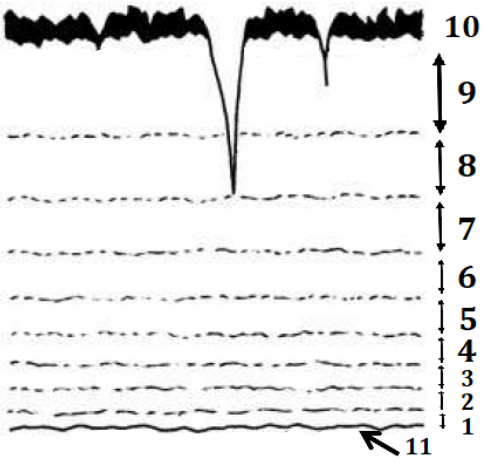


Figure 1 - 1.1 – Schematic representation of a cross-sectoral plan of a cork oak plank. The phellogen produces cork layers that develop radially with an annual physiological rhythm; nine annual growth rings are represented here (1, most recent; 9, older one). Cork bark (10) is exposed to the atmosphere. The phellogen (11) is in contact with the most recent growth ring (1).

So, cork quality is the result of the individual genetic makeup and growth conditions, both abiotic (e.g. water availability) and biotic (e.g. pathogens that comprise tree growth or that compromise cork homogeneity).

Within this task you are asked to identify the cork producing tree (task 1 - 1) and to identify key characteristics of cork quality (task 1 - 2).

Materials and equipment

- Five Petri dishes with plant material (A - E), one per specimen (each individual represents one species) containing:
    - Leaves, fruits
    - Small Petri dish with branches ultrathin cuts (for Sudan red staining)
- Cork micro slices for positive control for Sudan red staining (labelled as "+")
- Photographs of the branches, fruits, leaves and trees for each species (A - E)
- Appendix 1 "Morphological characteristics of the biological material"
- Microscope glass slides, 1 box
- Glass coverslips,1 box
- Tweezers, 1 piece
- 20 µL Micropipette, 1 piece
- 20 µL Micropipette tips, 1 box
- Deionized water in 10 mL test tube, 1 piece (labelled as "**H_2O**")
- Sudan red reagent in 10 mL test tube, 1 piece (labelled as "**Sudan Red**")
- Ethanol 70% in 10 mL test tube, 1 piece (labelled as "**EtOH**")
- Pasteur pipettes, 3 pieces
- 24 well plate, 1 piece
- 500 mL plastic beaker for waste, 2 pieces
- Timer also to be used in Task I.3, 1 piece
- Optical microscope, 1 piece
- Stereomicroscope - shared (one per two working groups)

If you spill a chemical or break a piece of glassware or destroy the biological material and you need a replacement, request the help of the lab assistant.

Any additional material from the list above mentioned will cost you 5 marks unless otherwise stated. You can only ask for one additional Petri dish with plant material and this will cost you 10 marks.

1 - 1.1. Identification of the cork producing tree

In your workstation you will find 5 Petri dishes with biological samples – named A, B, C, D and E - from five different trees, one of them is the cork producing tree. Please be very careful when handling the thin sections.

In this task you are asked to identify the 5 species to which each specimen belongs to, following a set of experimental activities.

Fill in the following table that will lead you to the characterization and identification of the 5 trees (A, B, C, D and E). For that you will need to use the biological samples in the plate, the photos and the Appendix 1 provided.

When filling in the table, indicate with a cross (X) the characteristics that best fit your observations and/or experimental results. It may be the case that there is more than one correct option for each specimen. Be sure to clearly indicate all that apply.

If you select incorrect options, this will cost you 20% of the marks per option.

If you do not select all the options that apply, this will cost you 10% of the marks per option.

1 - 1.1.1. Instructions for the experimental procedure

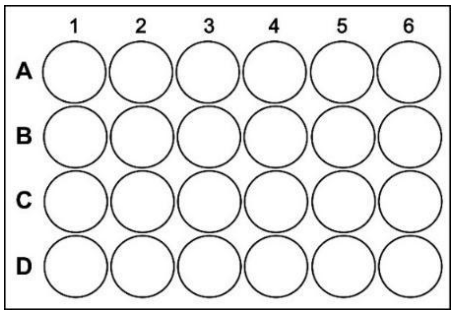
Read the table carefully before you start your observations.
To avoid multiple observations of the same material (getting in and out of the plate), observe all the characteristics under the optical microscope or stereomicroscope that are asked to fill the table.

Observation of the biological material provided AND photographic handout;

1. Leaf trichomes ("hairs") observation under the optical microscope (magnification 100X): **just put the leaf on the top of a microscope slide - use the whole leaf (not a fragment); do not use water nor a cover slip** - turn on the microscope and observe at lower magnification. Observe both sides of the leaf (upper and lower). Observe all the leaves available, and within each leaf observe multiple areas.
2. Water droplet test: select one leaf and use a micropipette to measure a drop of 10 µL of water. Gently apply, without pressing, the drop on each of the surfaces of the leaf (upper and lower). Apply at least 10 drops per leaf surface, in differents parts of the same leaf. Be sure to apply one drop on the middle vein and another drop on a surface that is not in contact with the middle vein.
3. Observe the water droplet under the stereomicroscope to determine its shape.
4. When analysing the lower surface, and after the droplet test, lean the leaf at 90° and check if the drop slips or changes shape. Answer the question based on the behavior of at least 6 out of 10 drops.
5. **Dry the droplets with paper and return the biological material to the respective Petri dishes.**
6. Sudan Red test: Use the 24 wells plate to stain the young stems slices. Use three wells per species. Pipette 0.5 ml of Sudan Red stain, 0.5 ml of 70% ethanol or 0.5 ml of water per well (as suggested in the figure below). You can find all these reagents in tubes of 10 ml and with the correct label. Use the provided tweezers to pick the cuts and pass them through the solutions.

Insert the slices in the 70% ethanol solution for 1-2 min, then insert them in the Sudan Red solution and incubate for 10 min; take the slices off and wash them in the previous 70% ethanol solution (1-2 min); pass the slices through distilled water (1-2 min) and mount them in a slide, with a drop of water and a coverslip.

Observe the preparation under the microscope. Look at several different pieces. In addition to the 5 tree samples, you also need to stain the positive control.



Proposed layout:
Wells A1 to A6 – use for the 70% ethanol solution;
Wells B1 to B6 – use for Sudan Red solution;
Wells C1 to C6 – use for water;
Wells D1 to D6 remain empty.
Use wells A1 to C1 for specimen A,
A2 to C2 for specimen B, and so on.
Use wells A6 to C6 for the positive control.

Read the Table and optimize the use of the microscope and stereo microscope.
The swap of biological material between Petri dishes will cost you 20 marks.

- **Enter the biological material dataset ID number in the answer sheet.**

Question 1.1.a

Following the instructions provided, fill Table 1.1.a in the answer sheet:

- **Enter your results under Question 1.1a. in the answer sheet.**

Question 1.1.b

On completing your observations, use the dichotomous key provided in the answer sheet to identify the species present in each Petri dish. On the answer sheet, in Table 1.1.b, make a correspondence between the Petri dish letter and the name of the tree

- **Enter your results under Question 1.1.b in the answer sheet.**

Question 1.1.c

In trees, in which leaf surface would you expect to find stomata?

- **Enter your answer under Question 1.1.c in the answer sheet.**

Question 1.1.d

In trees, what is the advantage of highly pubescent leaves?

- **Enter your answer under Question 1.1.d in the answer sheet.**

Question 1.1.e

If you are asked to determine if these trees are monocotyledonous or dicotyledonous, which plant organ would you use?

- **Enter your answer under Question 1.1.e in the answer sheet.**

Question 1.1.f

You were asked to perform the Sudan Red staining in young stems of the several specimens. You probably notice a dark layer in the most external part of the sections. Can you provide the most probable function of this layer?

- **Enter your answer under Question 1.1.f in the answer sheet.**

TASK 1 - 2.: SELECTION OF THE PERFECT CORK PLANK TO MAKE PREMIUM WINE BOTTLE STOPPERS

Introduction

Now that you have succeed to identify which sample belongs to a tree of *Quercus suber*, commonly known as the cork oak, we need your help in determining which sample of cork planks should be used for wine bottle stoppers.

Cork planks from three different trees, which represent three different types of cork, were collected for your analysis (Figure 1 - 2.1). Please take some moments to analyse the texture, the porosity and imperfections present in your cork samples. Cork planks enriched in lesions, or with the inappropriate width, are not suitable for making wine bottle stoppers.

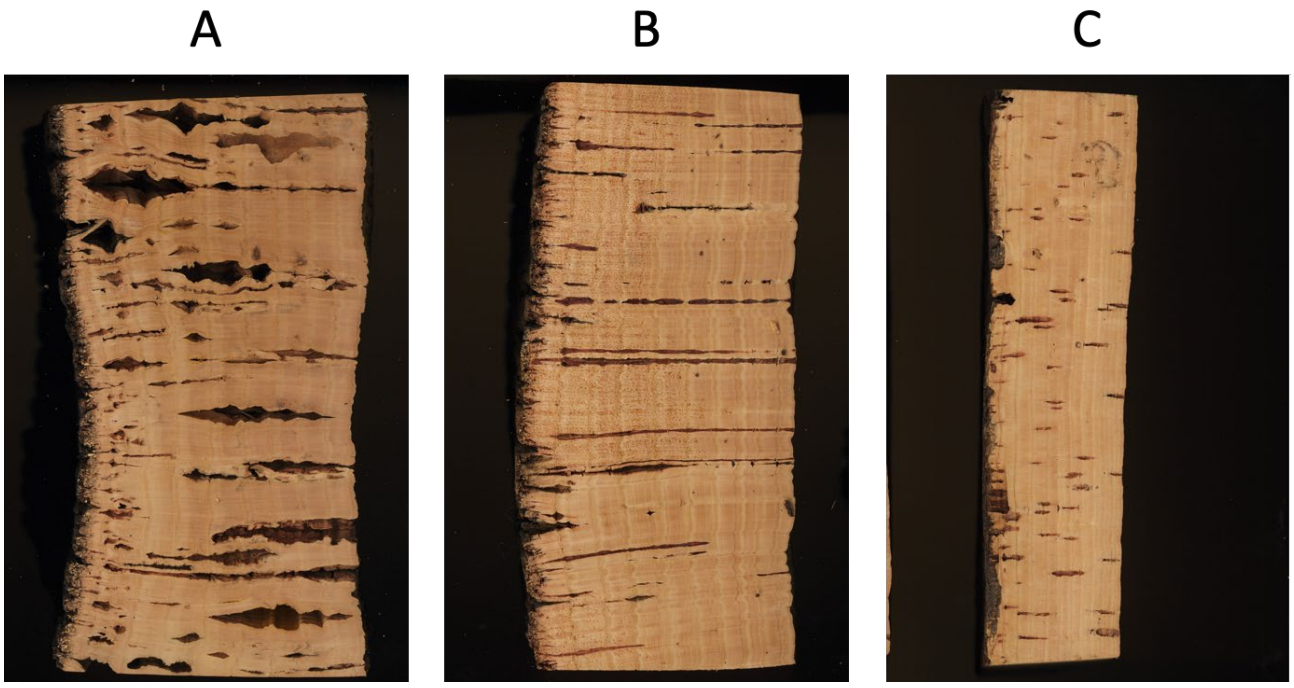


Figure 1 - 2.1 – Images obtained from the lateral area of each plank are shown below (labeled A to C).

It is possible to observe the environmental effects on each particular tree. Cork cells do not grow on Winter, they only grow in Spring and Summer. The growth rate depends on the physiological conditions of the tree and the amount of water available. During Spring, you may observe more cell divisions, which result in larger cells with thinner cell walls. During Summer there are fewer cell divisions, which result in cells with cell walls differently assembled (tougher and darker).

The environmental conditions are reflected in each tree growth rings and the analysis of the width of the tree rings can give you further information about the growing conditions, namely climate effects, insect attacks, fire and light availability.

In this task, you need to determine the average fraction of the cork material with imperfections of 3 cork planks shown in Figure 1 - 2.1 (one sample of each cork plank is available in the laboratory in case you want to briefly observe these imperfections). The sample with a suitable thickness and lowest fraction of imperfections should be the best for bottle stoppers production.

Materials and equipment

- Computer, 1 piece.

- Cork sets for hands-on observation of cork - shared. One cork set with 3 samples is available per Laboratory.

1 - 2.1. Cork plank quality control: quantification of imperfections

Follow the procedure described below:

1. Open the programs ImageJ and OpenOffice, by clicking twice in their respective symbols that are available on the Desktop. In OpenOffice, chose the option Spreadsheet.
2. In ImageJ, open files named A to C (command File>Open) that are on the Desktop (Folder Images_EUSO). These image files have been taken with a digital scanner and saved in RGB color format.
3. Extract the information in each channel by applying the command Image>Color>Split Channels. This command will extract the information in the Red, Green and Blue Channels into three different images.
4. To each image apply the command Image>Lookup Tables>Grays (to change the color of each frame to Gray). Then apply the command Image>Adjust>Brightness/Contrast (to adjust the intensity of the pixels in each image) and choose the option Auto. Finally choose the option File>Save As>Tiff (to save each image in a TIFF format). **Alter the name of each image to any chosen designation that clearly identifies the colour.**
5. For each plank (samples A to C) you get three images. For each sample, decide which image to use in the next steps.

Question 2.1.a

Which channel(s) allows you the best observation of the growth rings?

- **Enter your results under Question 2.1.a in the answer sheet.**

6. After choosing the image you want to analyze, **save it with a different name such as "Image_1 - tif"**. Then open the image with the command File>Open and choose the button in the toolbar that corresponds to the Rectangle Selection (1st button from the left side). Select the area of the cork you want to analyze. Do not include the black background.
7. Copy the information inside the selection by choosing the command Edit>Copy to System.
8. Choose the option File>New>System Clipboard. This step will paste the information that you have copied into a new RGB file.
9. Choose the option Image>Type>8-bit to convert the file from a RGB Color format into a 8-bit format.
10. Choose the option File>Save As>Tiff (to save the selected region in a TIFF format). **Save as Image_A_crop_1.tif.**
11. Choose the option Image>Adjust Threshold and define the intensities of the pixels in the "Threshold" window in order to include the dark areas you want to quantify. If you are happy with the selected regions choose "Apply". The selected regions will be colored with white and a pixel intensity of 255. The other regions will be colored with black and a pixel intensity of 0.

12. Choose the option Analyze>Set Measurements and define what you want to measure and the number of decimal places.
13. Choose the option Analyze>Measure and record the values obtained in the window "Results". This is the area of the entire image you have in pixels^2 (pixel x pixel).
14. Close the window "Results".
15. In order to save an image with the area with white pixels, use the option File>Save As>Tiff. Save as Image_A_crop_1 _threshold.tif
16. Choose the option Analyze>Analyze Particles and decide which parameters you will need. We propose that you choose the option "Outlines" in "Show:" (to observe the regions that have been selected) and that you click the option "Display Results" and "Add to Manager" (to save the results in a file that you can open using the OpenOffice spreadsheet also available on the Desktop). Then click "OK".
17. You will be able to select each assembled Region of Interest (ROI) in the window "ROI Manager" and see where it is in the window with your image. Select all the ROI and save the file in the same folder you have been saving all your ImageJ files. The values of the area for each ROI are shown in the window "Results" in pixels^2.

Do not forget to organize and save your files so that it is possible to confirm that you have executed correctly the proposed protocol. This information will be considered in the classification of questions 2.1.b to 2.1.e.

Question 2.1.b

How many files have you produced with the protocol you followed to determine the percentage of area with imperfections in the cork samples A to C? Please include the files you have produced with the spreadsheet based software that is available in your computer.

- **Enter your results under Question 2.1.b in the answer sheet.**

Question 2.1.c

Which region of the cork samples A to C have you considered in the determination of the percentage of area with imperfections? Draw **with waterproof marker** the region you considered in the answer sheet.

- **Enter your results under Question 2.1.c in the answer sheet.**

Question 2.1.d

Determine the percentage of area with imperfections in the different cork samples A to C. Which of the samples has the lowest percentage of area with imperfections? You will need to use the OpenOffice spreadsheet available in your computer.

- **Enter your results under Question 2.1.d in the answer sheet.**

Question 2.1.e

Take in consideration the area with imperfections, draw a bar plot with the percentages of the dark regions present in the 3 cork planks. You should use the values measured in the previous question.

- **Enter your results under Question 2.1.e in the answer sheet.**

Question 2.1.f

How many growth rings can you easily observe in the plank A?

- **Enter your answer under Question 2.1.f in the answer sheet.**

Consider Figure 1 - 2.2 for the next questions, where you may detect 10 different layers that correspond to 10 consecutive growing years.

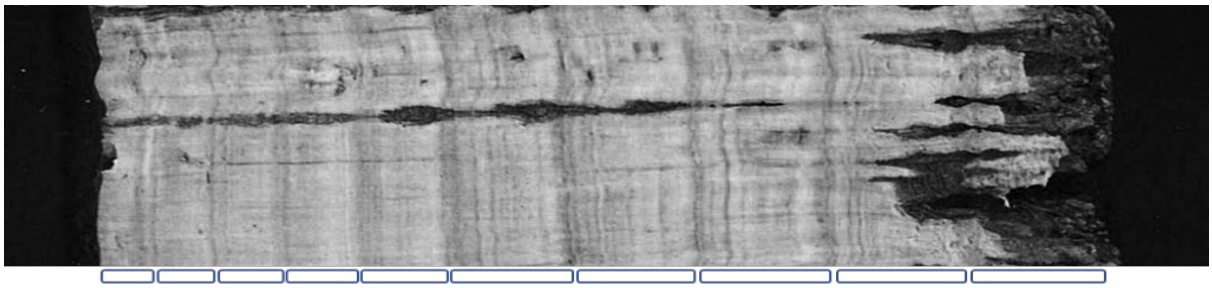


Figure 1 - 2.2 Image of the lateral area of one of the planks that you have analyzed. Numbers represent annual growth rings.

Question 2.1.g

Why do you think there are darker and lighter areas within the growth rings? Choose the best hypothesis.

- **Enter your answer under Question 2.1.g in the answer sheet.**

Question 2.1.h

In which year do you think it rained more?

- **Enter your answer under Question 2.1.h in the answer sheet.**

Question 2.1.i

Indicate which regions do you think correspond to Spring and Summer growth.

- **Enter your answer under Question 2.1.i in the answer sheet.**

Question 2.1.j

Which layer/growth ring has been exposed to the atmosphere?

- **Enter your answer under Question 2.1.j in the answer sheet.**

Task 1 - 3.: Determination of Total Phenolic Content and Evaluation of

CORK QUALITY

Introduction

Due to its unique physical and chemical characteristics, cork is an excellent seal for table wines, sparkling wines and liqueurs among others. As seen in the previous task (1 - 2), not all types of cork can be used in the manufacture of wine bottle stoppers.

Cork planks with too much permeability, or with the inappropriate thickness, are not suitable for making wine bottle stoppers. This is because cork aromas can have an effect on the sense of smell and taste of wine.

The complexity of cork aromas is sometimes associated with the appearance of sensory defects in wine. This may arise as a consequence of the presence of exogenous chemicals of microbiological origin. Although the percentage of appearance of aroma defects associated with the use of cork stoppers is very low, producers of cork stoppers have been making efforts to apply appropriate quality control methods. Among these, in the cork stoppers sensorial analysis it is important to evaluate the presence of 2,4,6-trichloroanisole (TCA), that cause the mould smell/taste in wine.

The formation of TCA occurs when microorganisms, such as fungi, come into contact with chlorine-based compounds, usually chlorophenols, as represented in Figure 1 - 3.1

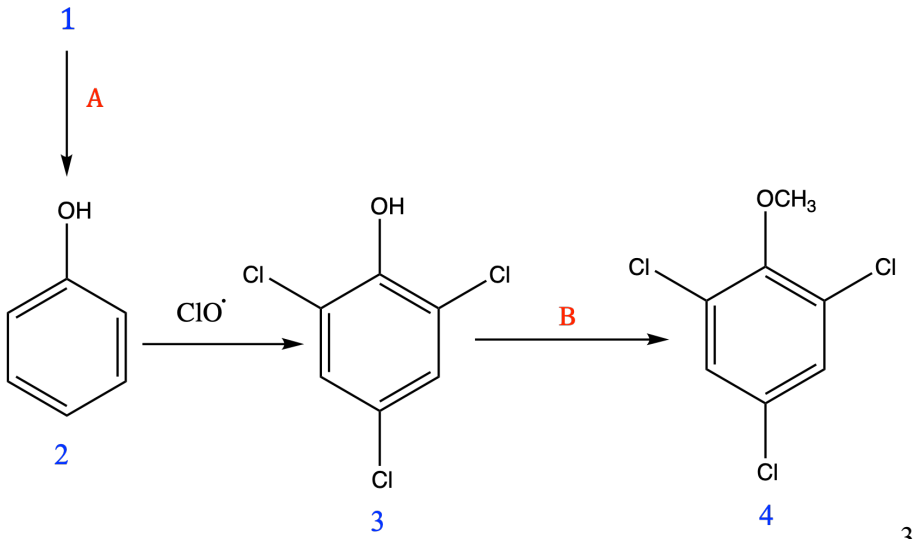


Figure 1 - 3.1 – Production pathway of 2,4,6-trichloroanisole (TCA).
1 - Lignin; **2** - Phenol; **3** - 2,4,6-Trichlorophenol (TCP); **4** - 2,4,6-Trichloroanisole (TCA); **A** - Fungi; **B** - Filamentous fungi biomethylation

Since phenol is itself a product of degradation of the polymeric structure of cork, the quantitative determination of phenol present in cork is an important parameter to determine, not only its quality, but essentially its possible applications.

In this task you will determine the amount of phenol present in cork samples and thus classify cork in terms of its quality to be used in the production of cork stoppers.

Phenolic quantification assay is based on Folin-Ciocalteu (Folin) reagent. The Folin reagent contains phosphomolybdic/phosphotungstic acid complexes. The method relies on the transfer of electrons in alkaline solution from phenolic compounds to form a blue chromophore constituted by a phosphomolybdenum/phosphotungsten complex. Its colour intensity depends on the concentration of phenolic compounds. The reduced Folin reagent is detectable with a spectrophotometer in the range of 600 to 710 nm.

In the quantitative analysis of phenols, the Lambert-Beer law will be used. It relates the concentration of a compound with its absorbance.

$$A = \varepsilon b C$$

This law predicts a linear relation between the absorbance reading (A) and the molar concentration of the compound (C), if (b), the cell or cuvette width, is kept constant. ε is a constant defined as the absorptivity and is characteristic of the compound and solvent media. Thus, it is possible to determine the concentration ($mg.L^{-1}$ or $mol.L^{-1}$) of a given compound in a solution through the absorbance of that solution if the cell length (cm) and the absorptivity ($L.mg^{-1}.cm^{-1}$ or $L.mol^{-1}.cm^{-1}$) are known.

