TEMPLATE for BEST PRACTICE EXAMPLES - SOLVED TASK

1. **Name of the task: Determination of the gender of molluscs**

2. **Why did you choose this task?**

Molluscs - bioindicators. Due to increased pollution with endocrine-disrupting substances, the gender of molluscs changes from male to female. Determining the gender of molluscs is a complex process, but this practice uses a simple color chemical reaction. Due to the different lipids accumulated depending on the gender of the mollusc, during the reaction the samples are stained with different colors (chromophores): female - pink, male - yellow. These differences can be assessed visually, a more detailed spectrophotometric analysis is performed by examining the absorption spectra. Pupils get to know molluscs species, their anatomy, learn to take mollusc mantle samples. In order to assess environmental pollution, the gender distribution of molluscs in water bodies is determined. The topic of the challenge engaged the pupils, corresponded to our educational facilities and allowed us to integrate different school subjects. This task is interesting, engaging and, most importantly, that by carrying out practical activities, it is possible to acquire new and deepen existing interdisciplinary (physics, chemistry, mathematics, art etc.) knowledge, to acquire practical and entrepreneurial skills.

3. **Subjects covered from STEAM areas:**

   Chemistry, Biology, Physics, Mathematics/ICT, Environmental Science, Art

4. **Target group (age range and size of the group):**

   Form 10th, age – 16-17 years old, 15 pupils.

5. **Duration of the activity:**

   2 lessons at school.

6. **Key words:**

Gender, molluscs, determination

7. **Key sentence describing context of the activity, followed by short description (200 words):**

To carry out STEAM activities for determination the gender of molluscs in order to assess the effects of environmental pollutants (integrated knowledge of biology, chemistry, physics, environmental studies, mathematics/ICT sciences, art).

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Project tasks:
1. Identify the species of molluscs and get acquainted with the anatomy (biology) of molluscs.
2. Determine the gender of molluscs using the following methods:
a) color chemical reaction (chemistry);
b) spectrophotometric analysis (physics).
3. Assess the impact of environmental pollutants (mathematics/ICT, environmental studies).

Usually, the gender of molluscs is determined by complex and expensive molecular genetic and histological methods. In this project, an innovative, but simple, fast and cheap colorimetric method is applied, it allows to visually assess the gender of molluscs based on the changed color of the solution, a more detailed analysis of the solutions is performed by analyzing absorption spectra with a spectrophotometer. Pupils become familiar with the anatomy of molluscs, learn to take samples of the mollusc mantle, which, when heated in a mixture of organic solvents, form one of two chromophores, depending on the mollusc's gender: when the reaction is performed with male molluscs, a bright yellow color is obtained (λ max 453 and 490 nm are measured with a spectrophotometer), which is formed as a result of 2-deoxyribose present in DNA and oxidized lipids, and in female molluscs, due to reaction with malondialdehyde, a pink color (λmax 532 nm) is formed. In order to assess environmental pollution, the gender distribution of molluscs in water bodies is determined. The predominance of the female gender leads to increased pollution with dangerous substances that disrupt the endocrine system.

8. **Description of the activity environment, including the list of materials and tools needed:**

Project activities were carried out in different educational spaces – specialized classrooms:

1. Biology and chemistry classroom: identification of the species of molluscs, anatomy of molluscs, sample preparation, colour chemical reactions, environmental studies.

2. Physics classroom: spectophotometric analysis, evaluation of physical parameters, quantitative and qualitative physical calculations, discussion and making conclusions.

3. IT and mathematics classroom: quantitative calculations and preparation of the presentation, tables and graphs using IT tools.

4. Art classroom: mixing of sample colours for getting the new colours.

Main products and tools: Molluscs, ruler, mortar and pestle, dissecting tray, plastic bags, vials, small and sharp knife to open molluscs, dissecting forceps, balance, ultrasonic bath, thermostatic bath, spectrophotometer, thiobarbituric acid, trifluoroacetic acid.