Materials and equipment

- Volumetric flask, 50 mL, 10 pieces
- Graduated beakers, 25 mL, 15 pieces
- Volumetric pipette 3.00 mL, 2 pieces
- Volumetric pipette 5.00 mL, 1 piece
- Volumetric pipette 10.00 mL, 1 piece
- Volumetric pipette 20.00 mL, 1 piece
- Volumetric pipette 25.00 mL, 1 piece
- Pipette filler bulb, 1 piece (general tray)
- 1000 µL micropipette, 1 piece
- 1000 µL micropipette tips, 1 box
- Disposable plastic Pasteur pipettes, 3 pieces
- 1 cm length/2 mL plastic cuvettes, 5 pieces
- 500 mL plastic beaker for waste, 2 pieces
- Waterproof marker, 1 piece
- Deionized water in 500 mL plastic wash bottle, 2 pieces (labelled as "**H_2O**") (can be refilled if needed without penalty)
- Stock solution of gallic acid (**0.00050 mol.L^{-1}**), 100 mL (labelled as "**Gallic Acid"**)
- Stock solution of Folin-Ciocalteu, 20 mL (labelled as "**Folin**")
- Stock solution of sodium carbonate (**7.5% w/w**) , 20 mL (labelled as "**Na_2CO_3**")
- Three samples of cork extracts (labelled as "**Lot A**"; "**Lot B**"; "**Lot C**"), 2 mL each
- Colorimeter, 1 piece
- TI-Nspire CX calculator, 1 piece
- Scientific calculator TI-30X, 1 piece (general tray)
- Timer also to be used in Task 1 - 1, 1 piece (general tray)

If you spill a chemical or break a piece of glassware and you need a replacement, please request the help of the lab assistant.
Any additional above mentioned material will cost you 5 marks unless otherwise stated. Additional samples will cost you 10 marks.

1 - 3.1. Calibration curve slope (m)

The first task is to determine the term εb from the Lambert-Beer law, that is the slope **m**, using a set of solutions of known concentrations (standard solutions) that will be prepared from a stock solution of gallic acid ($5.0x10^{-4}$ $mol.L^{-1}$). Gallic acid is traditionally used to determine the Total Phenolic Content (TPC) in various materials.

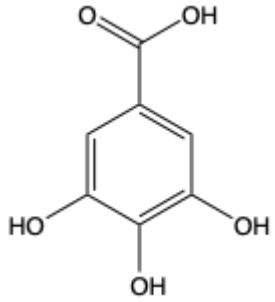


Figure 1 - 3.2 – Chemical structure of Gallic Acid (GA)

Prepare 50 mL of each standard solution using the volumes indicated in the Table 3.1 by using the pipette filler bulb (see in Appendix 2) and the appropriate pipette.

Table 3.1

Standard solutions prepared from the stock solution

Standard	**Stock solution 0.00050 $mol.L^{-1}$ Volume to add in mL**
S1	3.00
S2	5.00
S3	10.00
S4	20.00
S5	25.00

Question 3.1.1.

Calculate the concentration of the standard solutions prepared by you (in $mg.L^{-1}$). Indicate the obtained value with 2 decimal places.

- **Enter your calculations and results in the answer sheet, under Question 3.1.1 in the Table.**

1 - 3.1.1. Samples for calibration curve

1. Using the micropipette carefully transfer 500 µL of each standard solution into a 25 mL beaker and add to each 500 µL of the Folin reagent, stir, **wait 3 minutes** and then add to each 500 µL of the Na_2CO_3 solution.
2. Prepare the blank solution following the instructions in point **1.** but using 500 µL of H_2O instead of the standard solution.
3. Stir all prepared solutions and leave them to stand for **30 minutes**.
4. Connect, wait approximately 5 minutes and calibrate the colorimeter (see instructions for Vernier Colorimeter in Appendix 3).
5. After points **3.** and **4.** read the absorbance of the solutions at the wavelength of 635 nm using the colorimeter. Indicate the obtained value with 2 decimal places.

Question 3.1.2.

- **Record the values in the Table of the answer sheet under Question 3.1.2.**

Question 3.1.3.

Plot the absorbance (A) versus the gallic acid concentration ($C_{\text{gallic acid}}$).

- **Register only the experimental points in millimeter paper under Question 3.1.3 in the answer sheet.**

Question 3.1.4.

From the A and $C_{\text{gallic acid}}$ data determine the *slope* of the straight line that best fits the experimental data points.

The straight line that best fits the experimental data points can be determined by the least squares fitting method. The least squares fitting method is based on the minimization of a function that computes the sum of the squares of the differences between the expected values of A and its corresponding experimental values.

To do this, start by calculating the sums according to the following example Table.

Example of the calculations for the determination of the slope using the least squares fitting method.

$(x_i)^2$		$x_i \times y_i$	
x_1^2		$x_1 \times y_1$	
....		....	
x_5^2		$x_5 \times y_5$	
$\mathbf{S}x^2$ = Sum of $(x_i)^2$		$\mathbf{S}xy$ = Sum of $(x_i \times y_i)$	

Calculate ***m*** as follows:

$$m = \frac{S_{xy}}{S_{x^2}}$$

- **Fill the Table in Question 3.1.4 in the answer sheet with the sums you need to calculate the slope**
- **Enter your calculations and results of *m* under Question 3.1.4 in the answer sheet**
- **Record the value of the absorptivity of gallic acid**

In the plot A versus $C_{\text{gallic acid}}$ and using the calculated value for ***m*** draw the straight line that best fits the experimental points.

- **Use the Plot of Question 3.1.3 in millimeter paper in the answer sheet to draw the straight line.**

1 - 3.2. Limit of Detection – LOD

The limit of detection, LOD, is a very important parameter that determines the degree of confidence with which a given concentration can be measured. When studying samples of concentration below the detection limit of a technique, it is usually necessary to add a sample concentration step.

For any given technique the LOD can be calculated as 3 times the maximum standard deviation (σ) of the measurement divided by the slope of the calibration plot according to IUPAC (International Union of Pure and Applied Chemistry).

$$\text{LOD} = 3\sigma / \text{slope}$$

Any signal measured in the real world has an associated uncertainty, that is a fluctuation around an average value, that affects the precision of the measurement. For instance, if a solution of a given concentration is prepared several times by weighing the same amount of compound and dissolving it in the same amount of solvent, different final concentrations will be obtained.

The standard deviation is related with the distance between the measured values and the average value of the measurements and is affected by the uncertainty that is involved in the preparation and in the measurement.

Determine the uncertainty in the measurements of the absorbance at 635 nm by preparing 5 replicates of the standard **solution 1**. For each one:

1. Measure 500 µL of each standard solution into a 25 mL beaker and add 500 µL of Folin reagent, stir, wait **3 minutes** and then add 0.5 mL of Na_2CO_3.
2. Prepare a blank following the instructions in point 1. but using 500 µL of H_2O instead of the sample.
3. Stir and leave to stand for **30 minutes**.
4. Calibrate the colorimeter with the blank (see instructions for Vernier Colorimeter in Appendix 3).
5. After this period determine the absorbance of the 5 solutions at 635 nm using the colorimeter. Indicate the obtained value with 2 decimal places.

❖ **Record the value in the Table in the answer sheet under Question 3.2.1**

In statistics, the standard deviation (σ) is a measure that is used to quantify the amount of variation or dispersion of a set of data values. A low standard deviation indicates that the data points tend to be close to the mean (also called the expected value) of the set, while a high standard deviation indicates that the data points are spread out over a wider range of values.

For a finite set of numbers, the standard deviation is obtained by taking the square root of the average of the squared deviations of the values from their average value.

For example, if we have obtained the values 2; 4; 4; 4; 5; 5; 7; 9 from 8 successive measurements, the average of the measurements is 5.

First, calculate the deviations of each data point from the mean, and square the result of each, to obtain the squared deviations:

$$(2-5)^2=9$$

$$(4-5)^2=1$$

$$(4-5)^2=1$$

$$(4-5)^2=1$$

$$(5-5)^2=0$$

$$(5-5)^2=0$$

$$(7-5)^2=4$$

$$(9-5)^2=16$$

*Calculate the mean of these values (called variance) and you will obtain 4. The **standard variation** is the square root of the variance σ = 2*

Question 3.2.2.

From the standard deviation and the slope you should determine the LOD of the total gallic acid content.

- **Enter your calculations and results in the answer sheet, under Question 3.2.2**

1 - 3.3. Cork extracts analysis and evaluation

The cork planks A, B, C, from previous task 1 - 2, were subjected to an extraction procedure and the obtained extract was concentrated 10 times and stored at -20 °C to ensure its conservation. Three samples of cork extracts (Lot A; Lot B; Lot C) are supplied to be analysed.

1. Carefully transfer 500 µL of the "Lot A" sample into a 20 mL beaker and add 500 µL of Folin-Ciocalteu reagent, stir, wait **3 minutes** and then add 500 µL of Na_2CO_3. Stir and leave to stand for **30 minutes**.
2. Repeat point 1. for each of the "Lot B" and "Lot C" samples.
3. Prepare a blank following the instructions in point 1. but using 0.5 mL of H_2O instead of the sample.
4. After this period of **30 minutes** determine the absorbance at 635 nm and record the value in the Table under Question 3.3.1..

- **Record the value in the Table in the answer sheet, under Question 3.3.1.**

Question 3.3.2.

Determine the total phenolic content present in the cork samples provided and register the values in the answer sheets.

- **Enter your calculations and record the value in the Table under Question 3.3.2 in the answer sheet.**

Question 3.3.3.

Is the detection limit of the method adequate to ensure direct measurement of the samples (i.e., without a concentration step)?

- **Answer under Question 3.3.3 in the answer sheet.**

Question 3.3.4.

Considering the provided information and the data gathered by your team in task 1 - 2 and 1 - 3, which plank would you choose as suitable for premium stoppers production?

- **Answer under Question 3.3.4 in the answer sheet.**

Introduction

In this task you will explore another important characteristic of cork and its application in building construction.

The ability of a material to conduct heat is called the **thermal conductivity**. The air which fills the cork's cellular structure makes it an excellent thermal insulator. As it provides a high level of thermal insulation, cork finds many uses, including in the building and aerospace industries. As an excellent thermal insulator, cork has a very low thermal conductivity.

Thermal conductivity

Heat is the energy transferred due to temperature difference (the energy is transferred from the higher to the lower temperatures). Heat transfer can occur via different mechanisms, namely conduction, convection and radiation. Conduction is a mechanism of heat transfer (or heat flow) within a body or between bodies in contact, that results from the transfer of kinetic energy through collisions taking place at a microscopic level. When a very small[1] quantity of heat, or thermal energy, dQ is transferred in a very small[2] time interval dt, the heat flow, also called **heat current,** is defined as: $H = \mathrm{d}Q/\mathrm{d}t$. The heat current through an body (see Figure 1 - 4.1) of length l (along the direction of the heat flow) and uniform cross-sectional area A, when the temperature difference between its two ends is T_1 - T_2 (T_1 and T_2 are the higher and lower temperatures, respectively), is governed by the law:

$$H = \frac{\mathrm{d}Q}{\mathrm{d}t} = kA\frac{T_1 - T_2}{l}$$

where k is a positive constant, which is a function of the constituting materials of that body, called **thermal conductivity**. As for the ratio (T_1 - T_2) / l it is the temperature difference per unit of length.

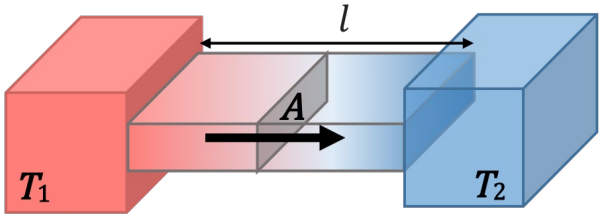


Figure 1 - 4.1 – Heat conduction through a bar of length l and cross sectional area A, placed between two bodies that are at temperatures T_1 and T_2, respectively. The temperature difference between the two sides of the bar causes a heat flow from the higher temperature side to the lower temperature side, as represented by the arrow.

The goal of this task is to determine the thermal conductivity of a thin sample of cork, by using the Lee's disc method, described under 1 - 4.1.

Warnings:

Do not touch the hot steam generator. Never direct the steam stream towards you or your colleagues. Use the forceps whenever you have to move the hot steam chamber. Use the pot holder whenever you have to touch materials at high temperatures (steam generator; Lee's disc and rubber tubes).

[1] dx denotes a very small amount of the quantity x.

[2] Note that, in general, the heat flow varies with time. Thus, to calculate an instantaneous measure of heat current, $H(t)$, a very small time interval around t should be used.

Materials and equipment (Figure 1 - 4.2)

- Steam generator (A) with a control slider (cs)
- Steam chamber (B)
- Brass disc (Lee's disc) (C)
- Acrylic stand for Lee's disc and steam chamber (D)
- A thin cork sample (E)
- Forceps (F)
- 2 calibrated temperature probes (thermistors) (G), to be connected to a TI-Nspire CX calculator, through a data logging Lab Cradle interface
- Texas Instruments TI-Nspire CX calculator with a data logging Lab Cradle interface (H)
- Thermal paste (to improve the thermal contact with the temperature probes) (labelled "**Heat Sink Compound Plus**") (I)
- A thick cork insulator block (J)
- A large cork insulating base (K)
- Rubber tubes (L)
- Pot holder (M)

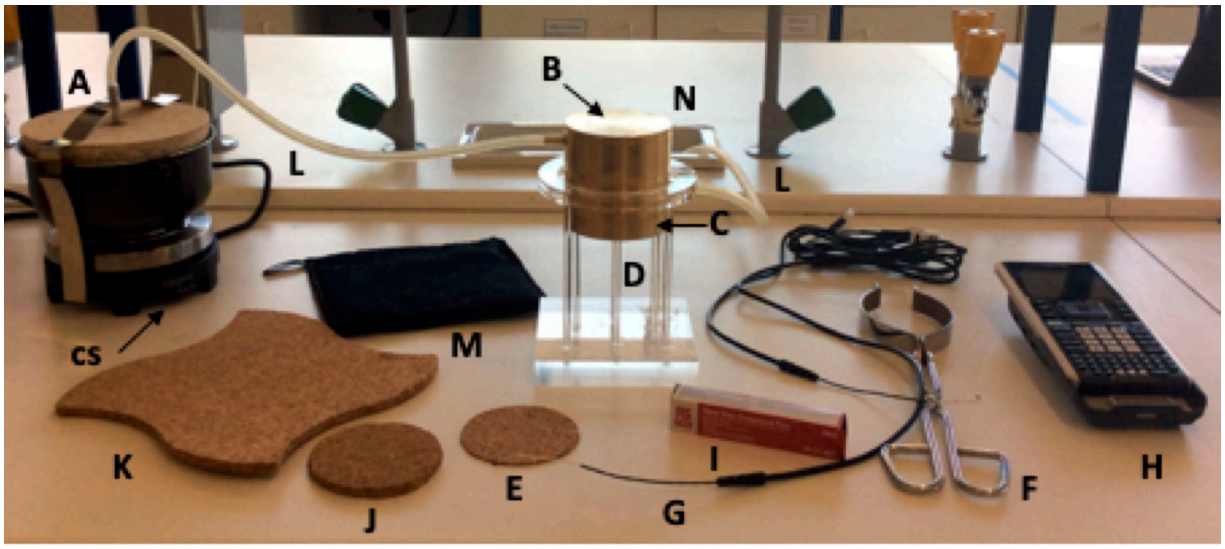


Figure 1 - 4.2 – General experimental materials and equipment.

The method comprises two parts using two different experimental setups.

1 - 4.1. Part 1 of Lee's Disc Method

Figure 1 - 4.3 illustrates the first of two experimental setups that you will use to measure the thermal conductivity of a cork sample, using the Lee's disk method. The cork sample (**CK**) is disc-shaped. You will place it between the cylindrical steam chamber (**SC**), and a brass disc – the Lee's disc (**LD)** – which are mounted on an acrylic stand base.

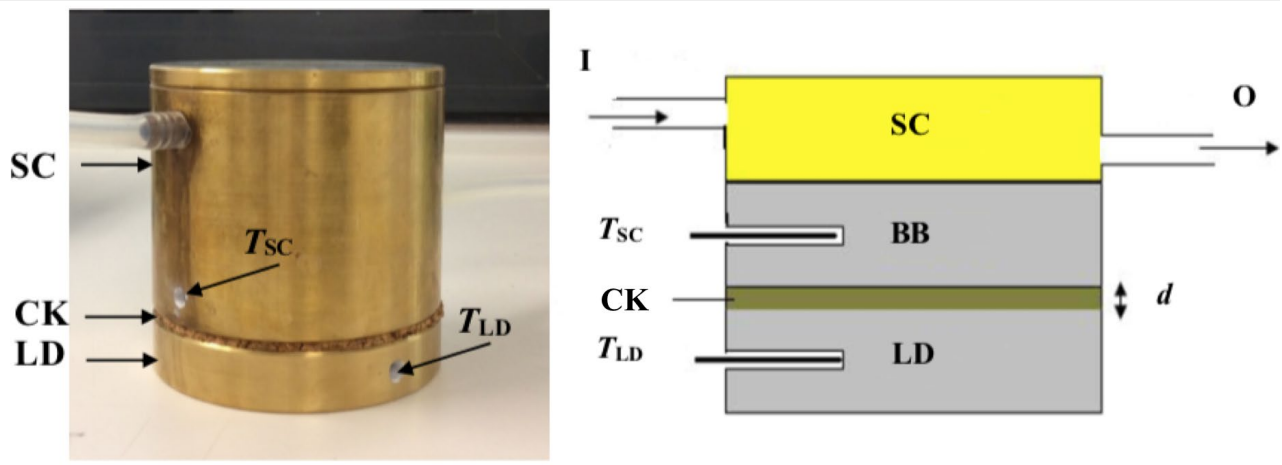


Figure 1 - 4.3 – Experimental setup for the first part of the Lee's disc method for determining the thermal conductivity of a cork sample. On the left is shown the apparatus itself, whereas in the right is illustrated, schematically, how data is collected. SC – Steam Chamber; CK – Cork Sample; LD – Lee's Disc; BB - Brass Base; T_{SC} – Temperature of the base of the steam chamber; T_{LD} – Temperature of the base of the Lee's Disc; I - Steam in; O - Steam out; d - sample thickness.

When the steam generator, connected to the steam chamber, is turned on, the chamber heats up. The temperature, T_{SC}, at the base of the steam chamber, just above the cork sample, can be measured by one of the thermistor (**T-SC**). A heat current, H_{in}, flows from the chamber to the Lee's disc through the cork sample, increasing the temperature of the Lee's disc. The second thermistor (**T-LD**), measures the temperature of the Lee's disc, T_{LD}, which is in contact with the bottom surface of the cork sample. When T_{LD} increases above room temperature, heat also flows (through conduction, convection and radiation) from the Lee's disc to the environment. Thus, a heat current, H_{out}, leaves the Lee's disc. As T_{LD} increases, H_{in} decreases and H_{out} increases. When $H_{in} = H_{out}$ a steady state is reached, and T_{SC} and T_{LD} remain constant at the values T_H and T_L, respectively.

Procedure

1. Consider the following data: the mass of the Lee's disc, m = 629 g; height of the Lee's disc, h = 1.5 cm; diameter of the Lee's disc, D = 8.0 cm and the thickness of the cork sample, d = 2.1 mm. Put this data in the answer sheet.

   ❖ **Enter the data (in SI units) in the Table under Question 4.1.1. in the answer sheet.**

2. Insert one of the temperature probes (**T-SC**) into the hole in the base of the steam chamber, and the other (**T-LD**) into the hole in the Lee's disc. To ensure a good thermal contact with the temperature probes, you should smear the tip of each probe with thermal paste, prior to inserting the thermistors in each hole.

3. Connect the two temperature probes to the Lab Cradle data logging interface – to see how to do this and how to operate with the TI-Nspire CX calculator software see Appendix 3. Start to monitor the temperatures T_{SC} and T_{LD} using the TI-Nspire CX calculator (**monitoring mode**).
4. The pan of the steam generator is already half filled with water and ready to operate. Do not remove the hose or the retaining clamp. Connect the flexible tube from the steam generator to the inlet of the steam chamber (located close to its top). Connect the steam outlet (located close to the base of the steam chamber) to the nearest lab sink. Connect the hot plate of the steam generator to the power supply and set the heating level to "3.5-4" using the control slider. If there is any problem with the steam generation, please call a lab assistant. You will not be penalised for this.
5. Put the steam chamber in contact with the Lee's disc. Turn on the steam generator and preheat the disc to the temperature, T, of 60ºC. This procedure is very fast. **Do not exceed T= 65ºC. Use the forceps to hold tight the chamber**, remove it from the acrylic stand and put it on top of the large cork insulating base (K in Figure 1 - 4.2). If the temperature exceeds 65ºC, wait until it cools back to that value, before proceeding to the next step.
6. Place the cork sample on the top of the Lee's disc. Put the steam chamber, **carefully and using the forceps**, on the top of the cork sample. The cork should appear between the two brass components of the setup (see Figure 1 - 4.3). Note that the complete ensemble is now back to the acrylic stand. Ensure that the temperature probes remain correctly placed.
7. Restart monitoring the temperatures T_{SC} and T_{LD} with the TI-Nspire CX calculator. When T_{SC} and T_{LD} remain unchanged, record the values T_H and T_L on the answer sheet. Notice that this procedure may take a long time.

Experiment 1 - 4.1 is finished and you should now turn the control slider to '0' (if you need to repeat this experiment call a lab assistant) and disconnect the temperature probe **T-SC** from the data logging Lab Cradle interface.

- **Enter your results in Table under Question 4.1.2. in the answer sheet.**

Question 4.1.3

In the answer sheet write the mathematical expression for the heat current, H_{in}, flowing into the Lee's disk at the steady state. The expression should be written as a function of k (the thermal conductivity of the cork sample) and of the appropriate symbols for other measured quantities.

- **Enter your results under Question 4.1.3. in the answer sheet.**

Question 4.1.4.

In the answer sheet, write the mathematical expression for a in $H_{in} = k\,a$. This expression for a should be written as a function of the appropriate symbols for measured quantities. From the measured values of those quantities, calculate an experimental value for a (give details of your calculations and express the value in appropriate units).

- **Enter your results under Question 4.1.4. in the answer sheet.**

1 - 4.2. Part 2 of Lee's Disc Method

The goal of this second part of the experiment is to measure how fast the Lee's disc cools down, i.e., its cooling rate, r, at the temperature when it reached the steady state. The cooling rate of the Lee's disc is a function of its temperature. When the disc is at a given temperature, T, its cooling rate is the ratio between a small temperature variation dT_{LD} around T, and the short time interval, dt, required for this variation to occur: $r(T) = dT_{LD} / dt$. At the steady state, the cooling heat current from the disc to the environment, H_{out}, can be related to the cooling rate by the expression:

$$H_{out} = mc\frac{dT_{LD}}{dt},$$

where m is the mass of the Lee's disc and c = 377 J / (kg K) is the specific heat capacity of the disk material. The specific heat capacity is the heat required to raise the temperature by one degree Celsius of 1 kg of the material.

In the following procedure you will rise the temperature of the Lee's disc about 5℃ to 10 ℃ above T_L and then let it cool down, while continuously measuring the temperature of the disc. The cooling down is due to heat flowing from the disc to the environment.

Procedure

1. Remove the cork sample from the previous setup, **using the provided pot holder and the forceps as protection**.
2. Set the power of the steam generator to "3.5-4" using the control slider. If there is any problem with the steam generation, please call a lab assistant. In this part of the experiment the TI-Nspire CX calculator should be used in acquisition mode. Select an appropriate sampling rate. Start collecting data from the temperature probe **T-LD** and wait until T_{LD} reaches a value about 5 ℃ to 10 ℃ above T_L.
3. Turn the control slider to "0" and disconnect it from the power supply. Using the pot holder as protection, withdraw the steam chamber from the top of the Lee's disc using the forceps and put it on top of the large cork insulating base (K in Figure 1 - 4.2). Put the insulation block J in Fig. 1- 4.2 on top of the disc and start recording the temperature T_{LD} over time.

Question 4.2.1.

Select adequate values from the collected data in order to extract the cooling heat current from the disc to the environment at the temperature of the steady state found in Question 4.1.2.

- **Enter your results in Table under Question 4.2.1. in the answer sheet.**

Question 4.2.2.

Plot the data of the Table (Question 4.2.1.) in the provided millimeter paper.

- **Plot the data in the provided millimeter paper.**

Question 4.2.3.

Use your plotted data to evaluate the cooling rate, r, at the temperature of the steady state found in Question 4.1.2. Present your calculations on the answer sheet, indicating which values you have

used.

- **Enter your calculations and results under Question 4.2.3. in the answer sheet.**

Question 4.2.4.

In the answer sheet, write the mathematical expression for the thermal conductivity of the cork sample, k, as a function of m, c, and any other quantities you got from your experimental data. Using that expression, calculate the thermal conductivity of the cork sample, k.

- **Enter your results under Question 4.2.4. in the answer sheet.**

1 - 4.3. Housing Thermal Insulation

Thermal resistance, R, of a slab is a measure of its thermal insulation against heat losses and can be defined as:

$$R = \frac{l}{k},$$

where k *is* thermal conductivity of the material and l is the slab's thickness. In buildings, walls are made of different material layers. When the inside and outside wall surfaces are at different temperatures, a heat current H will flow through all the wall layers.

Question 4.3.1.

Derive a mathematical expression for the total thermal resistance, R_{total}, of a wall with two layers of thickness l_1 and l_2, from materials with different thermal conductivities, k_1 and k_2, respectively, as a function of those quantities only.

- **Enter your calculations and results under Question 4.3.1. in the answer sheet.**

Question 4.3.2.

To prevent losses through thermal conduction, a house with walls made of 20 cm thick concrete and a 2 cm thick plaster drywall, an insulating layer of 1 cm cork board was added. Consider that the concrete side is facing the exterior of the house at a temperature of 0 ºC and that inside the house the temperature is kept at 20 ºC. Calculate the energy wasted by heat conduction during one hour through a wall with an area of 50 m^2 for the two following cases:

i) a naked (concrete+plaster, uninsulated) wall;

ii) an insulated (concrete+plaster+cork) wall.

Consider the following thermal conductivities (given in the SI units W K^{-1} m^{-1}): concrete: 1.10; plaster: 0.17; cork: use the value found in Question 4.2.4.

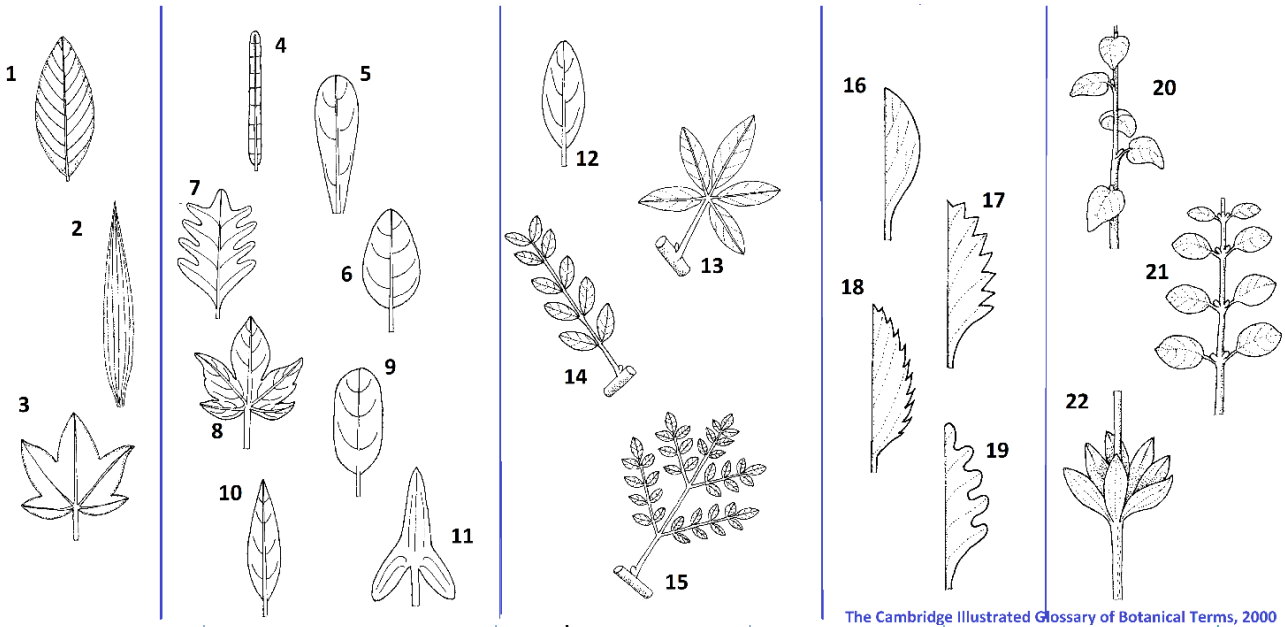
- **Enter your calculations and results under Question 4.3.2. in the answer sheet.**

APPENDIX 1

Morphological observation of the biological material, photos and figures

1.1. Leaf classification according to their morphology and arrangement.

A - Venation **B - Shapes** **C - Arrangement** **D- Margins** **E -Arrangement on the stem**



The Cambridge Illustrated Glossary of Botanical Terms, 2000

	7 – Pinnatipartite	15 – Bipinnately compound	19 - Lobed	
	8 – Palmately lobed			
	9 – Oblong			
	10 – Lanceolate			
	11 – Sagittate			

1.2. Trichome classification according to their morphology and arrangement

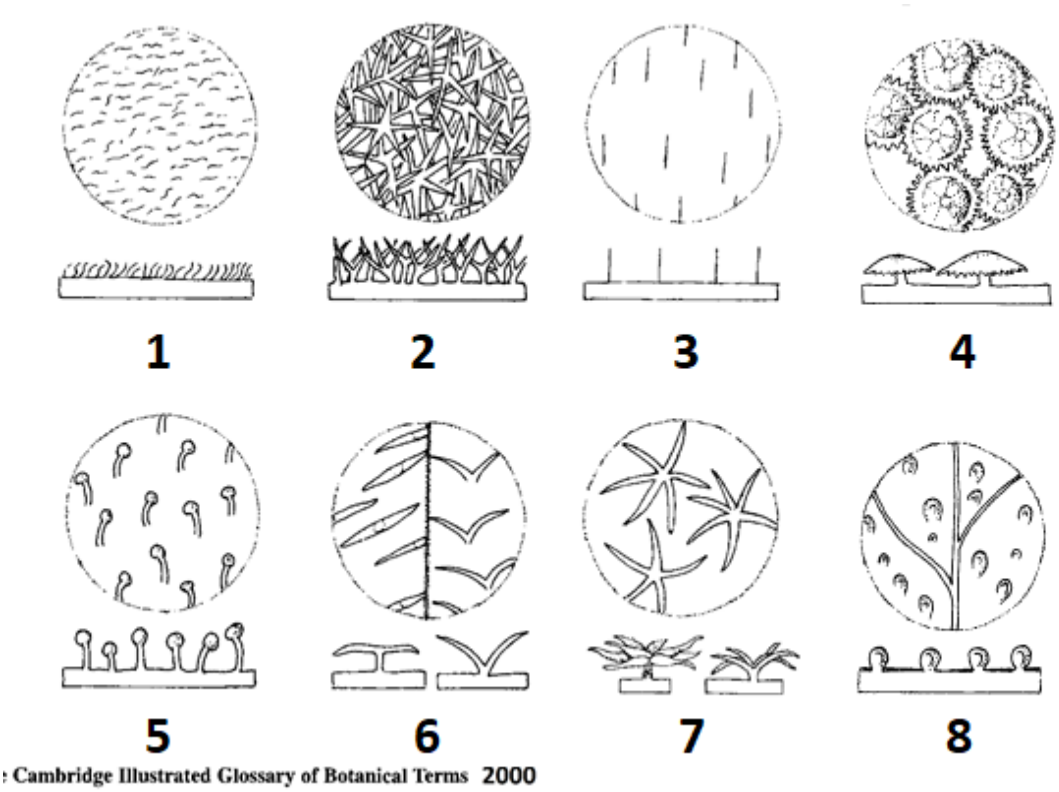
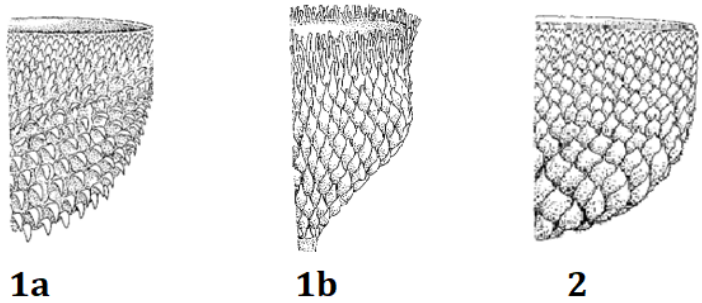


Figure Legend for Trichome classification

Arrangement	Shape
1 – Pubescent	5 – Glandular
2 – Pannose	6 – Bifid (2 types)
3 – Hirsutullous	7 – Stellate (2 types)
4 – Peltate	8 – Postulate

1.3. Cupule classification according to its morphology.

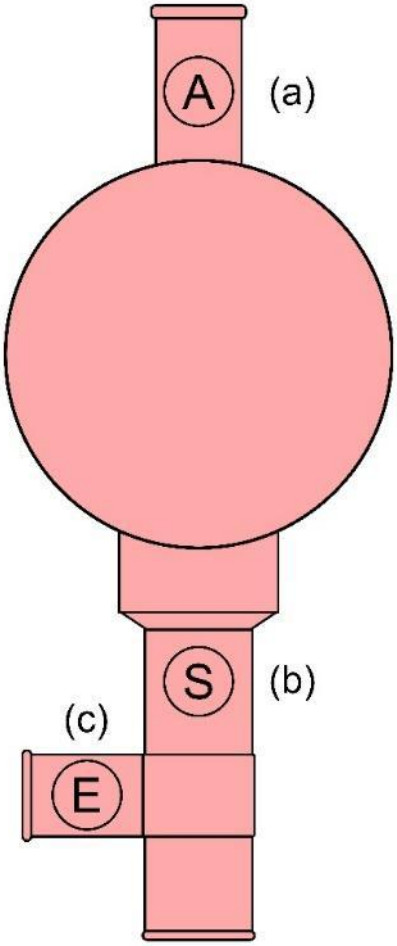


APPENDIX 2

PIPETTING

Pipette safety instructions

- Mouth pipetting is forbidden!
- Insert the top of the pipette in the bottom of the pipette filler carefully so as not to break the glass pipette.
- Do not allow the liquid to be drawn into the bulb.



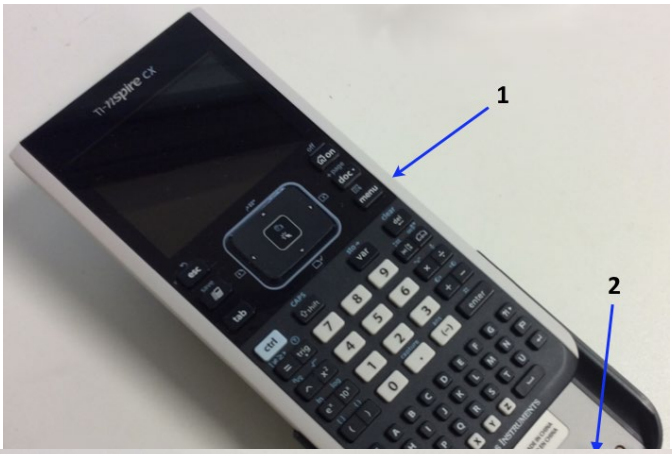
Pipette filler bulb: (a) Air valve (expels air from the bulb), (b) Suction valve (draws solution into the pipette), (c) Empty valve (drains solution from the pipette).

APPENDIX 3

Ti-Nspire

3.1. Collecting data with the data logging Lab Cradle interface connected to a calculator with the TI-Nspire CX Software.

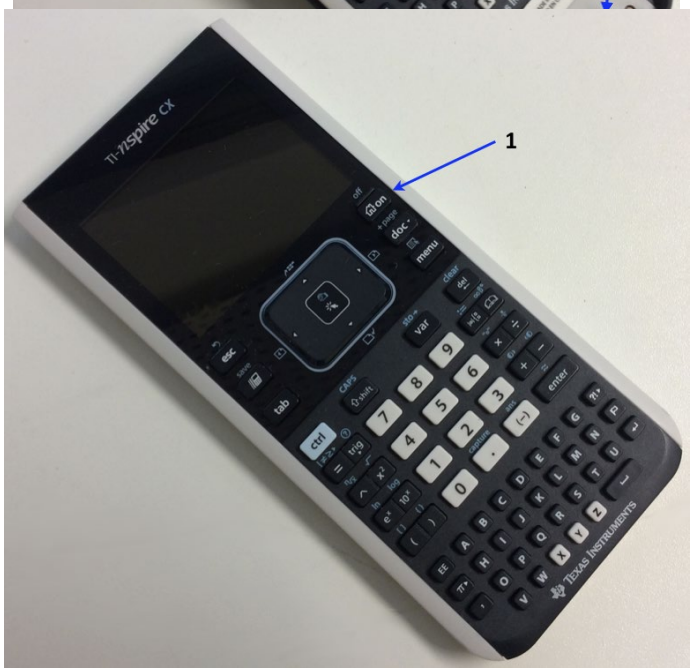
1. Connect the calculator to the interface



1 – Calculator

2 – Interface

2. Turn on the calculator.

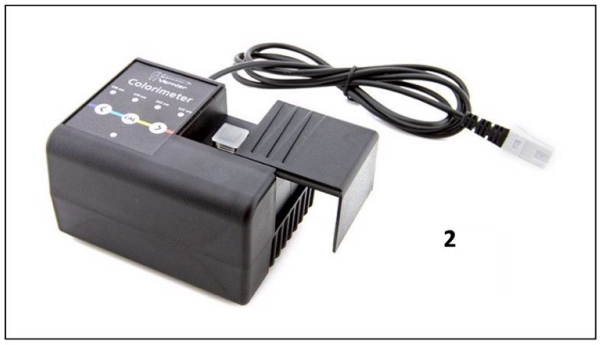


1 – Switch On/Off

3.2. Instructions for Vernier Colorimeter

The Vernier Colorimeter is designed to determine the concentration of a solution by analysing its colour intensity. The Colorimeter measures the amount of light transmitted through a sample at a user-selectable wavelength.

There are two models: model **1** and model **2**.

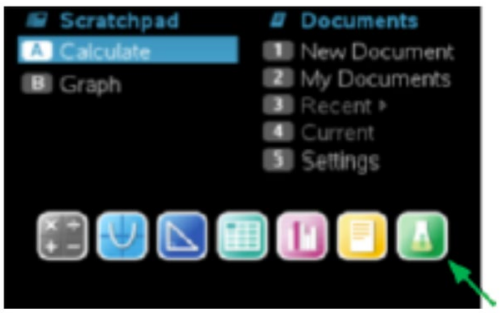


Using the Colorimeter

The Colorimeter is easy to use and maintain. Simply connect it to your data collection interface (TI graphing calculator), configure your software (Vernier LabPro®), and you are ready to make measurements. For best results, let the system stabilize at the desired wavelength for 5 minutes prior to calibration or data collection.

General procedure to follow when using the Colorimeter

1. Connect the Colorimeter to the interface in ch1 or ch2 or ch3.
2. Turn on the TI Nspire
3. Use the cursor with the Touchpad and press the icon 



4. The software will identify the colorimeter and load a default data collection setup.

5. Press the " < " or " > " button on the Colorimeter to select the correct wavelength setting for your experiment (430 nm, 470 nm, 565 nm, or 635 nm).
6. Calibrate the Colorimeter. **Note**: The Colorimeter needs to be powered about 5 minutes before calibrating. One of the four green wavelength indicator lights will be turned on when it is powered.

   a. Open the Colorimeter lid.
   b. Insert a cuvette, for your blank cuvette (100% transmittance or 0 absorbance). **Important:** Line up one of the *clear* sides of the cuvette with the arrow at the *top* of the cuvette slot. Close the Colorimeter lid.
   c. Next, press the CAL button to begin the calibration process. Release the CAL button when the red LED begins to flash. The absorbance should now be 0.00 or 0.01.

d. When the LED stops flashing, the calibration is complete and your unit is ready to collect data.

7. Collecting data.

a. Place the cuvette with a sample into the Colorimeter cuvette slot. **Important:** Line up the side of the cuvette with an arrow with the arrow at the *top* of the cuvette slot.

b. Read the absorbance value

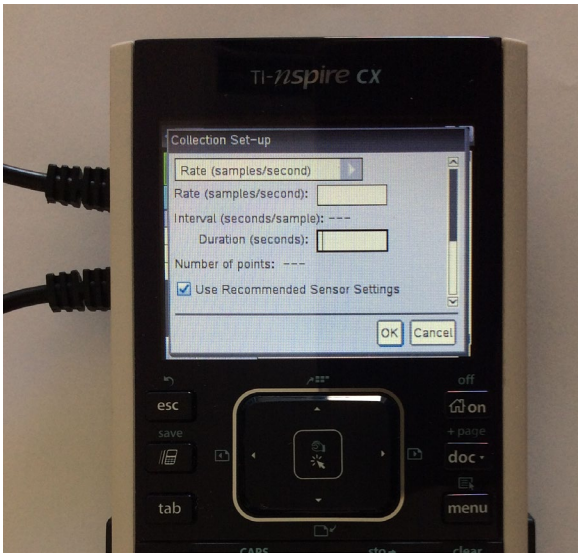
3.3. Instructions for the temperature sensors

1. Connect the sensor / sensors to interface. Use the first input channels.

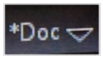
2. The program automatically detects the sensor/sensors. It starts up, by default, in **monitoring mode**, as shown in the following image:

3. To move from the monitoring mode to the **acquisition mode** you have to click on button  using the central touchpad. Data will start to be saved.

4. To adjust the sampling rate and define the acquisition duration, click on  and make the appropriated choices.



5. The collected data can be visualized in a meter by selecting  , in a plot by selecting  or in a table  .

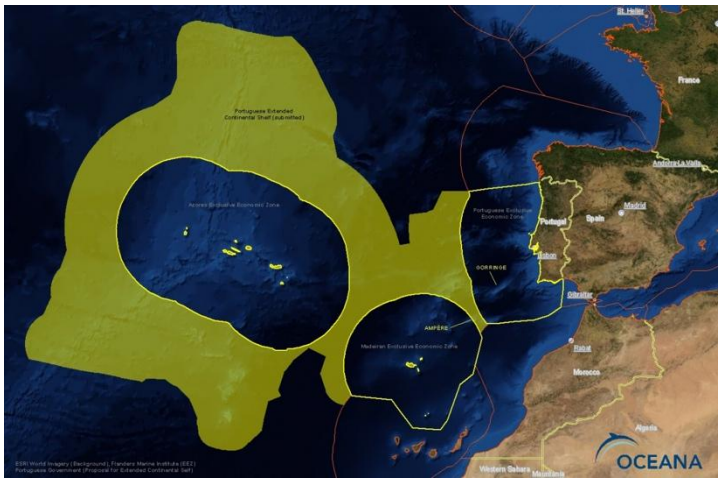
6. If you want to save your data use the popup  above the displaying data window, choose File and Save or Save as.

7. You can read data points in a plot by clicking on the graph to position the cursor at the desired point.

TASK 2 MARINE RESOURCES

Introduction

Currently Portugal has an Exclusive Economic Zone (a sea zone) of 1.7 million km^2, the third largest in the European Union and the 11th in the world. In August 2017 Portugal began to defend the proposed extension of the continental shelf beyond the 200 nautical miles, which can double the size of Portugal maritime territory, from the current almost two million to almost four million km^2. Given that Continental Portugal has a little more than 92 thousand km^2 of area, the extension of the maritime territory in another 350 miles will mean that the sea area will be 40 times greater than the terrestrial one.



Portugal Exclusive Economic Zone. Continental Portugal 327,667 km^2; Azores and Madeira Islands (953,633 km^2; 446,108 km^2). Total: 1,727,408 km^2 (picture from Oceana, https://eu.oceana.org)

Why is the maritime territory so important? One answer lies in the richness of marine resources that may be available, with a great impact in areas such as energy, biodiversity and biotechnology. That was exactly what Vasco and Isabel discovered in their Summer research internship in a Marine Research Center in the Lisbon area. During their internship, they went on a expedition to observe the installation of an open sea wave generator prototype. Vasco and Isabel became curious about the process of generating energy from waves. They also realized the presence of a small ecosystem in the vicinity of the wave generator and collected several biological samples to analyze different aspects of their biodiversity and possible applications.

You will show Vasco and Isabel how we can get energy from waves by exploring a point absorber wave-generator model. You will also show them how we can explore the marine biodiversity with a series of experiments using the biological samples that they collected and still discover a potential biotechnological application.

This Task consists of 3 individual subtasks which yield the following:

Task 2 - 1 – Wave-generator	120 Marks
Task 2 - 2 – Marine Ecosystems: Biodiversity and Resources	120 Marks
Task 2 - 3 – Biotechnological Potential of Green and Red Algae.	120 Marks

TASK 2 - 1.: WAVE-GENERATOR

Introduction

The Marine Centre researchers gave Vasco and Isabel an overview about wave energy.

"Winds, blowing over the ocean surface, generate waves. Waves propagate water oscillations to vast ocean areas. As a consequence, floating objects at the ocean oscillate up and down and forward and backward with water. Waves carry a huge energy. A variety of devices aimed at extracting energy from waves are under development. The so called point absorber wave-generator is designed to harness energy from vertical oscillations of the water surface in the open ocean. This energy is used to drive electromechanical energy converters generating electrical power."

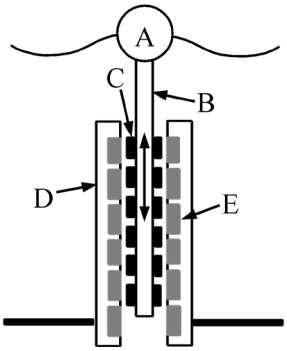


Figure 2 - 1 – Scheme for a vertical cross-section of a point absorber wave-generator: heaving buoy (A), translator (B), magnet (C), stator (D), coil (E).