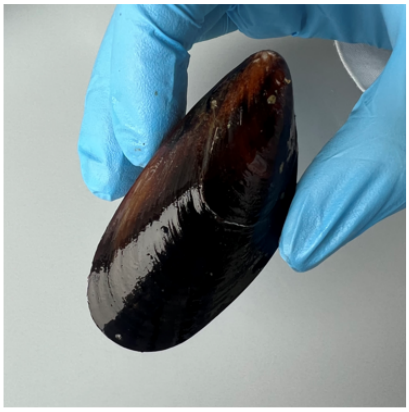
9. **Step by step, detailed description of the activity, including teaching and learning strategies:**

1. Collecting molluscs. Unio pictorum, Anodonta cygnea, Dreissena polymorpha were found in Vente Horn. Mytilus chilensis was buyed in market. Pupils learn to identify mollusc species.

Dreissena polymorpha

Unio pictorum

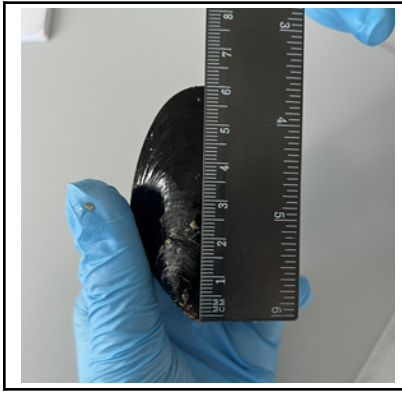
Anodonta cygnea



Mytilus chilensis

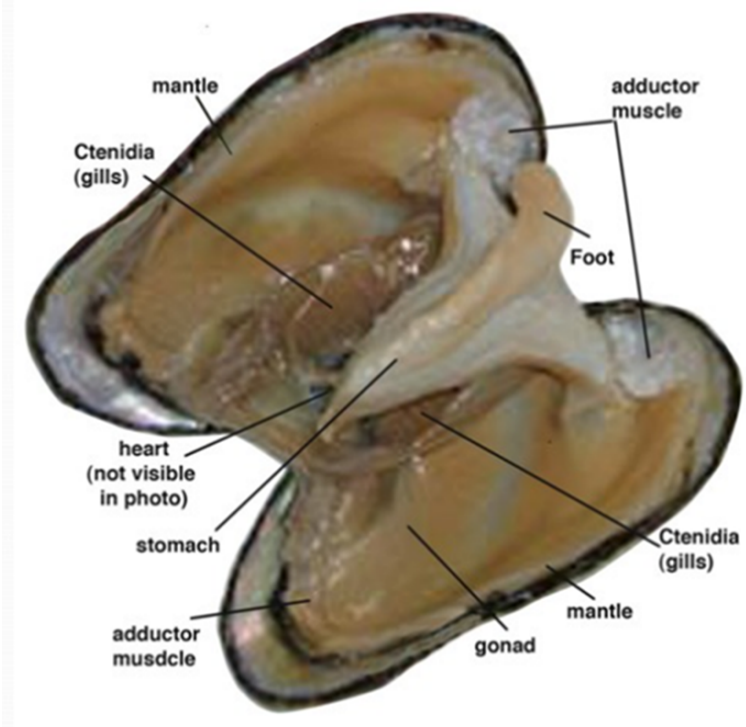
2. Sample transportation and storage. Separated by species and placed in bags, they were transported to the laboratory in a cooler. They were then stored in a -20°C freezer for 12 days. Molluscs are thawed before the experiment.

3. Shell lenght and width measurements. Measure the lenght and width of each mollusc shell, in mm. Record this on datasheet.



Species	Lenght, mm	Width, mm
Dreissena polymorpha	2±0.3	1.1±0.2
Unio pictorum	5±0.4	3±0.3
Anodonta cygnea	6.4±0.2	4.2±0.2
Mytilus chilensis	6.5±0.1	3.5±0.2

4. Anatomy of molluscs. Open each mollusc by cutting through its adductor muscles. Examine the organs.



5. Sample preparation. The mantle is taken from larger molluscs. From the smaller ones, their entire mass is taken. The taken part (about 50 mg of wet weight) is crushed in a mortar and placed in test plates and test tubes.

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2 ml of 20% trifluoroacetic acid solution and 0.5 ml of 0.75% thiobarbituric acid solution are added to test tubes with mollusc mantle.