Vasco and Isabel would like to better understand how a point absorber wave-generator works! So, you are going to help them by performing some experiments. To prepare those experiments, you need first to learn the basic physics underlying the process.

Changes in the magnetic environment of a closed conducting loop can cause a voltage induced in the loop and an induced current to flow in the loop – electrical power is generated. This physical phenomenon is called electromagnetic induction.

The magnetic field of a cylindrical bar magnet of neodymium type is very strong close to the magnet poles (cylinder bases) and decreases rapidly with the distance. If we move such a magnet up and down, in the direction of the cylinder axis, through the surface of a conducting loop, the changes in the magnetic environment of the loop will generate some electrical power. A higher power may be obtained if the magnet moves quickly through a short coil. With an assembly of coils and magnets even more power can be generated! In a point absorber wave-generator there are two main structures (see Figure 2 - 1): the tubular stator and the translator. The stator contains a fixed array of coils, while a set of permanent magnets move up and down with the translator, through the stator centre, as a heavy buoy coupled to the translator top oscillates with the waving of the water surface.

The point absorber wave-generator model that you are going to assemble will have only **one static coil** and **two joined bar magnets** driven by the **mechanical oscillations of a vertical mass-spring system** (instead of the vertical water surface oscillations). This model will allow you to show to Vasco and Isabel how a point absorber wave-generator operates and the impact of different oscillations on the power generated. However, the mechanical oscillations of the mass-spring system need first to be analysed. This is the purpose of the first experiment.

2 - 1.1. Characterizing the mass-spring system

The mass-spring system is composed by an elastic spring suspended vertically from its top end and by a given mass (M) attached to the spring bottom end (see Figure 2 - 2). The mass is at rest when the system is in equilibrium. By stretching the spring vertically, energy is transferred to the system. The amount of transferred energy is

$$E_{\text{elast}} = \frac{1}{2} k A^2 ,$$

where k is the spring constant and A is the stretching imposed to the spring (and the deviation of the mass from its equilibrium position). When the system is released, the mass starts to oscillate up and down. The time interval for motion repetition, i.e. the motion period (T), can be evaluated by measuring the time interval between the mass departure from an extreme position and its next return to that same position. Since both the suspended mass and the spring oscillate together, T depends on both their masses. For a given spring of mass (m), the period of elastic oscillations is governed by the effective mass parameter,

$$M_{\text{ef}} = \frac{m}{3} + M .$$

Materials and equipment

- Standing structure mounted on a standing base;
- Pointer pair;
- Clamp with hook;
- Spring ($m = (14.0 \pm 0.1)\ g$);
- A plastic tube with a suspending thread containing a pair of bar magnets, labelled as "**M1**" (M1 = (117.5 ±0.5) g), and a brass mass with two suspending hooks, labelled as "**M2**" (M2 = (200.0±0.5) g);
- Square ruler;
- Stop watch.

Figure 2 – 2 – Equipment used in 2 - 1.1: standing structure (A) mounted on a standing base (B), pointer par spring (C), clamp with hook (D), spring (E), plastic tube with a suspending thread containing a pair of bar magnets (M1), brass mass (M2), stop watch (F) and square ruler (G).

Data from this experiment will be presented in Table 1.1 provided on the answer sheet. The data in the column labelled as "Set *i* " will characterize a given set of experimental conditions. The last 4 rows of this Table will not be filled during data acquisition. They will be filled when analysing the acquired data (Questions 1.1.2 and 1.1.4).

Question 1.1.1

The data acquisition for each set of experimental conditions (**defined below from 1.1.1a to 1.1.1d**) should proceed as follows:

- Attach the mass to the spring suspended vertically from the hook fixed at one of the vertical rods of the standing system.
- Use the pointer pair attached to the vertical rod and the square ruler. Mark the position of the mass bottom with the upper pointer and adjust the position of the other pointer to a distance *A* below. Record the value for the distance between the pointers (*A).*
- Stretch down the spring by *A,* pulling it vertically down. Then, release the mass-spring system (do not apply any push to the system) in order to let it describe free vertical oscillations (without lateral deviations). Start the stopwatch to measure the time interval for 10 periods (t_{10}). Record the value t_{10} in the Table 1.1 on the answer sheet, in the line labelled as (#1) – this is the first of 3 measurements of t_{10} measured under the same conditions.
- Repeat the previous procedure two more times to improve statistics. Record the results for these assays (#2 and #3) in Table 1.1 on the answer sheet.

Question 1.1.1a

Take M1 for the first set of experimental conditions ("Set 1"). Set an amplitude *A* for the oscillations of 3 cm.

❖ **Record the data for "Set 1" in Table 1.1 on the answer sheet.**

Question 1.1.1b

Keep the mass M1 in the mass-spring system. Set a new amplitude for the oscillations of 2 cm.

❖ **Record the data in the column for "Set 2" in Table 1.1 on the answer sheet.**

Question 1.1.1c

In your mass-spring system replace M1 with M2. Set an amplitude for the oscillations of 3 cm.

❖ **Record the data in the column for "Set 3" in Table 1.1 on the answer sheet.**

Question 1.1.1d

Suspend M1 from M2, set an amplitude of 3 cm for the oscillations of this set of masses.

❖ **Record the data in the column for "Set 4" in Table 1.1 on the answer sheet.**

Question 1.1.2

For each set of experimental conditions calculate: the t_{10} mean value of ($\underline{t_{10}}$), the oscillations period

(T) and the effective mass parameter (M_{ef}).

❖ **Fill the last 3 rows of Table 1.1 on the answer sheet with your results.**

Question 1.1.3

Which of the following relations (where k is a spring constant) best fits your results?

(1) $T = \frac{k}{2\pi}\frac{A}{M_{\text{ef}}}$

(2) $T = \frac{2\pi}{k} A\, M_{\text{ef}}$

(3) $T^2 = \frac{4\pi^2}{k} M_{\text{ef}}$

(4) $T^2 = \frac{4\pi^2}{k}\frac{M_{\text{ef}}}{A}$

(5) $T^2 = \frac{4\pi^2}{k} A\, M_{\text{ef}}$

(6) $T^2 = \frac{2\pi}{k}\frac{M_{\text{ef}}}{A}$

❖ **Circle the number of the correct answer in the answer sheet, box 1.1.3.**

Question 1.1.4

Extract from the different data sets the k values in N/m.

❖ **Fill the last row of Table 1.1 on the answer sheet with your results.**

Question 1.1.5

Calculate the mean value for the spring constant k.

❖ **Write your result in the answer sheet, box 1.1.5.**

2 - 1.2 Generating an AC voltage

In this experiment you will assemble your wave-generator model and analyse the influence of the amplitude and period of the oscillations on the output voltage ($V_{gen}(t)$). This voltage is an AC voltage, characterized by a period ($T_V$or time interval for repetition) and by a maximum absolute value or voltage amplitude (V_0).

Your spring-mass system is subject to some damping (the amplitude of the oscillatory motion decays in time) due to air friction. However, this damping can be neglected in the short time intervals in which you will analyse voltages.

Materials and equipment

- The setup used in experiment 2 - 1.1;
- A 6000 turns coil (throughout the work the 6000 winding turns of the coil will be used);
- Three finger clamp to fix the coil to the standing structure through an universal clamp;
- Two conducting cables with one banana connector and one alligator connector (banana-alligator conducting cables);
- One voltage sensor/probe to be connected to a data logging Lab Cradle interface;
- A data logging Lab Cradle interface to be connected, using a mini USB to USB cable, to a computer;

- Computer, one piece;
- Mechanical pencil, one piece.

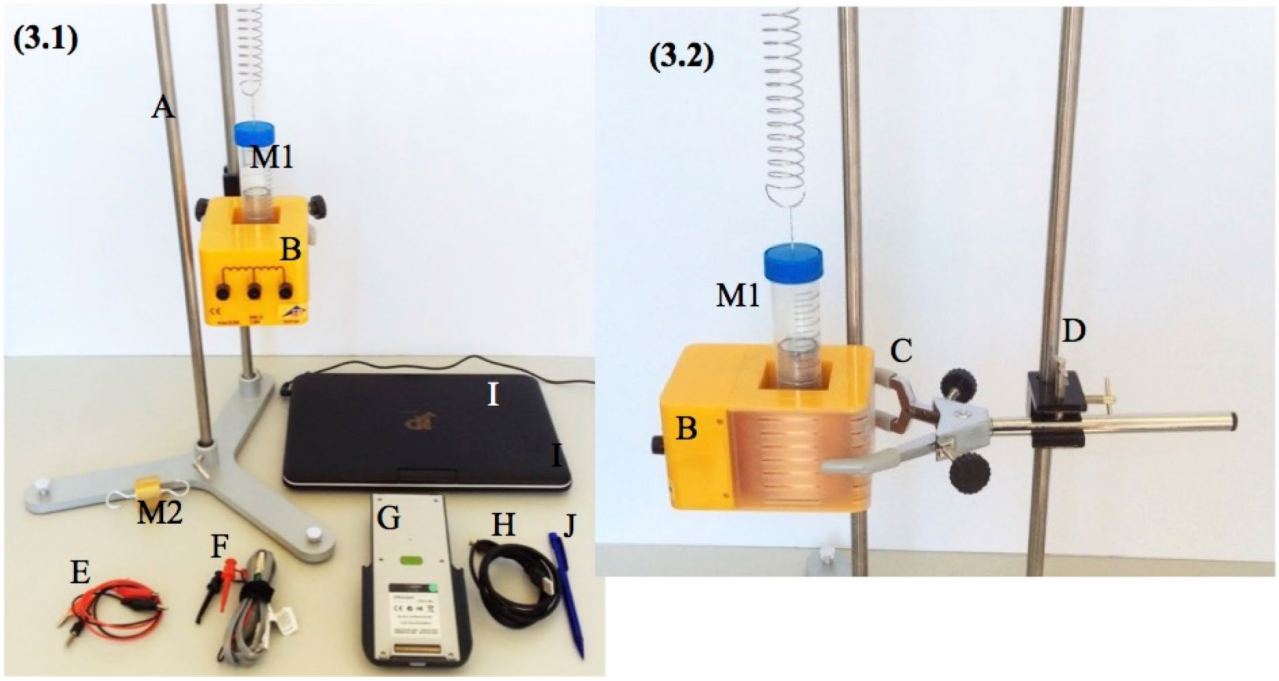
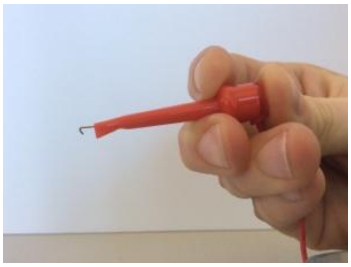


Figure 2 - 3 – (3.1) Materials used in experiment 2 - 1.2 and (3.2) coil placement detail: setup used in 2 - 1.1 (A), coil (B), three finger clamp (C), universal clamp (D), plastic tube with a pair of magnets (M1) in their rest position, bras mass (M2), conducting cables (E), voltage probe (F), Lab Cradle interface (G), mini USB to USB cable (H), Computer (I) and mechanical pencil (J).

Your point absorber wave-generator model will be driven by the oscillations of the mass-spring system. To assemble the model, you will start with the mass-spring set up used in the experiment 2 - 1.1. Append to this set up the 6000 turns coil by fixing it with a three finger clamp and a universal clamp to the vertical standing rod (see Figure 2 - 3.2). This coil should be fixed below the suspended spring. The spring axis and the coil central empty space axis should lie on the same vertical. From the spring bottom end you will suspend M2 and/or M1 (the plastic tube containing two bar magnets).

To test your wave-generator's capabilities you will first investigate its open circuit output voltage under different mechanical oscillation conditions. For that purpose, plug the voltage probe to the winding terminals of the 6000 turns of the coil (use the banana-alligator conducting cables). The access to the metallic voltage probe terminals proceeds as shown:



Connect the voltage probe to the **lab cradle** interface (**not connected** to the TI-nspire calculator**)** and connect this interface to the computer. Login in the computer, using the username and password written in the instruction sheet that is next to it, and launch the software TI-Nspire CX CAS.

Instructions for operating with the data acquisition system and software are given in Appendix 4. During the experimental activities keep the software in the Graph view. Proceed as follows: 1. pull down the spring; 2. start data acquisition; 3. wait for the reference horizontal line to appear on the screen; 4. release the spring starting the voltage generation. The horizontal line will provide you with the reference (0 V) for your voltage measurements. By clicking on the acquired plot and using the arrow keys, you can read the coordinates of the points on your graph. To change the axes limits, write a new value over the current one. In the menu File you can find the key to save your data for a later review.

Question 1.2.1

For each mechanical oscillation condition (**defined below from 1.2.1a to 1.2.1c**), characterized by a period (T) and an amplitude (A), proceed as follows:

- Adjust the position of the spring suspending hook (and of the coil fixing clamp, if needed) so that: when the system is at rest, **one of the magnets inside the tube is above the top of the coil and the other is inside the coil central empty space** (see Figure 2 - 3.2); the bar magnets can oscillate vertically along the coil axis (through the coil central empty space).
- Stretch down the spring by A. In order to set A, you can make use of the scale marked on the plastic tube and of the reference provided by the top end of the coil. Start a data acquisition for about 3 seconds (choose a sampling rate of 200 samples/second). When the reference line appears on the chart, release the spring in order to get vertical oscillations. You will observe on the computer screen the output voltage ($V_{gen}(t)$).

Question 1.2.1a

Set the first mechanical oscillation condition (C1) using the mass M1 in the mass-spring system and stretching down the spring by 3 cm. Analyse the output voltage of the wave-generator model in this C1 condition.

- **Sketch the graph of $V_{gen}(t)$, starting from the reference and for a time interval of about 1 s, in box 1.2.1a on the answer sheet (include scales in the axes).**
- **In your sketch indicate the voltage period (T_V) and voltage amplitude (V_0).**
- **Record the values of M, A, T_V and V_0 for C1 in Table 1.2 on the answer sheet.**

Question 1.2.1b

Set the mechanical oscillation condition (C2) by keeping M1 in the mass-spring system and stretching down the spring by 2 cm. Analyse the output voltage of the wave-generator model in this C2 condition.

- **Record the values of M, A, T_V and V_0 for C2 in Table 1.2 on the answer sheet.**

Question 1.2.1c

Set the mechanical oscillation condition (C3) by suspending M1 from M2, in the mass-spring system and stretching down the spring by 2 cm. Analyse the output voltage of the wave-generator model in this C3 condition.

- **Record the values of M, A, T_V and V_0 for C3 in Table 1.2 on the answer sheet.**

Question 1.2.2

- **Indicate in Table 1.2 the values of the oscillation period (T), for each of the three mechanical oscillation conditions (C1, C2 and C3).**

Question 1.2.3

(1) The period of the wave-generator model output voltage is given by ... [$T_V = aT$ or $T_V = T$ or $T_V = 1/T$ or $T_V = T/a$, where a is a number greater than 1].

(2) The amplitude of the wave-generator model output voltage (V_0) increases with ... [T or T/A or A/T or A].

- **Complete the two previous sentences with the option enclosed in square brackets that better fits your results under the Question 1.2.3..**

2 - 1.3. Storing electrical energy

In order to use the power produced by the wave generator (harvested from the mechanical oscillations) an energy take-off system is required. In this task you will construct a simple take-off system for your point absorber wave-generator model. This system integrates a bridge rectifier circuit and a capacitor.

Materials and equipment

- The equipment used in task 2 - 1.2;
- One breadboard;
- Thin conducting wires (jumpers);
- One red LED;
- 4 germanium diodes;
- A 1000 Ω resistor;
- A 10000 μF electrolytic capacitor (that can be charged up to 16 V).

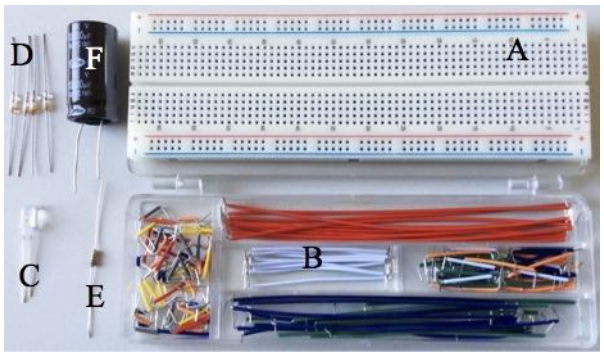


Figure 2 - 4 – Equipment for mounting the take off circuit: breadboard (A), jumpers (B), LED (C), diodes (D), resistor (E) and capacitor (F).

The breadboard (see Figure 2 - 5) is a base which provides you an easy way to assemble the take-off circuit. The connections between the circuit elements are established by inserting their pin terminals and connecting wires (jumpers) into appropriate holes of the breadboard. Some of

breadboard holes are electrically connected by metal strips on the bottom of the breadboard, as marked in the Figure 2 - 5 by the black lines – aside from horizontal rows of 5 pins, connected sets of 5 pins run vertically along the sides.

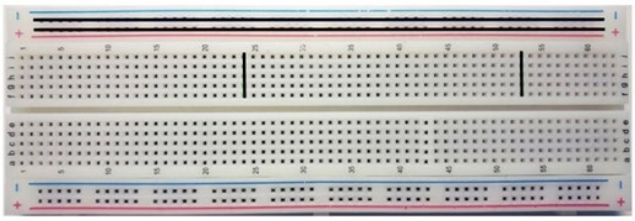
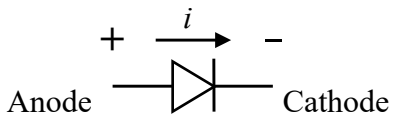


Figure 2 - 5 – Breadboard for the electric circuit assembly. The black lines show which holes are electrically connected. Please note that breadboard is symmetric between the top and the bottom part.

A diode is an electric polarized component with two terminals – an anode and a cathode (see Figure below). Current can only flow through the diode if the anode is positively polarized and the cathode is negatively polarized (direct polarization) and if the external voltage is above a given threshold (about 0.35 V for germanium diodes you will use). If the polarization is reversed the diode behaves as an "open switch". The electric symbol for a standard diode is displayed in the Figure below, where the direct polarization and current direction are also shown. Diode packages (glass for your germanium diodes) have a thick painted bar near one end, indicating the cathode.

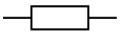
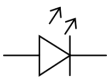


A capacitor is a circuit component that can be charged, storing electrical energy. When a capacitor is at the voltage V_C, it stores the energy

$$E_{\mathrm{C}} = \frac{1}{2} C V_{\mathrm{C}}^2 ,$$

where C is the capacitance, which represents the capacitor ability to store electrical charge.

Below, you find a Table with the schematic symbol for a polarized capacitor and for other circuit components you are going to use, as well as important information about these components.

Circuit component	Symbol	Description
Inductor		An inductor is a coiled conductive wire. Changes in the current across an inductor, cause variations in the coil magnetic environment which involve energy transfer.
Resistor		A resistor is a conductor with a given resistance. For a given applied voltage, the current through the resistor is directly proportional to its resistance.
Polarized capacitor		To charge a polarized capacitor, it should be placed correctly, with the "+" terminal, which is marked on the device, at the higher potential.
Light emitting diode		This is a type of diode that emits light when a current flows through it. The longer LED terminal is the anode and it should be polarized positively for the LED to emit light.

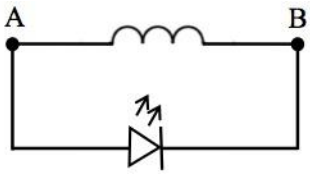
The Bridge Rectifier

Your point absorber wave-generator model may supply an alternating current (AC) which periodically reverses direction. A direct current (DC), which flows only in one direction, must be used to store electrical energy in a capacitor (otherwise, the capacitor will charge and discharge periodically). A bridge rectifier is a circuit that converts AC current into DC current using diodes.

When measuring the voltage between two points, connect the black terminal of your voltage probe to the lower potential point and the red one to the higher potential point.

Question 1.3.1

Connect a LED to the 6000 turns coil (use the banana-alligator conducting cables) as shown below.



Connect one of the voltage probes at point A and the other at point B - Use M1 and impose $A = 3$ cm (condition C1 also used in 1.2.2a) to the mass-spring system. Start the data acquisition for 10 s.

- **Sketch the graph of $V_{gen}(t)$, starting from the reference and for a time interval of about 3 s, in box 1.3.1 on the answer sheet (include scales in the axes).**
- **In your sketch indicate the time intervals corresponding to the two first LED flashes.**

Question 1.3.2

Compare the sketches in box 1.2.1a and box 1.3.1. Classify as true (T) or false (F) each of the following statements. Wrong answers will be penalised (-0.5 marks).

(1) When the wave-generator model is connected to the LED, the period of $V_{gen}(t)$ decreases.

(2) When the wave-generator model is connected to the LED, the mass-spring system is continuously suffering damping caused by the magnetic environment created by the coil.

(3) When current flows through the LED some mechanical energy from the mass-spring system is transferred to the coil electromagnetic field.

(4) Unlike the mass-spring system, the sea waves are a continuous source of mechanical energy.

- **Circle the right sentences in the the answer sheet, box 1.3.2**

Question 1.3.3

From your data, is it possible to find which is the threshold voltage for the LED conduction? If yes, indicate that threshold.

- **Provide your answer in the the answer sheet, box 1.3.3.**

Question 1.3.4

Disconnect the LED from the wave-generator model. Assemble the bridge rectifier circuit (be careful to do not break the diodes glass package near the diode terminals) according to the following circuit diagram (use the breadboard) and connect it to the 6000 turns coil (also shown in the diagram). If you need you can get one (and only one) extra diode but you will lose points (see below).

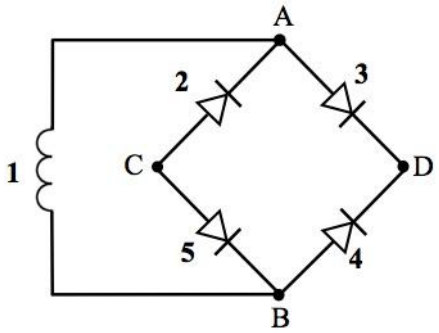


Figure 2 - 6 – Circuit diagram for the bridge rectifier circuit. The circuit is composed by the wave generator coil (1) and four diodes (2, 3, 4 and 5).

Question 1.3.4a

In addition to the provided 4 diodes you may use 1 extra diode (to replace a broken diode) under request to the physics lab assistant. No more than one extra diode can be used and if you use it you will lose 2 points if you ask to use this spare diode.

- **If an extra-diode is used, you should state it in the answer sheet, box 1.3.4a. Both you and the lab assistant will sign that statement. You will lose 2 points.**

Question 1.3.4b

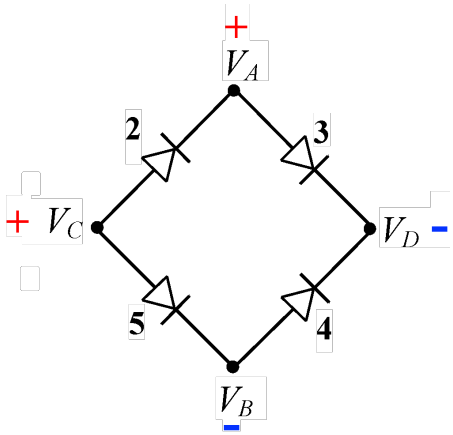
Connect the voltage probe to analyse for 2 seconds the voltage between points A and B ($V_{gen}(t)$, starting from the reference) generated when using M1 in the mass-spring system and by imposing $A = 3$ cm to the spring. Repeat the procedure but this time connecting the voltage probe between points C and D ($V_{bridge}(t)$).

- **Sketch the graphs of $V_{gen}(t)$ and $V_{bridge}(t)$ starting from the reference and for a time interval of 2 s (include scales in the axes).**

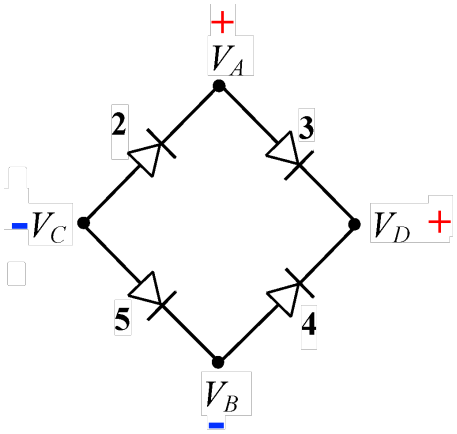
Question 1.3.5

In the following diagrams signs (+) and (-) indicate voltage polarizations. Which diagram/diagrams correctly relate the bridge DC output voltage (between points C (V_C) and D (V_D)) with the bridge AC input voltage (between points A (V_A) and B (V_B)?

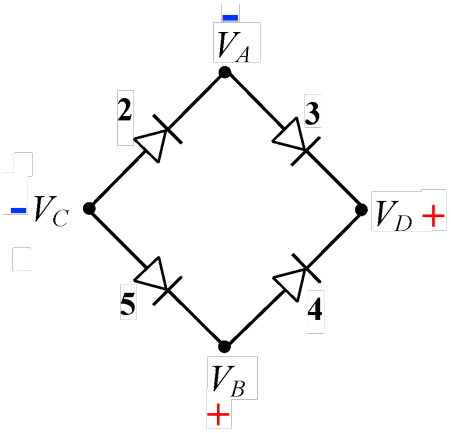
(1)



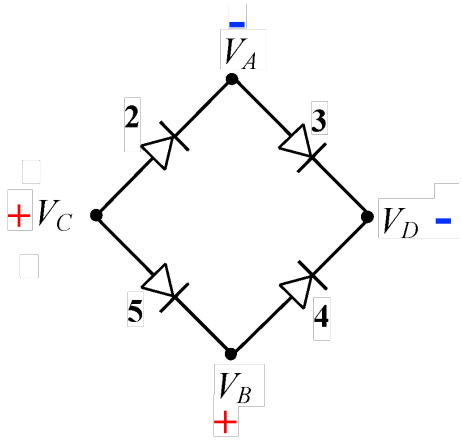
(2)



(3)



(4)



❖ **Circle the number/ numbers of the correct diagram/diagrams in the the answer sheet, box 1.3.5.**

Capacitor charging

You will use an electrolytic capacitor as the electrical energy storage device. This device has the capacity to store charge when a voltage is applied to its terminals. In the following experiments you are going to perform with the wave-generator model, only a small amount of charge is stored. Therefore, it is safe to discharge the capacitor by connecting its terminals with a conducting wire. Use this procedure whenever you need to have your capacitor discharged.

Question 1.3.6

Connect the RC series – the electrolytic capacitor in series with the resistor (for limiting current) – to the bridge rectifier as shown in Figure 2 - 7.

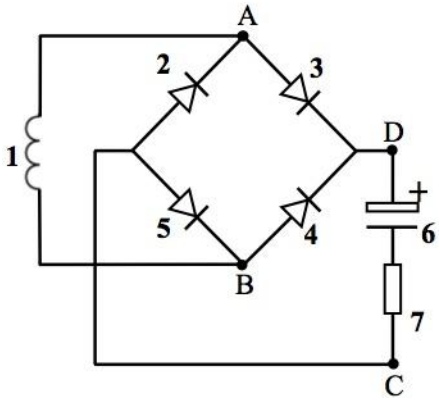


Figure 2 - 7 – Circuit diagram for connecting the series of the electrolytic capacitor (6) and the resistor (7) to the bridge rectifier.

Question 1.3.6a

Discharge the capacitor. Connect the voltage probe in order to analyse for 10 s the voltage between points A and B ($V_{gen}(t)$). Use M1 and impose $A = 3$ cm. Save your data.

- **Sketch the graphs of $V_{gen}(t)$ starting from the reference and for a time interval of 3 s, in box 1.3.6a on the answer sheet (include scales in the axes).**

Question 1.3.6b

Discharge your capacitor. Connect the voltage probe in order to analyse for 10 s the capacitor voltage ($V_C(t)$). Use M1 and impose $A = 3$ cm.

- **Sketch the graphs $V_C(t)$ starting from the reference for a time interval of 10 s, in box 1.3.6b on the answer sheet (include scales in the axes).**

What is the value (V_{CT}) reached by $V_C(t)$ after the first oscillation period?

- **Indicate the value of V_{CT} in the plot and write the numerical value below the plot in the answer sheet, box 1.3.6.**

Question 1.3.7

Note that the maxima of $V_{gen}(t)$ have decreasing values in time, i.e. that voltage amplitude decays over time. The V_0 decay is approximately exponential:

$$V_0(t) = V_1 \exp(-\gamma t) + V_2 \;\Leftrightarrow\; \ln(V_0 - V_2) = -\gamma t + \ln(V_1),$$

where V_1, V_2 and γ are constants. γ is the damping ratio and it characterizes the rate of energy loss.

From your experimental data extract the value of V_2 and of a set of (V_0, t) pairs.

- **Record the values in Table 1.3 on the answer sheet.**

Question 1.3.8

Use the TI-Nspire software (see Appendix 4) to create two "manual columns" with the values recorded in 1.3.7. Create a "calculated column" with the values of $ln\ (V_0 - V_2)$ and fulfil the last

column of table 1.3.7. Draw the plot of $ln\,(V_0 - V_2)$ as a function of time.

- **Fill Table 1.3 with the values calculate for $ln\,(V_0 - V_2)$.**

Question 1.3.9

With the TI-Nspire software (see Appendix 4), adjust your plotted data to a linear fit line.

- **Write the linear fit parameters and the quality fit parameter r^2, given by the software, in the answer sheet, box 1.3.9.**
- **Present your value for γ in the answer sheet, box 1.3.9.**

Question 1.3.10

As V_0 drops, A (the amplitude of the mechanical oscillation of the mass-spring system) also drops and the mass-spring system loses energy. Both are approximately characterized by the same damping constant (γ):

$$A(t) = A_0 \exp(-\gamma t),$$

where A_0 is the initial amplitude.

The efficiency of your wave-generator model working in the above condition for the storage of energy (η) is the ratio between the energy stored in the capacitor and the energy lost by the mass-spring system.

Calculate the energy stored in the capacitor (E_C) and the energy lost by the mass-spring system (ΔE_{elast}) during the first oscillation period. Determine the corresponding energy conversion efficiency (η).

- **Provide your answer in the answer sheet, box 1.3.10.**

Question 1.3.11

In the open sea, waves continuously transfer energy to the translator. Do you see any simple way to do the same with your mass spring system? Implement your idea to increase $V_C(t)$ up to 3 V. Then connect the RC series to the LED to make it light up. Does the LED light up for a while? If it does, call the physics lab assistant to show him/her.

- **Provide your answer in the answer sheet, box 1.3.11. If the answer is yes, you and the lab assistant should sign.**

TASK 2 - 2.: MARINE ECOSYSTEMS: BIODIVERSITY AND RESOURCES

You are now asked to characterise the populations within the ecosystem found in the vicinity of the wave generator. You will use those biological samples that were collected in open sea by Isabel and

Vasco.

This task includes an agarose gel electrophoresis that takes at least 1 h. To finish your work on time, we strongly advise starting with the section 2 - 2.1.: "Bivalve taxonomic identification".

2 - 2.1. Bivalve taxonomic identification

Introduction

Isabel and Vasco were particularly curious about the mussels attached to the wave generator's structure. They tried to classify them through images using a book guide and found that the mussels should belong either to the species *Mytilus galloprovincialis* (*M. galloprovincialis*) or *Mytilus trossulus* (*M. trossulus*), which can easily interbreed and produce hybrids. It is very difficult to distinguish *M. galloprovincialis* from *M. trossulus* using just morphologic characteristics (Figure 2 - 2.1), but they can easily be discriminated based on the analysis of their DNA. So, Isabel and Vasco are asking for your help to distinguish the samples. They discovered that there is a significant difference between *M. trossulus* and *M. galloprovincialis* in a gene that encodes an adhesion protein. This protein is used for making the byssal threads, which mussels use to attach themselves to a substrate: in *M. galloprovincialis*, there is a deletion in DNA that codes for the adhesion protein gene.

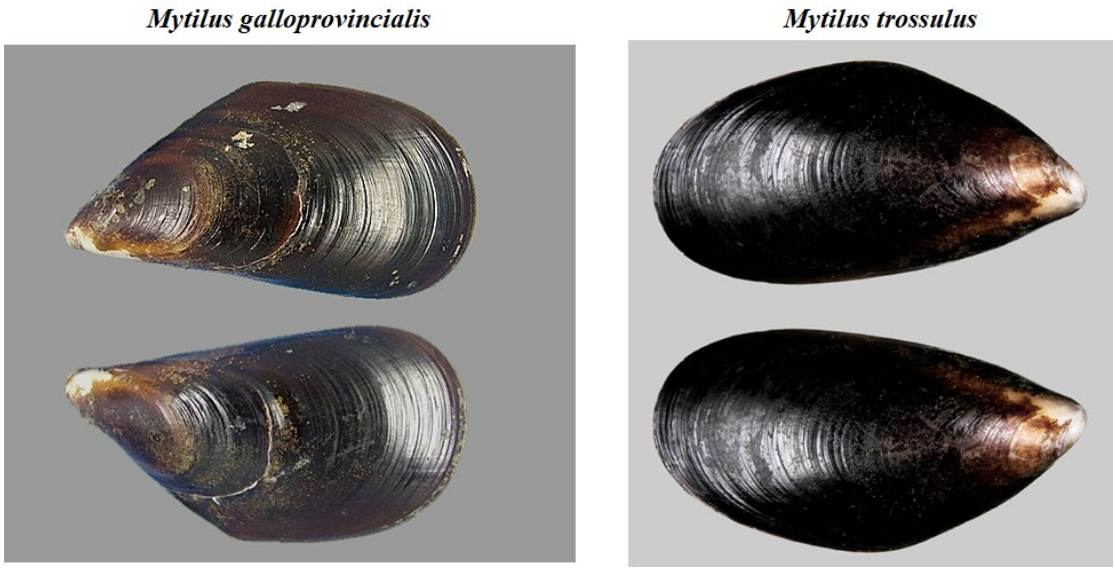


Figure 2 - 2.1 – Photograph of *Mytilus galloprovincialis* and *Mytilus trossulus*.

If necessary, use the Glossary at Appendix 5 (words in the Glossary are indicated by an asterisk " * ").

Due to the deletion in this gene in one of the species, it is possible to discriminate both species by PCR*, using specific primers*: *M. trossulus* yields a fragment that is longer (more base pairs (bp)), whereas *M. galloprovincialis* yields a shorter fragment (less bp). This difference is clearly distinguishable by agarose gel electrophoresis.

Isabel and Vasco have previously performed the PCR* reaction using DNA from 3 different mussels (A, B and C) collected at the generator and a mixture of two pairs of primers* (2 forward and 2

reverse). One pair of primers* is specific to amplify a fragment of the gene that codes for the adhesive protein - amplification may give rise to products of different lengths, depending on the mussel species. The other pair of primers* amplifies another DNA sequence that is conserved in both species, giving rise to the shorter single product that is used as a positive control of the reaction.

You have these PCR* products in tubes identified by A, B and C, kept in ice. To visualize and sort your PCR* products you need to do an agarose gel electrophoresis with a DNA stain (visible under UV light).

In addition to your PCR* products, you will also be running a DNA molecular weight ladder* as a size marker.

You are asked to analyze the PCR* products resulting from amplification of the DNAs from mussels that were attached to the generator, helping Isabel and Vasco to identify the mussels they collected.

Materials and equipment

- Ice box with 5 tubes: PCR products from DNA A, B or C (labelled as "**A**", "**B**" and "**C**"), DNA ladder (labelled as "**L**") and Orange G loading buffer (labelled as "**LB**")
- 10 empty 1.5 mL tubes, in the tube holder
- Micropipettes: 1000 µL, 200 µL and 20 µL, 1 piece each
- 20-200 µL Micropipette tips, 1 box
- 1000 µL Micropipette tips, 1 box
- Electrophoresis gel apparatus with an 1.5% (w/v) agarose gel and TBE 0.1X buffer
- Erlenmeyer with electrophoresis buffer TBE 0.1X Buffer solution (labelled as "**TBE 0.1X**")
- Microcentrifuge, one for each two teams
- UV transilluminator, to share with the other teams (located in another room - to use with the help of a Lab assistant)

Any additional above-mentioned material will cost you 5 marks. Additional DNA samples or agarose gel will cost you 10 marks each.

2 - 2.1.1. Preparation of the samples containing the PCR products for electrophoresis

1. Prepare the PCR* products (A, B and C) and the DNA ladder according to the following scheme. Label 4 clean 1.5 mL tubes, and prepare the following mixtures:

   Tube I - 15 µL of PCR product from DNA A + 3 µL of Orange G loading buffer*

   Tube II - 15 µL of PCR product from DNA B + 3 µL of Orange G loading buffer*

   Tube III - 15 µL of PCR product from DNA C + 3 µL of Orange G loading buffer*

   Tube IV - 15 µL DNA ladder + 3 µL of Orange G loading buffer*

2. Mix the solutions well using the pipette and ask an assistant for help to pulse-centrifuge the reaction mixtures using the microcentrifuge.

2 - 2.1.2. Loading the samples in the agarose gel

In your work station you will find an electrophoresis apparatus with an 1.5% (w/v) agarose gel submersed in electrophoresis buffer.

If the gel is not entirely submersed, pour some more buffer in the tank. The wells must be totally submersed.

Load the 18 µL of each sample from tubes I to IV (DNA + loading buffer*) into the wells of your gel, by slowly pouring the sample in the middle of the well. After loading the sample, slowly remove the pipette tip from the well keeping the dispenser pressed. Reject that tip and use a new one to repeat the procedure for the next sample, loading it in the adjacent well. Repeat the procedure for all samples, including the DNA ladder*. Be sure to carefully load each sample in a different well! Figure 2 - 2.2 indicates **how to load the wells**.

Be careful not to damage your gel!

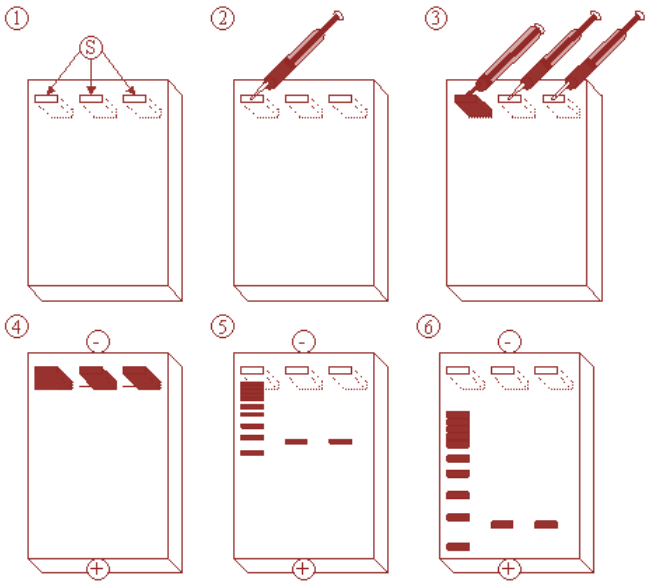


Figure 2 - 2.2 – Schematic representation of the electrophoresis process. S – slots/wells.

2 - 2.1.3. Running Electrophoresis

Before running the electrophoresis, make sure that all samples were loaded, the gel is submersed in the buffer, and that the wells of the gel are near the black electrode. Put the lid over the tray, as it was previously and then plug in to run the electrophoresis. Check for air bubbles at the side of the (red) electrode otherwise call lab assistant for help. After a few minutes you should see an orange band migrating away from the well, running towards the red electrode at end of the gel. See Figure 2 - 2.2.

Question 2.1a

Complete the legend of the gel (the positions of each sample (I to IV)), considering that well # 1 is the one at your left. **Figure 2 - 2.2 indicates how to load the wells.**

Enter the legend of your gel Question 2.1.a in the answer sheet.

2 - 2.1.4. Analysis of the gel

After 1 hour, ask for assistance to check if you can stop the electrophoresis. By the third hour of the task, you should go to photograph your gel. By that time, the schematic representation will be given to you, even you do not succeed in running the gel. Incomplete running/no running will be penalized.

After finishing the electrophoresis, ask for an assistant to accompany you to an UV transilluminator. There, you can check the presence of DNA bands in your gel.

1. The assistant will photograph your gel and will attach the photo to your answer sheet.

Question 2.1b

A photo of your gel will be attached to the answer sheet.

- **The photo of your gel will be attached under Question 2.1.b in the answer sheet.**

2. At that time, the assistant will give you a schematic representation of the gel. This image corresponds to the same PCR products ran in the same conditions as yours. Use this image to answer the Questions 2.1c-2.1g in the answer sheet. Consider that both species are present in the samples collected.

Question 2.1c

According to the image (schematic representation) provided, identify the number of the well in which PCR products from DNA of *M. trossulus* were loaded.

- **Select the correct option in Question 2.1.c in the answer sheet.**

Question 2.1d

According to the image provided, identify the number of the well in which PCR products from DNA of *M. galloprovincialis* were loaded.

- **Select the correct option in Question 2.1.d in the answer sheet.**

Question 2.1e

From these results do you think that Isabel and Vasco have collected an hybrid mussel (a cross-breed between *M. trossulus* and *M. galloprovincialis*)?

- **Select the correct option in Question 2.1.e in the answer sheet.**

Question 2.1f

Which PCR products are appropriate to choose the answer to question 2.1e?

- **Select the correct option in Question 2.1.f in the answer sheet.**

Question 2.1g

Based on the information of the DNA Size Ladder in Appendix 5, fill in the table, indicating the approximate molecular weight (number of base pairs) of each band, in each sample (use Figure 3 in Appendix 5).

- **Enter your answer to Question 2.1.g in the answer sheet.**

Question 2.1h

For a more accurate resolution of a DNA sample, which agarose gel concentration should you prepare?

- **Select the correct option in Question 2.1.h in the answer sheet.**

Question 2.1i

Regarding DNA charge, choose the correct option.

- **Select the correct option in Question 2.1.i in the answer sheet.**

2 - 2.2. Marine bacteria diversity associated with the wave energy device

In addition to helping the harvesting of multicellular living beings, Isabel and Vasco also collected samples by scraping structures from the wave generator. Their research group was interested in analyzing the different bacterial populations that could be fixed to the wave generator device.

Materials and equipment

- One 1.5 mL tube with marine bacteria sample (labelled as "**MB**"), on the tube holder
- Neubauer chamber with cover slip, 1 piece
- Optical microscope, 1 piece
- Micropipettes: 1000 µL, 200 µL and 20 µL, 1 piece each
- 20-200 µL Micropipette tips, 1 box
- 1000 µL Micropipette tips, 1 box (shared with Task 2 - 3)
- Immersion Oil (labelled as "**Immersion Oil**"), 1 piece
- Calculator, 1 piece
- Hand tally counter , 1 piece
- One plate with marine bacteria "A" (labelled as "**A**")
- One plate with marine bacteria "B" (labelled as "**B**")
- One Gram-stained slide with isolate "A" (labelled as "**A**")
- One Gram-stained slide with isolate "B" (labelled as "**B**")

- Sterile loop, 10 pieces
- One 1.5 mL tube with H_2O_2 (3%, v/v), on the tube holder (labelled as "**H_2O_2**")
- Clean glass slides, 1 box
- Tweezer, 1 piece
- Oxidase discs inside a small Petri dish, 3 pieces (labelled as "**OX**")
- One glass tube containing 5 mL of bacterial culture "A" (labelled as "**A**")
- One glass tube containing 5 mL of bacterial culture "B" (labelled as "**B**")

Any additional above-mentioned material will cost you 5 marks. Additional bacteria samples or Gram stained slides will cost you 10 marks. Breaking the Neubauer chamber will cost you 10 marks.

2 - 2.2.1. Enumeration of bacterial cells

You are now asked to help Isabel and Vasco! First, you are going to determine the number of bacterial cells collected from the surface of the wave energy device. To do that, two experimental approaches (Neubauer chamber and cell viable count) will be used. At the end you should be able to discuss the results in terms of the numbers of bacteria counted with the two methodologies used.

2 - 2.2.1.1. Total bacterial cells (Neubauer chamber)

A sample ("MB") collected from the wave energy device was previously diluted to a suitable concentration for cell counting (1:10).

Preparation of the slide and cell counting

1. Start by putting the clean glass cover on the Neubauer chamber central area.
2. With the aid of a micropipette, mix sample by pipetting up and down.
3. With the aid of a micropipette, introduce the sample (200 µL) into the Neubauer chamber. To do that, adjust the tip of the micropipette to the edge of the chamber (into the left groove of the Neubauer counting chamber) and gently release the liquid until the chamber is full (avoid introducing air bubbles).
4. Place the chamber on the platform of the microscope focusing initially with a 100× magnification (an entirely central square divided into 25 small squares should be observed).
5. Change the magnification to 400×. You may need to reduce the amount of light by closing the diaphragm of the condenser to be able see the cells. Perform the count in 5 small squares (each one divided into 16 smaller squares) within the central large square (Figure 2 - 2.3.).
6. Count the cells with the help of the hand tally counter.

Follow the criteria: 1) If cells fall on a line, include in your count those on the top and left-hand lines and exclude those on the bottom and right-hand line (Figure 2 - 2.3.); 2) Cells may have the propensity to aggregate. You should avoid counting these aggregates.

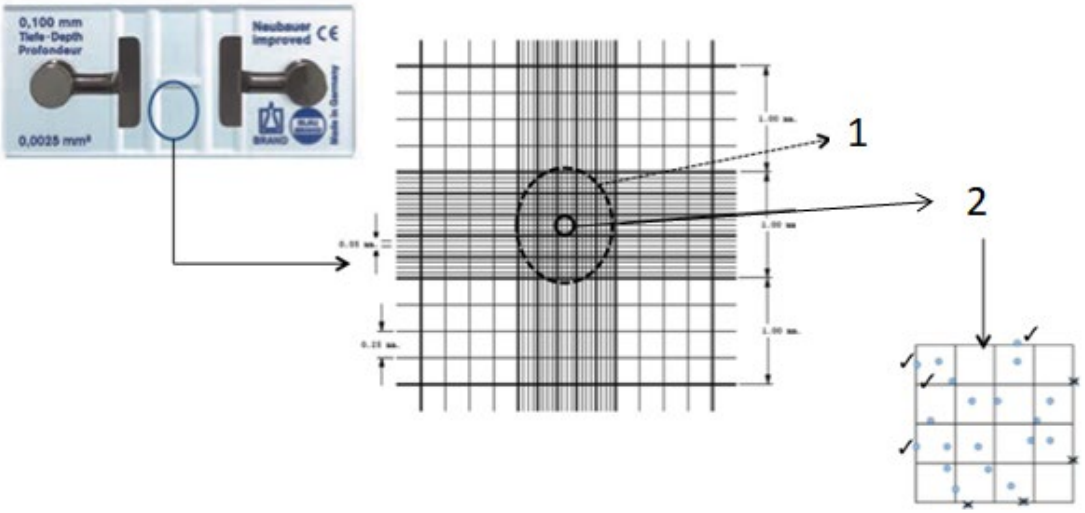


Figure 2 - 2.3 – General features of the counting chamber to be used. 1 - Central large square (100× magnification); 2 - Small square (400× magnification) (divided into 16 smaller squares)

Question 2.2.a.

Use the results obtained to fill the table and calculate the total number of cells per mL.

❖ **Write your answer under Question 2.2.a in the answer sheet.**

2 - 2.2.1.2. Estimation of viable bacterial cells

The same sample "MB", collected from the wave energy device, was serially diluted with sterile seawater. A spread plate method was employed for the estimation of viable bacteria. Marine agar was used as the culture medium; the plates were inoculated (100 µL) followed by their incubation at room temperature for 1 week. Colonies were counted for each dilution and the results are presented in the Table below.

	Serial dilutions of the sample "MB"		
	10^{-3}	10^{-4}	10^{-5}
Count	210	20	2

Question 2.2.b.

Calculate the total number of viable cells per mL of sample, expressing the results as colony forming units (CFU) per mL (original solution). Record your calculations.

❖ **Write your answer under Question 2.2.b in the answer sheet.**

2 - 2.2.2. Gram staining of bacteria associated with the wave energy device

Gram staining is a staining technique that differentiates bacteria into two major groups, namely Gram–positives and Gram–negatives. The procedure is based on the ability of bacteria to retain color of the stains used. Gram–negative bacteria are decolorized by alcohol, losing the color (purple) of the first stain (crystal violet). Gram–positive bacteria are not decolorized by alcohol and will remain as purple. After decolorization, a counterstain is used to impart a pink color (safranin) to the decolorized Gram-negative organisms (Figure 2 - 2.4.).

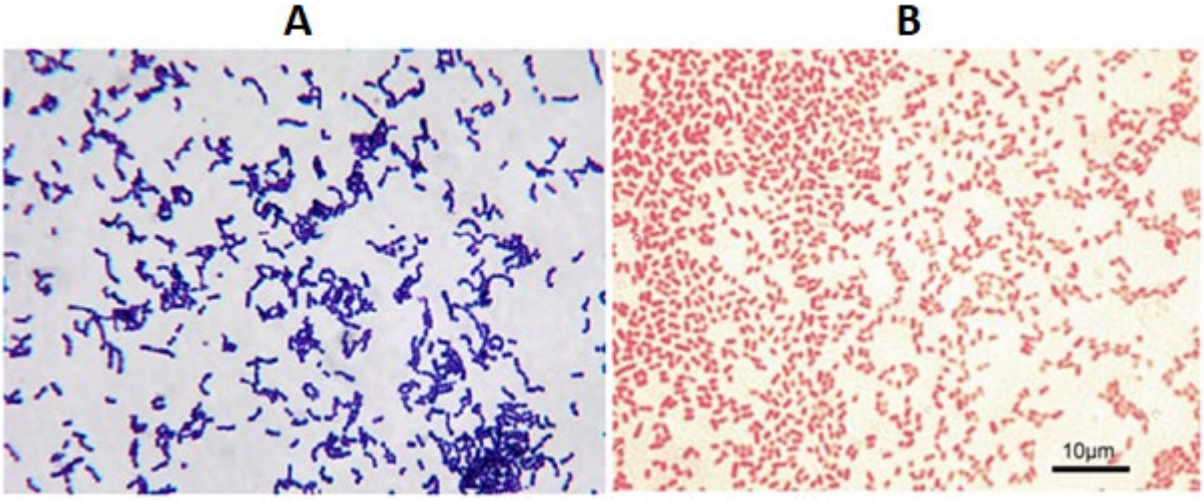


Figure 2 - 2.4 – Gram staining of Gram-positive (A) and Gram-negative (B) bacteria. Photo obtained from mycrobe.org

Pure cultures of the most abundant bacteria isolated from the wave energy device were streaked and cultivated on marine agar plates. Two of the most representative are available in your laboratory bench (designated by isolates "A" and "B"). The Gram staining of each isolate was previously performed (slides "A" and "B"). Observe the two slides under the microscope.

2 - 2.2.2.1. Microscope observation of the slides

Focus and observe the slides using the objectives of lower magnification (10×, 40×). For oil immersion (100×), add the oil directly to the smear and re-focus the slide with the fine adjustment knob.

Question 2.2.c.

Use a pen to draw pictures of the bacterial cells (morphology and the arrangement) observed under the 100× objective. Indicate the Gram reaction. For the interpretation of your results take in consideration the representative photos of Gram-positive and Gram-negative bacteria in Figure 2 - 2.4.

- **Write your answer under Question 2.2.c in the answer sheet.**

2 - 2.2.3. Biochemical characterization of bacteria associated with the wave energy device

To perform the biochemical tests use the two marine bacterial isolates ("A" and "B") previously selected, that are cultivated on marine agar plates.

2 - 2.2.3.1. Catalase test (antioxidant assay)

This test is used to demonstrate the activity of the enzyme catalase, needed to convert hydrogen peroxide (H_2O_2) into oxygen and water. The presence of the enzyme is determined when a bacterial inoculum introduced into H_2O_2 causes an immediate effervescence (release of O_2 observed by the visualization of bubbles).

1. With a sterilized loop place a small amount of the bacterial culture from the plate on a clean glass slide.
2. Add a few drops of H_2O_2 (3%, v/v) and watch for the production of bubbles in the suspension (existence of effervescence; release of O_2). If no bubbles are produced the test is negative for catalase activity. Compare your results with reference photos in Figure 2 - 2.5.
3. Perform these procedures for both cultures "A" and "B".

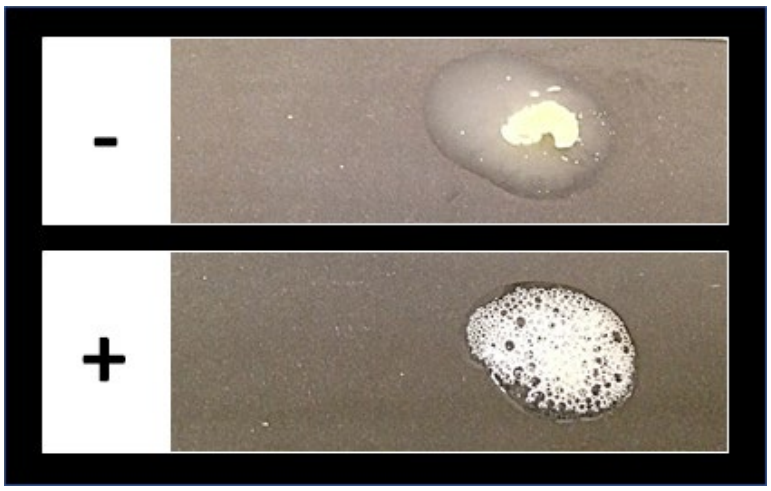


Figure 2 - 2.5 – Catalase test, showing negative (-) and positive (+) results.

Question 2.2.d

Record the results of your experiments in the Table using a (+) for a positive test and a (-) for a negative test.

❖ **Write your answer under Question 2.2.d in the answer sheet.**

2 - 2.2.3.2. Oxidase test

This test is used to demonstrate the activity of the enzyme cytochrome *c* oxidase, which is involved in the final step of the respiratory electron transfer chain. The presence of the enzyme is determined when a bacterial inoculum causes the oxidation of the substrate (tetramethyl-*p*-phenylenediamine dihydrochloride) to indophenol (a dark purple colored product).

1. With a sterilized loop add a portion of the bacterial culture on an oxidase disc (OX). For that place the disc on a slide with a tweezer, and then transfer a small amount of the bacteria culture from the plate using the loop.
2. Observe the result (positive test indicated by the development of a dark purple colour; bacterial

isolate produces the enzyme). The reaction is observed within 2 minutes at room temperature. Compare your results with reference photos in Figure 2 - 2.6.

3. Perform these procedures for both cultures “A” and “B”.

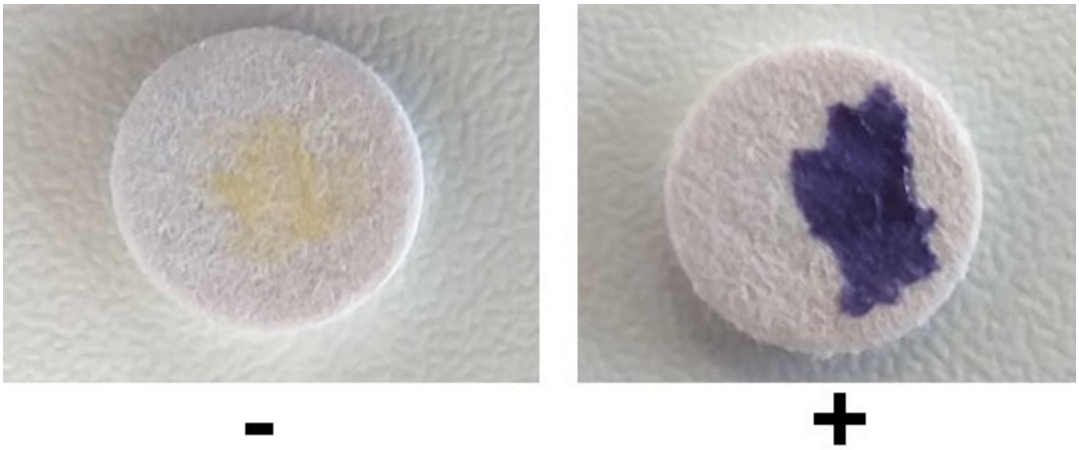


Figure 2 - 2.6 – Oxidase test, showing negative (-) and positive (+) results.

Question 2.2.e

Record the results of your experiments in the Table using a (+) for a positive test and a (-) for a negative test.

- **Write your answer under Question 2.2.e in the answer sheet.**

2 - 2.2.3.3. Biofilm formation

A procedure to determine the ability of bacteria to produce biofilms is the so-called “static microcosms” bioassay. Biofilms are observed at the air-liquid interface within a glass tube containing 5 mL of culture medium (marine broth) previously inoculated with 50 µL of a freshly-grown bacterial culture. Tubes are allowed to incubate for 1 day, without shaking, at 22 ºC, after which biofilm production at the air-liquid interface can be detected by naked eye or, alternatively, with the assistance of a loop.

1. Carefully observe each of the two static microcosms (glass tubes) provided, inoculated with Isolate A and Isolate B. Is there any sort of cellular mass identifiable at the air-liquid interface by naked eye?
2. Touch the air-liquid interface of each static microcosm (Isolate A and B) with a sterile loop, and record whether or not cellular material can be recovered from the surface of the liquid medium. If yes, the microcosm can be scored as biofilm-positive.
3. Perform these procedures for both cultures “A” and “B”.

Question 2.2.f

Record the results of your experiments in the Table using a (+) for a positive test and a (-) for a negative test.

- **Write your answer under Question 2.2.f in the answer sheet.**

Question 2.2.g.

Which of the options is a disadvantage of the direct microscopic count of bacterial cells?

- **Select the correct option in Question 2.2.g in the answer sheet.**

Question 2.2.h.

In a viable plate count technique, each … represents a … from the bacterial sample.

- **Select the correct option to fill the blanks (...) in Question 2.2.h in the answer sheet.**

Question 2.2.i.

What is the name of the period between inoculation of bacteria in a culture medium and the beginning of its multiplication?

- **Select the correct option in Question 2.2.i in the answer sheet.**

Question 2.2.j

What is the composition of a bacterial cell wall?

- **Select the correct option in Question 2.2.j in the answer sheet.**

Question 2.2.k

Which cell component is differentiated by the Gram staining technique?

- **Select the correct option in Question 2.2.k in the answer sheet.**

Question 2.2.l.

Which two cell components exist in bacteria?

Select the correct option in Question 2.2.l in the answer sheet.

Question 2.2.m.

What is catalase?

- **Select the correct option in Question 2.2.m in the answer sheet.**

Question 2.2.n.

Why may biofilm production by marine bacteria be advantageous?

- **Select the correct option in Question 2.2.n in the answer sheet.**

TASK 2 - 3.: BIOTECHNOLOGICAL POTENTIAL OF GREEN AND RED ALGAE

Introduction

Heavy metals are major pollutants in marine, ground, industrial, and even treated waters. Sorption of heavy metals onto live or dead biological materials (biosorption) is a potential method for removing or recovering toxic and precious metals from wastewater. Successful metal biosorption can be achieved by a variety of biological materials including microalgae and seaweeds.

Here you will help Vasco and Isabel to evaluate the biotechnological potential of two seaweeds that were collected close to the wave generator prototype. You will test the seaweed's potential as new materials for metal biosorption. Zinc will be the tested metal.

The green algae *Ulva* sp. and the red algae *Gymnogongrus* sp. were collected. After collection, algae were carefully freed of foreign matter, extensively washed, oven dried at 60 ºC for 72 hours, and then grounded. Algae were stored in a fresh bench and in a stoppered flask.

Figure 2 - 3.1 – Green algae *Ulva* sp. and red algae *Gymnogongrus* sp.

2 - 3.1. Biosorption isotherms

You will determine the Zn (II) ions uptake capacity by the red and the green algae. The objective is to determine which algae have the greatest potential to be used in a water treatment station to treat water contaminated with heavy metals. For this you will need to evaluate how much zinc can be absorbed by the algae. You will prepare zinc solutions with different concentrations and use samples of green and red algae, stir them for some time in the presence of the different solutions and then quantify the final Zn (II) ions in solution.

Materials and equipment

- 50 mL glass bottles with 0.05 g grounded dried Green Algae, 6 pieces
- 50 mL glass bottles with 0.05 g grounded dried Red Algae, 6 pieces
- Volumetric pipettes: 100.00 mL, 25.00 mL, 20.00 mL, 1 piece each
- Volumetric pipettes: 50.00 mL, 10.00 mL, 2 pieces each
- Pipette: 5.00 mL, 3 pieces
- Disposable plastic Pasteur pipettes, 3 pieces
- Pipette filler bulb, 1 piece (general tray)
- Micropipettes: 1000 µL, 200 µL and 20 µL, 1 piece each
- 20-200 µL Micropipette tips, 1 box with 96 tips
- 1000 µL Micropipette tips, 1 box with 96 tips (shared with Task 2 - 2.2)
- 25.00 mL burette, 1 piece
- 250 mL volumetric flask, 5 pieces
- 10 mL volumetric flask, 4 pieces

- 500 mL plastic beaker for waste, 2 pieces
- 100 mL Erlenmeyer flask, 2 pieces
- 25 mL beaker, 6 pieces
- 4 cm ⌀ funnel, 1 piece
- Magnetic stirrer, 2 pieces
- Magnetic stirrer bar, 12 pieces
- 1 cm length/2 mL plastic cuvette with covers, 30 pieces
- 1.3 $g.L^{-1}$ Zincon solution, 1.5 mL, labelled as "**Zincon"**
- Primary Zn (II) (II) standard solution, 500 mL, labelled as "**Sol P**"
- 0.010 $mol.L^{-1}$ EDTA solution, 50 mL, labelled as "**0.010 $mol.L^{-1}$ EDTA**"
- 0.0050 $mol.L^{-1}$ $MgCl_2$ solution, 50 mL, labelled as "**0.0050 $mol.L^{-1}$ $MgCl_2$**"
- Eriochrome black T solution, 5 mL, labelled as "**ErioT**"
- Sodium carbonate-bicarbonate (Na_2CO_3/$NaHCO_3$) Buffer solution, pH 10.5, 20 mL, labelled as "**Na_2CO_3/$NaHCO_3$ pH 10.5**"
- Sodium borate saline ($Na_2B_4O_7$) Buffer solution, pH 9.0, 5 mL, labelled as "**$Na_2B_4O_7$ pH 9**"
- Deionized water in 500 mL plastic wash bottle, 2 pieces (labelled as "**H_2O**") (can be refilled if needed without penalty).
- Colorimeter, 1 piece
- TI-Nspire CX calculator, 1 piece
- Scientific calculator TI-30X, 1 piece (general tray)
- Clock in the wall, (2 per lab)

In case you spill a chemical, or break a piece of glassware, ask a replacement to a supervisor.

Any additional above mentioned laboratory material will cost you 5 marks unless otherwise stated. An additional algae sample or solution will cost you 10 marks.

2 - 3.1.1. Samples for equilibrium Zn(II) quantification

Preparation of Zn (II) standards with known concentration

Using the Primary Zn (II) stock solution (**Sol P**), prepare 5 diluted solutions according to the values indicated in the table below in 250 mL volumetric flasks.

*Dilution of Primary Zn (II) Solution (**Sol P**)*				
2.5×	*5×*	*10×*	*25×*	*50×*

Question 3.1.1

Calculate the volume of **Sol P** needed to prepare each of the standard diluted solutions of Zn (II).

- **In Table 3.1.1 in the answer sheet, write the volumes of Sol P you used for the preparation of each Zn (II) standard**

The concentration of Sol P is unknown until it is determined in section 2 - 3.1.2.

1. Label the 250 mL volumetric flasks with the dilution (***2.5***×, ***5***×, ***10***×, ***25***×, ***50***×).
2. By using the pipette filler bulb (see in the Appendix 7) and the appropriate pipette, transfer the necessary volume of **Sol P** to each of the 250 mL volumetric flasks.
3. Add deionised H_2O to the flask to about one cm below the mark line. Fill the flask to the mark line using a Pasteur pipette.

Using 6 green and 6 red algae samples:

1. Use 6 of the given 50 mL bottles containing 0.050 g of green or red algae and label them with the type of algae and the value of the zinc solution standards (**Sol P**, ***2.5***×, ***5***×, ***10***×, ***25***×, ***50***×).
2. Add 50 mL of each of the zinc solution standards to the appropriate flask, including **Sol P**. You will need to prepare a total of 12 bottles, 6 bottles of different concentration (**Sol P**, ***2.5***×, ***5***×, ***10***×, ***25***×, ***50***×) for each algae.

Use the same 50 mL pipette for all solutions. You do not need to clean the pipette between solutions. Start by taking 50 mL from the highest dilution (50x) to the green and red algae flasks then take 50 mL from the next dilution (25x) and proceed accordingly until the lowest dilution (SolP) (this corresponds going from the lowest to the highest concentration).

Pipetting order			
Red algae		Green algae	
Flask	**Volume mL**	**Flask**	**Volume mL**
50×	50	*50*×	50
25×	50	*25*×	50
10×	50	*10*×	50
5×	50	*5*×	50
2.5×	50	*2.5*×	50
Sol P	50	*Sol P*	50

3. Add a magnetic stirrer bar to each one of the 50 mL bottles and stir them continuously for **not less than 90 minutes (clock in the Lab wall)** at room temperature using the magnetic stirrer.

Do NOT switch on the hot plate on the magnetic stirrer!
The experiment must be done at room temperature.

While you wait for not less than 90 minutes proceed to section 2 - 3.1.2 for the determination of the Zn(II) concentration of solution P.

4. After 90 minutes stop the agitation and wait to allow the algae particles to rest at the bottom of the flask. Proceed to **2 - 3.1.4.** for metal quantification.

2 - 3.1.2. Determination of the concentration of a standard solution of Zn (II) (Sol P)

The primary standard solution of Zn (II) ion (**Sol P**) was prepared by dissolving a precise amount of $ZnSO_4.7H_2O$ in H_2O in a 500 mL volumetric flask. The amount of $ZnSO_4.7H_2O$ weighted was written in the flask label, but unfortunately the label of the solution was damaged and it is not possible to read ~~know~~ the weight of $ZnSO_4.7H_2O$ used to prepare the solution.

Your task is to determine which of the four (a to d) was weighted to prepare the solution:

a) 12.50 mg
b) 125.0 mg
c) 1.250 g
d) 12.50 g

For this you will perform a complexometric determination of Zn (II) with EDTA by back titration with $MgCl_2$. You will determine an approximate concentration of Zn (II) in $mol.L^{-1}$ and $mg.L^{-1}$ in **Sol P** and therefore you must choose the nearest value from the above list.

You will use a chemical reagent that forms a complex with Zn (II) ions to determine the concentration of zinc in solution. The complexation agent is the anion of ethylenediamine tetraacetic acid, abbreviated as EDTA. This ion has the ability to "wrap" itself around positive metal ions in water solution. This process is called chelation or complex formation. The chelation reaction between EDTA and many metal ions has a very large equilibrium constant, 1 mol of EDTA will react with 1 mol of (Zn^{2+}).

For Zn (II) ions in water the reaction with EDTA is as follows :

$$Zn^{2+}(aq) + EDTA^{4-}(aq) \quad \rightleftarrows \quad [Zn(EDTA)]^{2-}(aq)$$

To detect the ending point of the titration a metallochromic indicator will be used. Metallochromic indicators are organic compounds that originate different colours when complexed with metal ions. In this experiment the indicator used is Eriochrome black T (**ErioT**). When free, this indicator has a blue colour whereas when complexed with Mg^{2+} the colour becomes violet.

An excess of EDTA is added to the standard Zn (II) ion solution (**Sol P**) to ensure that all Zn (II) ions are complexed. You will also add 20 µL of **ErioT** and the solution turns blue because **ErioT** is free in solution.

The excess of EDTA will then be back titrated with a solution of known concentration of $MgCl_2$. The magnesium will react with EDTA.

$$EDTA^{4-}(aq) + Mg^{2+}(aq) \rightleftarrows [Mg(EDTA)]^{2-}(aq)$$

As soon as all the excess of EDTA is complexed with magnesium, the excess of magnesium added will react with **ErioT** forming a pink complex.

$$Mg^{2+}(aq) + ErioT(aq) \rightleftarrows Mg\text{-}ErioT(aq)$$

At this point the colour of the solution changes from **blue** to **violet**. The number of moles of $MgCl_2$ added until the indicator changes colour, is equal to the number of moles of EDTA in excess in relation to the Zn (II) ions.

Since the total number of moles of EDTA added to the solution and the number of moles of $MgCl_2$ used in the back titration is known, it is possible to calculate the number of moles of Zn (II) ions and the concentration of **Sol P**.

2 - 3.1.3. Titration of Sol P

Use the funnel to fill the burette above the 0.00 mL mark with 0.0050 $mol.L^{-1}$ $MgCl_2$. Drop the excess of liquid (into the 500 mL plastic beaker for waste) so that the burette is filled precisely to the 0.00 mL mark.

Note: **Be careful** not to have any air bubbles trapped in the valve of the burette.

Prepare **two** 100 mL Erlenmeyer flasks

In each of the 100 mL Erlenmeyer flasks add the following solutions:

1. With a 5.0 mL pipette - transfer 5.0 mL of **Sol P** solution to the Erlenmeyer.
2. With a 10.0 mL pipette - add 10.0 mL of 0.010 $mol.L^{-1}$ EDTA solution.
3. With a 5.0 mL pipette - add 5.0 mL of sodium carbonate-bicarbonate Buffer solution pH 10.5.
4. With the 20 µL micropipette – add 20 µL of **ErioT** solution.

Important notes on the titration:

- You should perform two titrations, a first titration to determine the approximate position of the end point (as soon as the solution turns from **blue** to **violet**) and a second titration to determine accurately the end point.
- On the first titration use the color of the solution of the second Erlenmeyer flask as a visual reference for the initial blue color of the solution, that will help you to determine the end point.
- You should add small amounts of $MgCl_2$ solution when in proximity of the end point.
- No drop of $MgCl_2$ solution after addition should remain on the walls of the Erlenmeyer. This could be a source of error.
- Stir the Erlenmeyer flask manually between additions of $MgCl_2$.

Question 3.1.3.a

Record the volume of $MgCl_2$ corresponding to the end point of the titration.

❖ **Enter the volume of $MgCl_2$ under Question 3.1.3a in the answer sheet.**

Question 3.1.3.b

Calculate the number of moles of Zn (II) ions in 1.0 L of **Sol P**. You should indicate the value with 5 decimal places (this quantity is known as the molarity of the solution and is expressed in $mol.L^{-1}$).

❖ **Enter your calculations and result under Question 3.1.3.b in the answer sheet.**

Question 3.1.3.c

Based on your titration results and calculations choose from the list below the correct mass of $ZnSO_4.7H_2O$ that was used to prepare **0.500 L** of the primary standard solution of Zn (II) ions (**Sol P**).

a) 12.50 mg
b) 125.0 mg
c) 1.250 g
d) 12.50 g

❖ **Circle your choice under Question 3.1.3.c in the answer sheet.**

Question 3.1.3.d

Using the value chosen in **Question 3.1.3.c**, calculate the mass of Zn (II) ions (in mg) in **1.0 L** of **Sol P**. You should indicate the value with 1 decimal place. This value is the concentration of **Sol P** in $mg.L^{-1}$

❖ **Enter your calculations and result under Question 3.1.3.d in the answer sheet.**

Question 3.1.3e

❖ **Calculate the initial Zn (II) concentration (C_i) in the 50 mL reaction flasks. Register the calculated C_i values in the answer sheet under Table 3.1.3.e and Tables 3.1.5.a and 3.1.5.b.**

Check your clock and be aware of the time of the biosorption experiment. To finish on time you should use any spare time left to read carefully section 2 - 3.1.4. and 2 - 3.1.5. prepare the material and perform all auxiliary calculations.

2 - 3.1.4. Metal Quantification

The concentration of free Zn (II) ions in the zinc-algae aqueous solutions is determined spectrophotometrically. Zinc forms a blue complex with zincon in a solution buffered to pH 9.0. The minimum detectable concentration is 0.02 $mg.L^{-1}$ of Zn (II). The absorbance of the blue zinc–zincon complex in solutions is read at 635 nm.

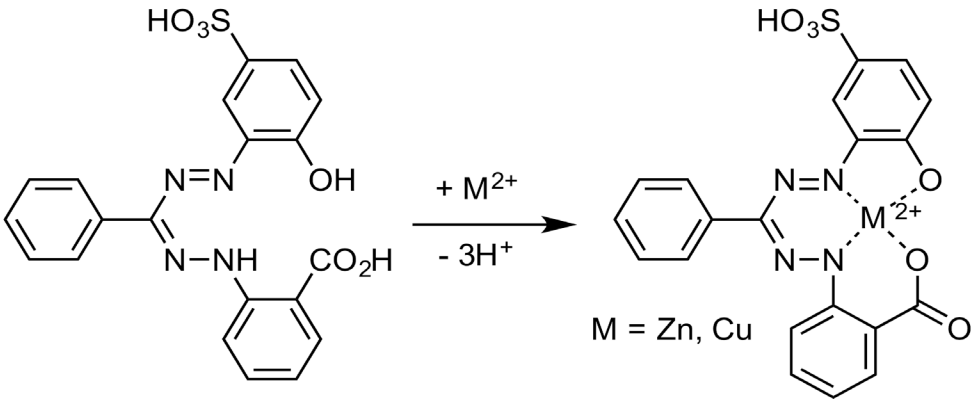


Figure 2 - 3.2 – Zincon complexation with divalent metal ions

- Since you span a large interval of Zn (II) concentrations you will need to dilute some samples before you can follow the protocol to measure absorbance.
- Using the four 10 mL volumetric flasks prepare diluted samples as follows:
  - Using the 200 µL micropipette transfer 200 µL aliquots of **Red Sol P, Red 2.5×, Green Sol P and Green 2.5× zinc-algae solution** to each of the four 10 mL volumetric flasks. Take the aliquots carefully to avoid taking algae particles with the sample.
  - Fill the four volumetric flasks to the mark line with **H_2O**. Do not forget to label the flasks with the indication of the dilution.

You are ready to proceed with the quantification protocol:

1. Prepare 12 plastic cuvettes (1 cm length/2 mL), 6 for each algae.
2. Using the appropriate micropipette take aliquots directly from the 50 mL zinc–algae solution flasks (avoid taking algae particles with the sample) or from the previously prepared dilutions in the 10 mL volumetric flasks and transfer them to the plastic cuvettes according to the table below. Add H_2O to the cuvette when indicated:

	50×	*25×*	*10×*	*5×*	*2.5×*	*Sol P*
Flask of origin	**50 mL Zinc algae solution**	**50 mL Zinc algae solution**	**50 mL Zinc algae solution**	**50 mL Zinc algae solution**	**10 mL volumetric flask**	**10 mL volumetric flask**
Volume to pipette	200 µL	100 µL	20 µL	20 µL	200 µL	200 µL
H_2O	0	100 µL	180 µL	180 µL	0	0
Total volume in cuvette	200 µL	200 µL	200 µL	200 µL	200 µL	200 µL

3. You should have a total of 12 cuvettes, 6 for each algae, each with a total volume of 200 µL.
4. Add reagents to the cuvettes in the following order with mixing between additions (use the cuvette cover):
   - 100 µL of sodium borate saline buffer solution, pH 9.0
   - 60 µL of zincon solution
   - 640 µL of H_2O
5. At this point each of your 12 cuvettes will have a total volume of 1000 µL. Connect and calibrate the colorimeter (see instructions for Vernier Colorimeter in Appendix 6). Use a cuvette filled with H_2O for the blank.
6. Read the absorbance of the blue zinc–zincon complex at 635 nm using the colorimeter.

Question 3.1.4

❖ **Complete columns A.1 (Green algae) and B.1 (Red algae) in Table 3.1.4 in the answer sheet with the measured absorbance for each cuvette.**

2 - 3.1.5. Data analysis – Biosorption Isotherms

The Lambert-Beer Law

According to the Lambert-Beer law the concentration of a compound is related with its absorbance:

$$A = \varepsilon b C$$

This law predicts a linear relation between the absorbance reading (A), measured at a specific wavelength, and the concentration of compound (C), if (b), the cell or cuvette width, is kept constant. ε is a constant called absorptivity and is characteristic of the compound.
For a given solution contained in a cuvette with a constant cell width, one can assume ε and b to be constant, k. This leads to the equation:

$$A = k.C$$

The following Lambert-Beer law curve is an example of a calibration curve obtained for a collection of Zn (II) standards of known concentration in a particular colorimeter.

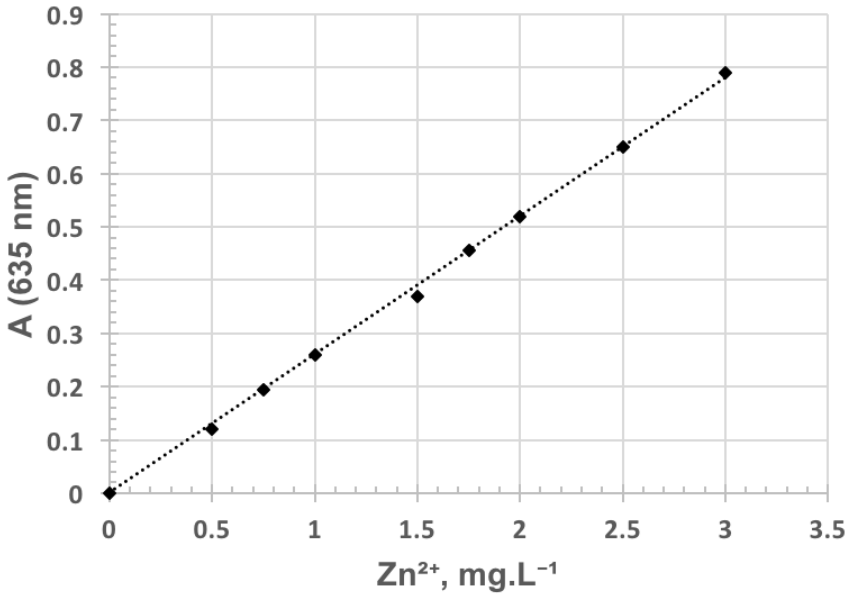


Figure 2 - 3.1.5 – Example of a Lambert-Beer law plot for a collection of Zn (II) standards of different concentration.

IMPORTANT: Each colorimeter has a calibration curve (*A=k.C*). Check the number (#) printed in your colorimeter (Col #) and use the corresponding value of *k* for your calculations. A list of *k* values for all colorimeters is available in Appendix 6.

Question 3.1.5.a

Using the relation between absorbance and concentration listed for your colorimeter calculate the concentration of Zn (II) in the cuvette.

- **Register the number of your colorimeter and the value of *k* (*A=k.C*) in the answer sheet.**
- **Using the Lambert-Beer law relation for your colorimeter, complete columns A.2 and B.2 in Table 3.1.4 with the Zn (II) concentration in each cuvette.**

Question 3.1.5.b

- **Calculate the final Zn (II) concentration (C_f) in the 50 mL reaction bottles for: SolP, 5x, 25x and 50x for both algae. Write the calculation for one of them. Use the appropriate sections of question 3.1.5.b in the answer sheet to indicate the calculations.**

Question 3.1.5.c

- **Calculate the final Zn (II) concentration (C_f) in the remaining 50 mL reaction bottles for each algae. Complete Tables 3.1.5.a and 3.1.5.b. and with all the calculated C_f values in the answer sheet.**

Question 3.1.5d

Zinc uptake capacity (q) is calculated after stabilization of Zn (II) concentration in solution. It is possible to know the quantity of Zn ions removed by algae by the difference between the initial (C_i)

and final (C_f) metal concentration. By dividing this value by the quantity of algae (C_A) used in the biosorption experiment, the uptake capacity is obtained:

$$q = \frac{(C_i - C_f)}{C_A}$$

where C_i and C_f are the Zn concentrations in mg.L^{-1} and C_A is the algae quantity in g.L^{-1}.

- **Calculate C_A and the Zn uptake capacity (q) for each algae. Register the values in the answer sheet under Tables 3.1.5.a and 3.1.5.b.**

IMPORTANT: If the majority of your q values are negative most probably you have the wrong concentration for Sol P. Consider using the value of 800.0 mg.L^{-1} and redoing the calculations of C_i.

2 - 3.1.6. Determine the maximum Zn (II) uptake capacity by approximation to a Langmuir isotherm

The evaluation of the biosorbent is carried out considering the equilibrium relations (adsorption isotherms). You will use the Langmuir sorption model for the estimation of maximum sorbate (metal) uptake (q_{max}). The Langmuir isotherm can be expressed as:

$$q = \frac{q_{max} b C_f}{(1 + b C_f)}$$

where q (the uptake capacity) is the amount of sorbate uptake, q_{max} is the maximum amount of sorbate uptake upon saturation of the surface, C_f the final concentration of the sorbate in solution (in mg.L^{-1}) and b a constant related with the affinity of metal ions to the adsorption sites (in L.mg^{-1}).

The Langmuir model can be verified using the linear transformation of the previous equation:

$$\frac{1}{q} = \frac{1}{q_{max}} + \frac{1}{q_{max} b C_f}$$

By plotting ($1/q$) versus ($1/C_f$), q_{max} and b can be determined from the *slope* ($m = 1/(q_{max}b)$) and the *Y-intercept* ($c = 1/q_{max}$) constants of a straight line ($y = mx + c$) that best fits the experimental points.

Question 3.1.6.a

Calculate $1/q$ and $1/Cf$. Plot ($1/q$) versus ($1/Cf$) for both algae.

- **Register the calculated values in the answer sheet under Tables 3.1.5.a and 3.1.5.b. Use the supplied millimetric paper sheet to draw the two plots of $1/q$ versus $1/C_f$ (draw the plots separately and do not forget to identify them).**

Question 3.1.6.b

In the ($1/q$) versus ($1/C_f$) plots draw the straight lines that best fit the experimental points for the

two algae.

- **Draw the straight lines that best fits the experimental points in the $1/q$ versus $1/C_f$ plot for the two plots (do not forget to identify them). Be aware that the *Y*-intercept must be a positive number. If you want to disregard the contribution of one or more experimental points to the straight line mark it with a cross within a circle ⊗, but a penalty will be applied.**

Question 3.1.6c

Determine the *slope* (m) and the *Y-intercept* (c) of the straight lines that best fit the experimental points ($y = mx + c$) for both algae.

- **Calculate the slope (m) and Y-intercept (c) for the green algae and the red algae.**

Question 3.1.6d

Finally, calculate q_{max} and b as follows:

$$q_{max} = \frac{1}{c}$$

and

$$b = \frac{c}{m}$$

- **Enter your calculations and results of q_{max} and b under Question 3.1.6d in the answer sheet.**

Question 3.1.6e

Based on your results for q_{max} and b which algae would you select to remove Zn ions from wastewater?

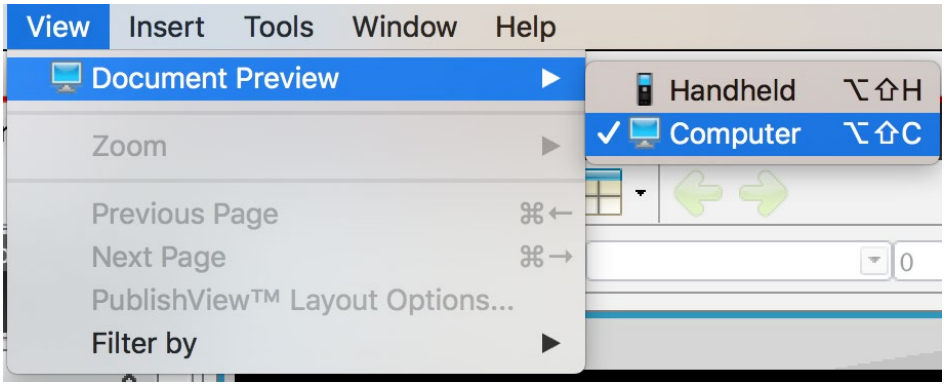
- **Enter your choice under Question 3.1.6.e in the answer sheet.**

APPENDIX 4

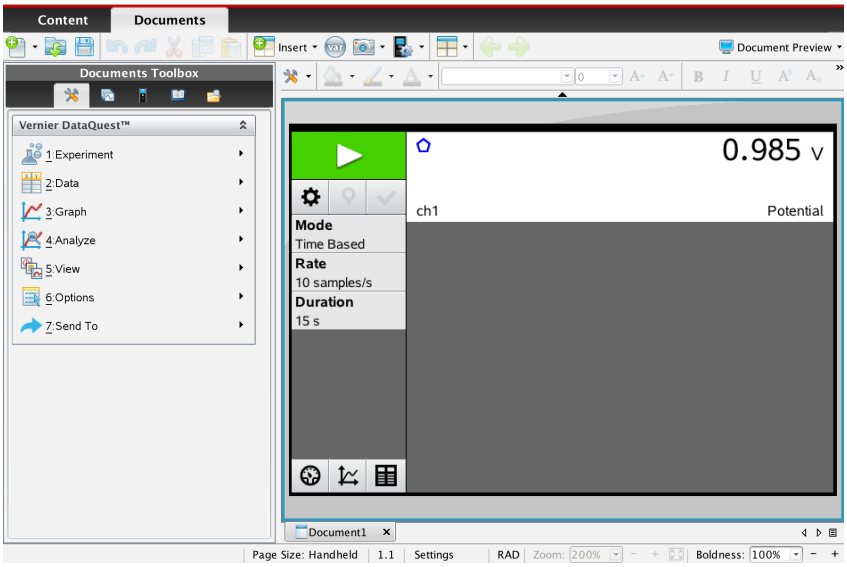
Ti-Nspire

4.1. Collecting data with the data logging Lab Cradle interface connected to a calculator with the TI-Nspire CX Software.

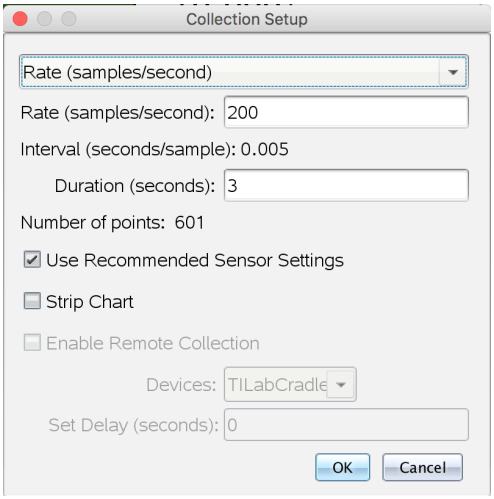
1. In the computer launch the application TI-Nspire CX. Choose the option “Trial Version”
2. In order to view the document in the computer mode make the following selection:



3. Connect the sensor/ sensors to the Lab Cradle
4. Connect the Lab Cradle to the computer using a mini USB to USB cable.
5. The program automatically detects the sensor/ sensors, showing you the following interface:



6. To adjust the sampling rate and define the acquisition duration by clicking on  and making your choices:



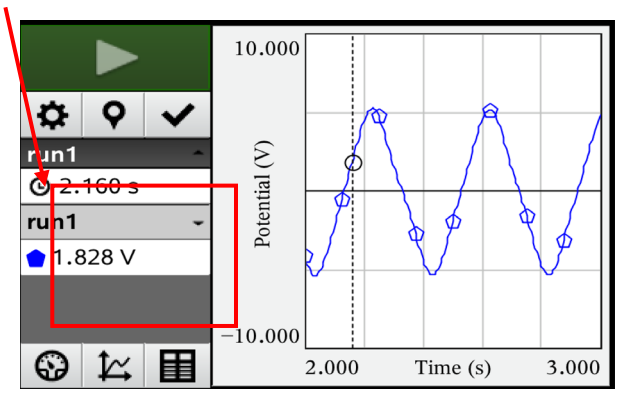
Note that if the sampling rate is too low your plots will not have sufficient points.

7. To start the collection click on 

8. The collected data can be visualized in a meter by selecting  , in a graph by selecting  or in a table  .

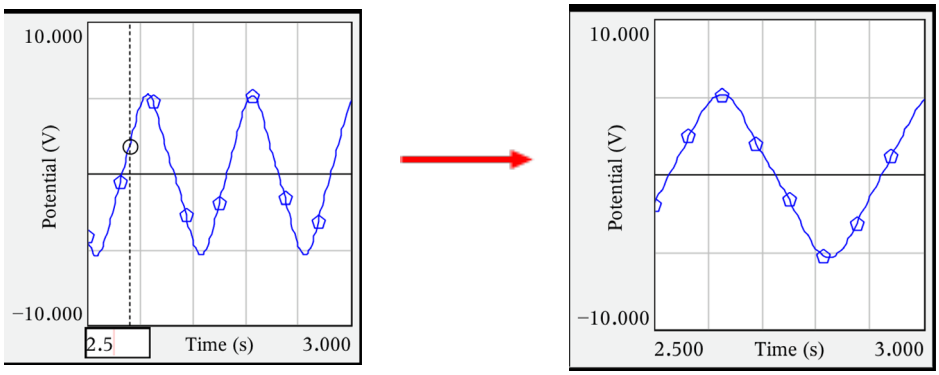
9. Save your document choosing the “Save” or “Save as” option in the menu “File” or by clicking on  .

10. You can read data points in a plot by clicking on the graph to position the cursor at the desired point:



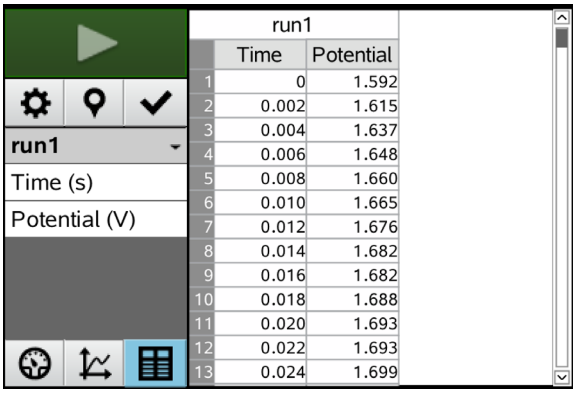
You can use the arrow keys to move the cursor.

11. To change the axes limits, write a new value over the current one:



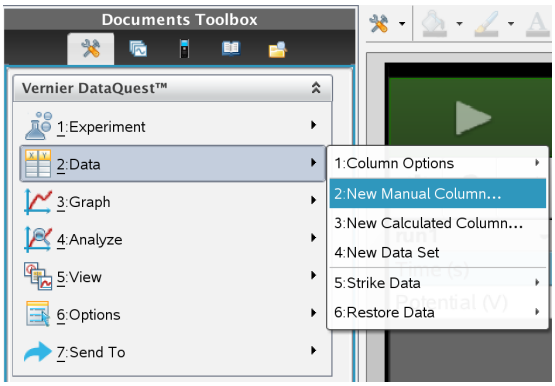
12. You can work out your data by: **appending columns to your table**, **making calculations with data from existing columns** and creating a new column with the results, **plotting data from existing columns**, **making curve fits to your plots**, etc. You can find some examples below.

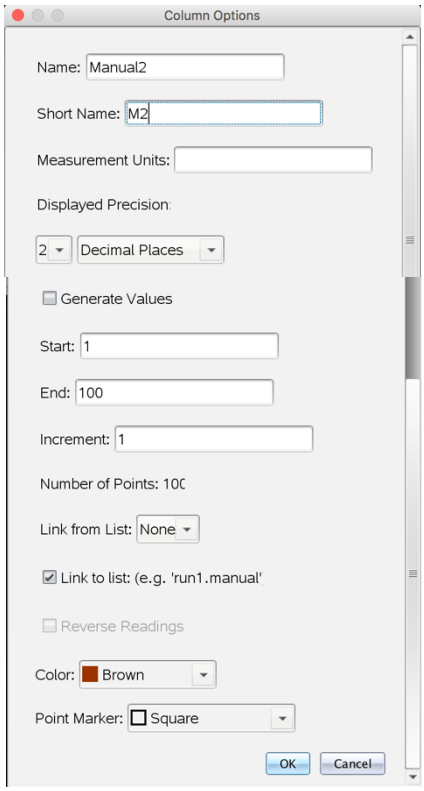
A set of collected data table looks like this:



13. **Append columns to the table to**:

- **fill manually**

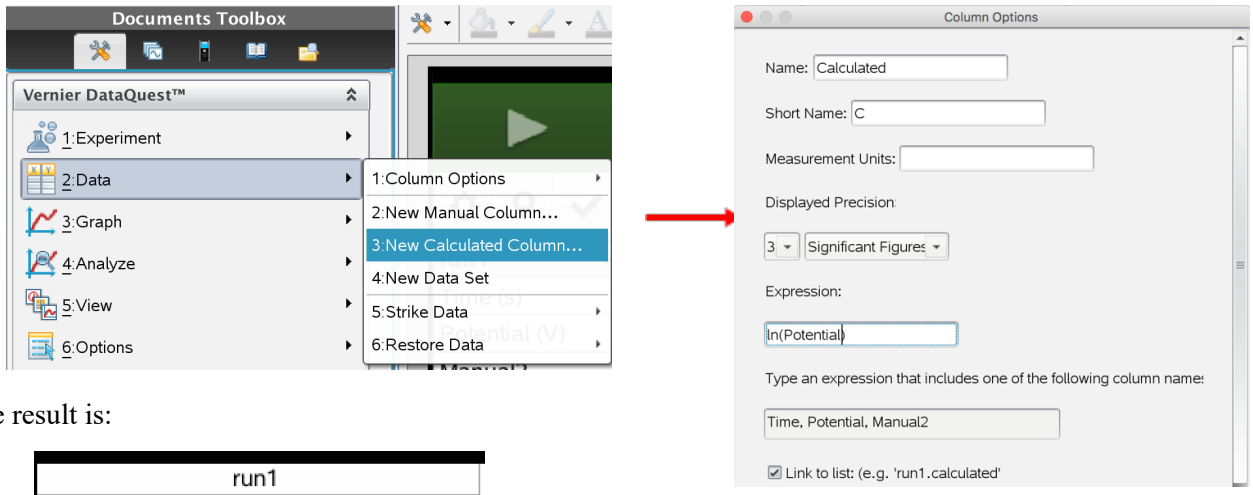




Then you can fill manually the new column:

run1			
	Time	Potential	Manual2
1	0	1.592	1.00
2	0.002	1.615	2.00
3	0.004	1.637	3.00
4	0.006	1.6484	
5	0.008	1.660	
6	0.010	1.665	

14. Calculate data from existing columns (in the example the ln function is applied to the numbers in the column named "Potential" and the results are listed in a new column named "Calculated")

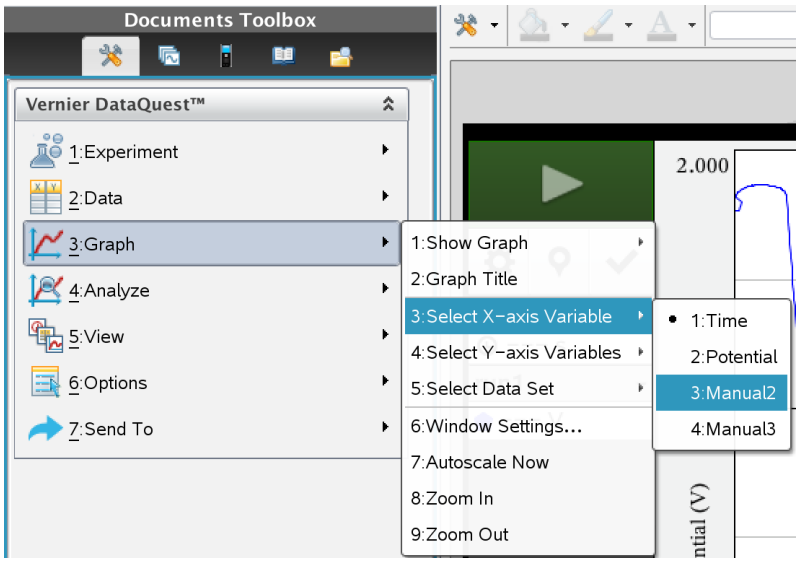


The result is:

run1				
	Time	Potential	Manual2	C
1	0	1.592	1.00	0.465
2	0.002	1.615	2.00	0.479
3	0.004	1.637	3.00	0.493
4	0.006	1.648	4.00	0.500
5	0.008	1.660		0.507
6	0.010	1.665		0.510
7	0.012	1.676		0.517
8	0.014	1.682		0.520
9	0.016	1.682		0.520

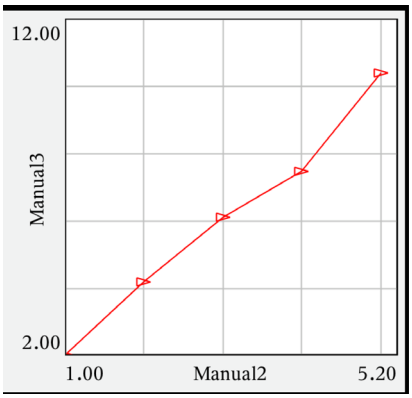
15. Plotting data in columns

Select  and then select the variables *x* and *y* you want to plot:

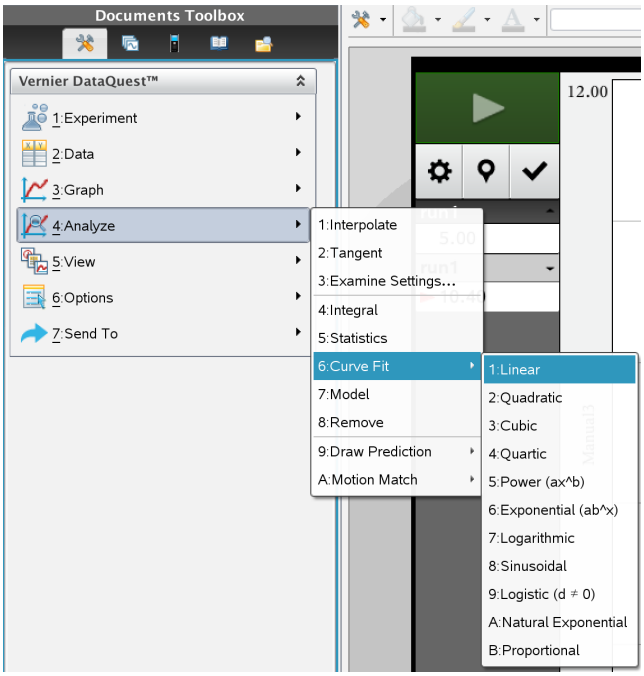


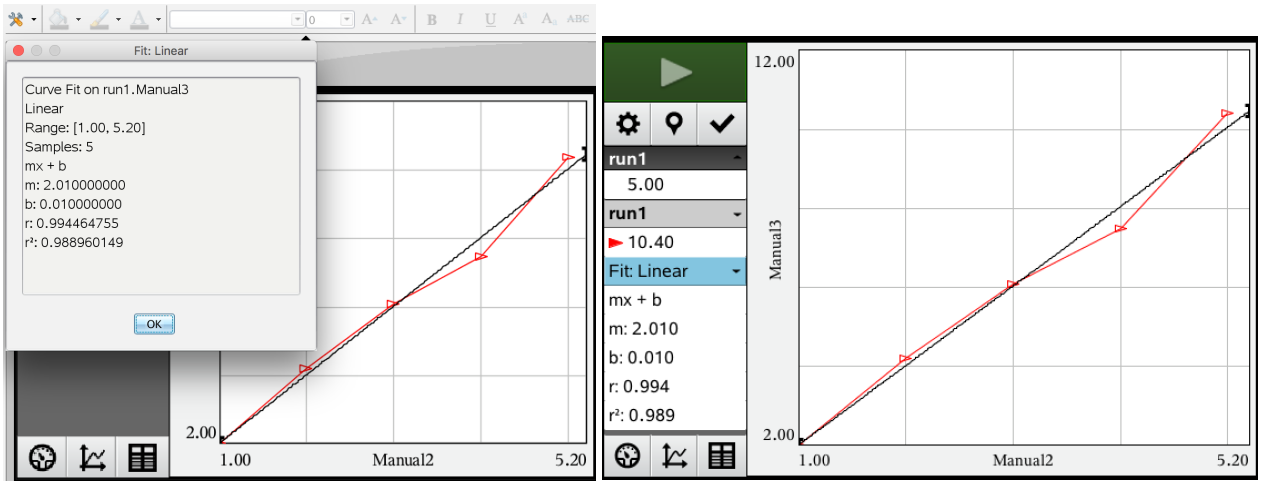
Manual2	Manual3
1.00	2.00
2.00	4.20
3.00	6.10
4.00	7.50
5.00	10.40

Manual3 as a function of Manual2 will give:



16. Making curve fits to plots





APPENDIX 5

GLOSSARY

PCR (Polymerase Chain Reaction)*- PCR is an *in vitro* amplification assay of DNA that produces exponentially large amounts of a specific sequence of DNA (template).

PCR amplification requires the presence of at least one DNA template strand, which can be any form of double-stranded DNA (in this case: DNA extracted from mussels).

Specificity comes from the ability to target and amplify a specific segment of DNA out of a complete genome, which is done with adequate primers* (Figure 1).

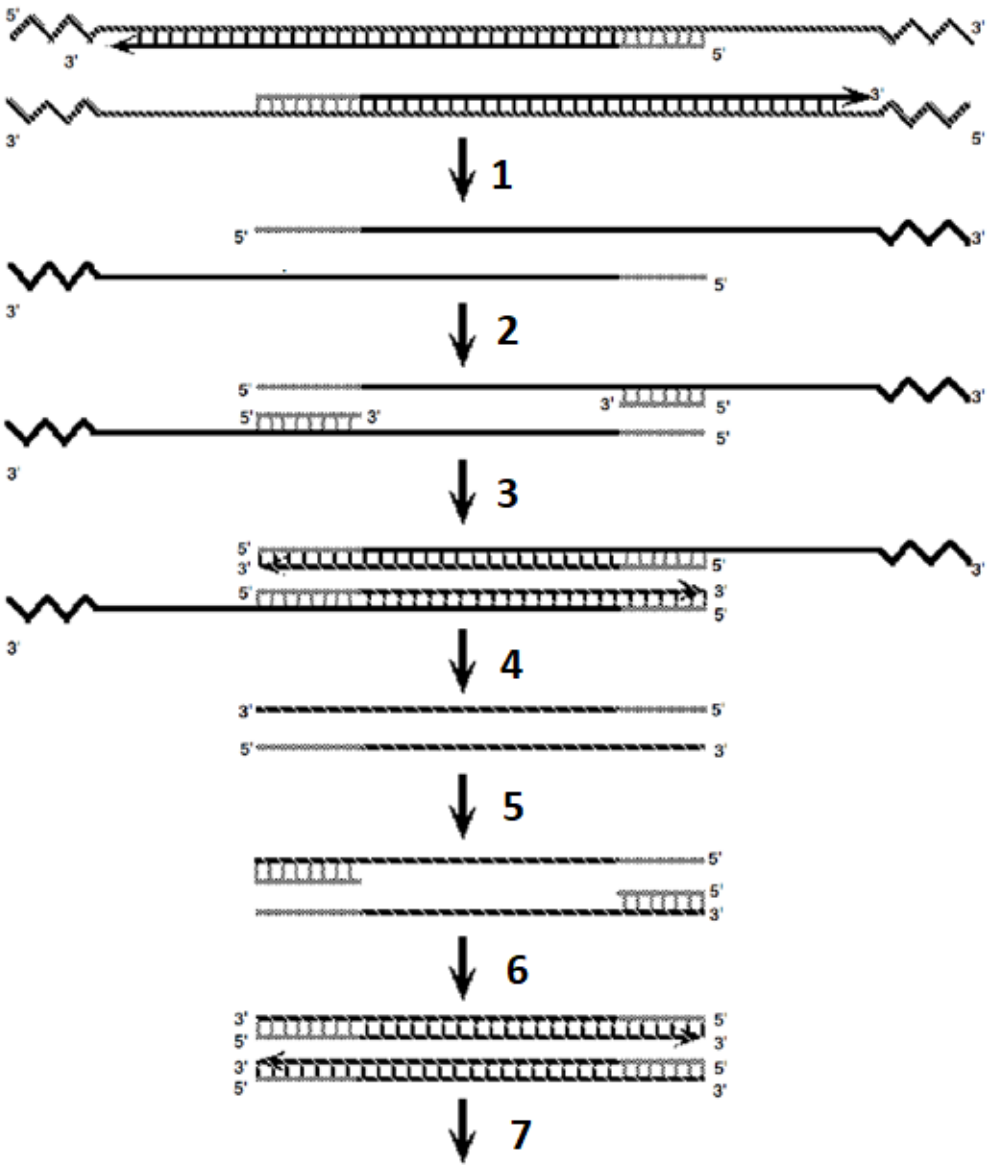


Figure 1 – An example of the amplification of a DNA template by PCR: 1) Denaturation of double strand DNA at high temperatures); 2) Annealing of the primers (hybridization with the complementary sequence); 3) Extension of new complementary DNA strands; 4) Denaturation of double strand DNA at high temperatures); 5) Annealing of the primers; 6) Extension of new complementary DNA strands; 7) Repetition of this reactions for several cycles.

Primers*- are short chains of DNA that are complementary to the DNA template we wish to copy using PCR. Forward primers bind to the downstream (at the 3' end) and reverse primers bind upstream (at the 5' end) of the target region of interest (Figure 2). For a PCR reaction to occur you need to use a suitable pair of forward and reverse primers to generate a specific fragment of DNA (Figure 2).

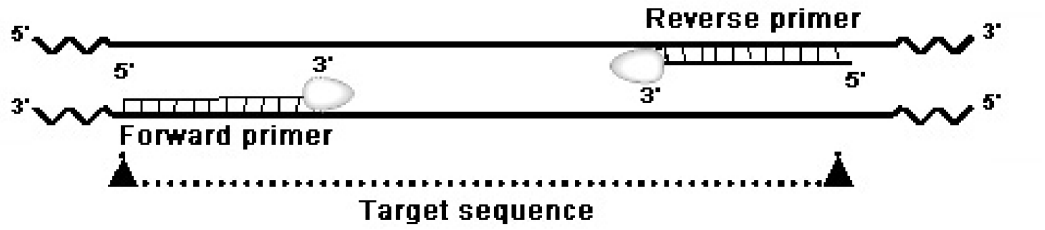


Figure 2 – Forward and reverse primers annealed to a target DNA sequence during PCR.

DNA molecular weight ladder*- Mixture of linear DNA fragments of known molecular weight used to estimate the size in base pairs (bp) of other DNA fragments. Schematic diagram of the expected band sizes of the DNA ladder used in this Task is indicated in Figure 3.

Figure 3 – DNA molecular weight ladder used. DNA bands and their corresponding molecular weight (number of base pairs) are indicated in the figure.

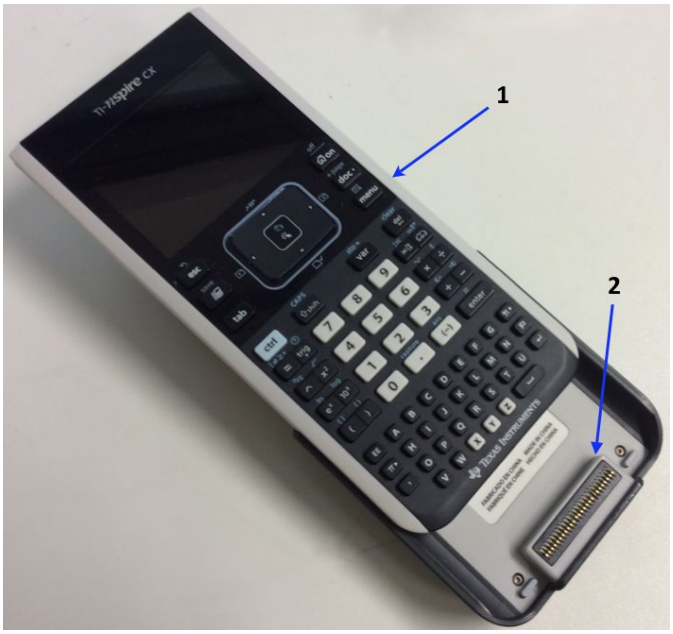
Orange G DNA Loading buffer*- Solution used to prepare DNA samples for loading on agarose gels. It contains a dye (orange) for visual tracking of DNA migration during electrophoresis and glycerol to increase the density of the sample.

APPENDIX 6

Ti-Nspire

3.1. Collecting data with the data logging Lab Cradle interface connected to a calculator with the TI-Nspire CX Software.

1. Connect the calculator to the interface



1 – Calculator

2 – Interface

2. Turn on the calculator.

1 

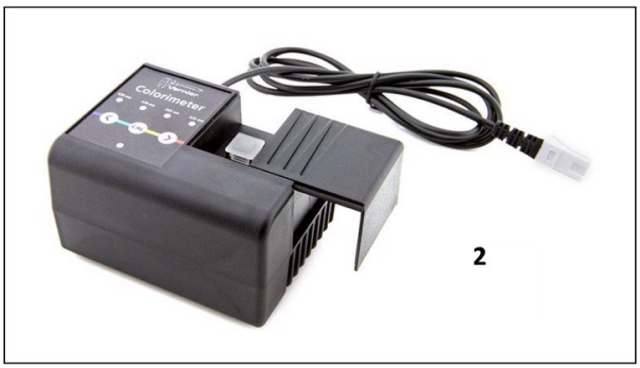
– Switch On/Off

3.2. Instructions for Vernier Colorimeter

The Vernier Colorimeter is designed to determine the concentration of a solution by analysing its colour intensity. The Colorimeter measures the amount of light transmitted through a sample at a user-selectable

wavelength.

There are two models: model **1** and model **2**.

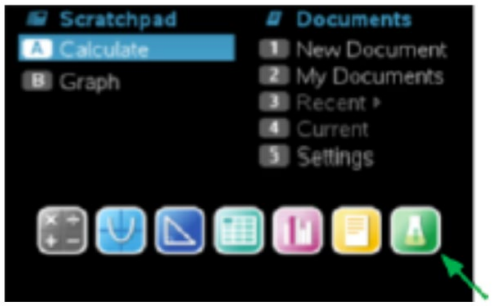


Using the Colorimeter

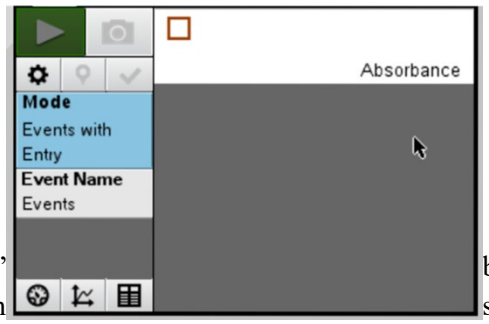
The Colorimeter is easy to use and maintain. Simply connect it to your data collection interface (TI graphing calculator), configure your software (Vernier LabPro®), and you are ready to make measurements. For best results, let the system stabilize at the desired wavelength for 5 minutes prior to calibration or data collection.

General procedure to follow when using the Colorimeter

1. Connect the Colorimeter to the interface in ch1 or ch2 or ch3.
2. Turn on the TI Nspire
3. Use the cursor with the Touchpad and press the icon 



4. The software will identify the colorimeter and load a default data collection setup.

5. Press the " < " or " > "  button on the Colorimeter to select the correct wavelength setting for your experiment (430 nm, 470 nm, 565 nm, or 635 nm).

6. Calibrate the Colorimeter. **Note**: The Colorimeter needs to be powered about 5 minutes before calibrating. One of the four green wavelength indicator lights will be turned on when it is powered.

   a. Open the Colorimeter lid.
   b. Insert a cuvette with the blank specified in your task. **Important:** Line up one of the *clear* sides of the cuvette with the arrow at the *top* of the cuvette slot. Close the Colorimeter lid.
   c. Next, press the CAL button to begin the calibration process. Release the CAL button when the red LED begins to flash. The absorbance should now be 0.00 or 0.01.
   d. When the LED stops flashing, the calibration is complete and your unit is ready to collect data.

7. Collecting data.
   a. Place the cuvette with a sample into the Colorimeter cuvette slot. **Important:** Line up the side of the cuvette with an arrow with the arrow at the *top* of the cuvette slot.
   b. Read the absorbance value

8. If the Colorimeter does not turn on - no green light - ask your lab assistant for a different cradle.

Lambert-Beer calibration law for Zinc(II) determination for Vernier Colorimeter

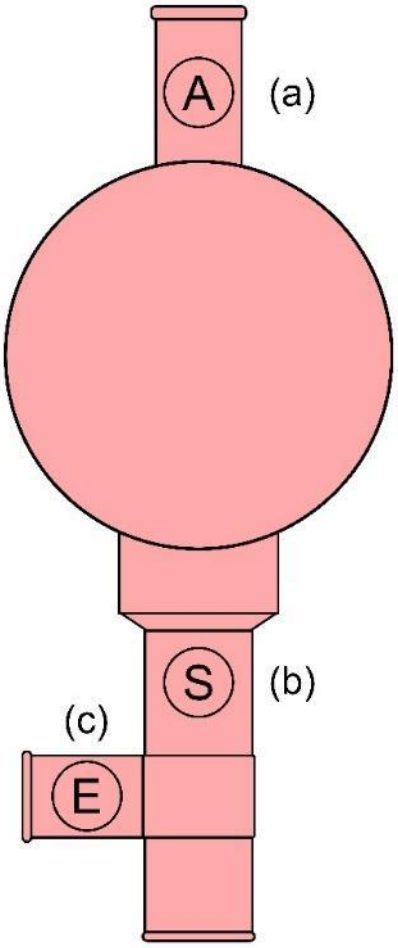
Col #	*k* (A = *k*.C) C in $mg.L^{-1}$	Co #	*k* (A = *k*.C) C in $mg.L^{-1}$	Col #	*k* (A = *k*.C) C in $mg.L^{-1}$
1	0.28	**13**	0.30	**27**	0.24
2	0.23	**14**	0.27	**28**	0.25
3	0.23	**15**	0.27	**29**	0.30
4	0.22	**16**	0.28	**30**	0.21
5	0.18	**17**	0.29	**31**	0.24
6	0.22	**19**	0.29	**32**	0.22
7	0.29	**20**	0.28	**33**	0.29
8	0.25	**21**	0.26	**34**	0.23
9	0.26	**22**	0.28	**35**	0.28
10	0.27	**23**	0.29		
11	0.26	**24**	0.29		
12	0.25	**26**	0.26		

APPENDIX 7

PIPETTING

Pipette safety instructions

- Mouth pipetting is forbidden!
- Insert the top of the pipette in the bottom of the pipette filler carefully so as not to break the glass pipette.
- Do not allow the liquid to be drawn into the bulb.



Pipette filler bulb: (a) Air valve (expels air from the bulb), (b) Suction valve (draws solution into the pipette), (c) Empty valve (drains solution from the pipette).