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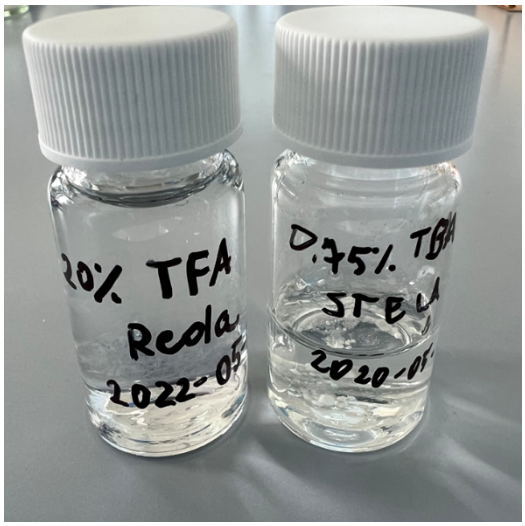
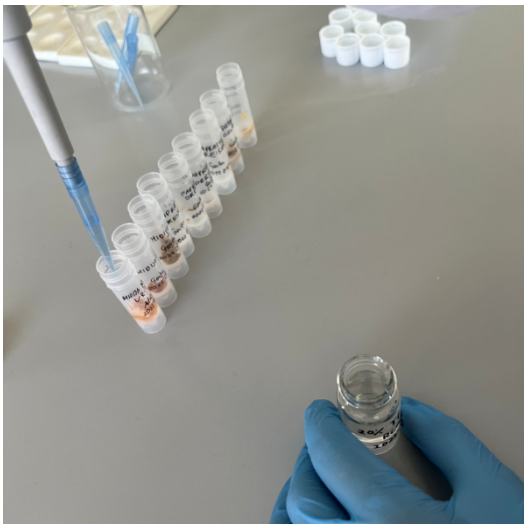
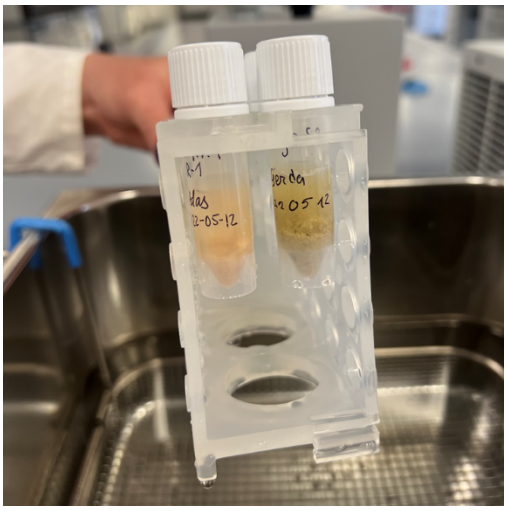


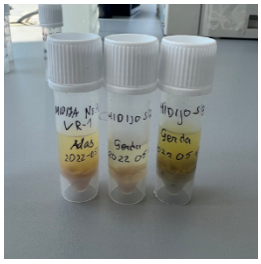


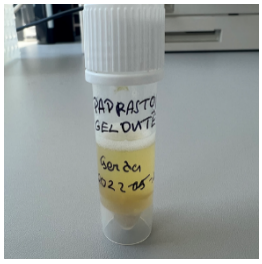
6. Colour chemical reaction. Prepared samples are placed in an ultrasonic bath for 15 min, 30°C, then in a thermostatic bath and heated to 90°C for 35 min until the color of the samples changes.

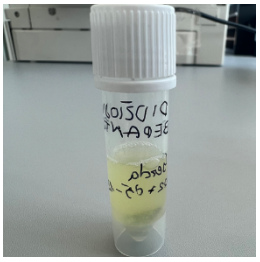
Samples in an ultrasonic bath

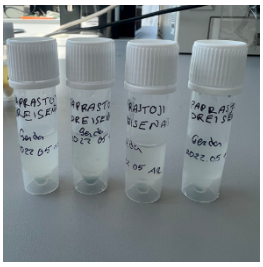


Samples taken from ultrasonic bath



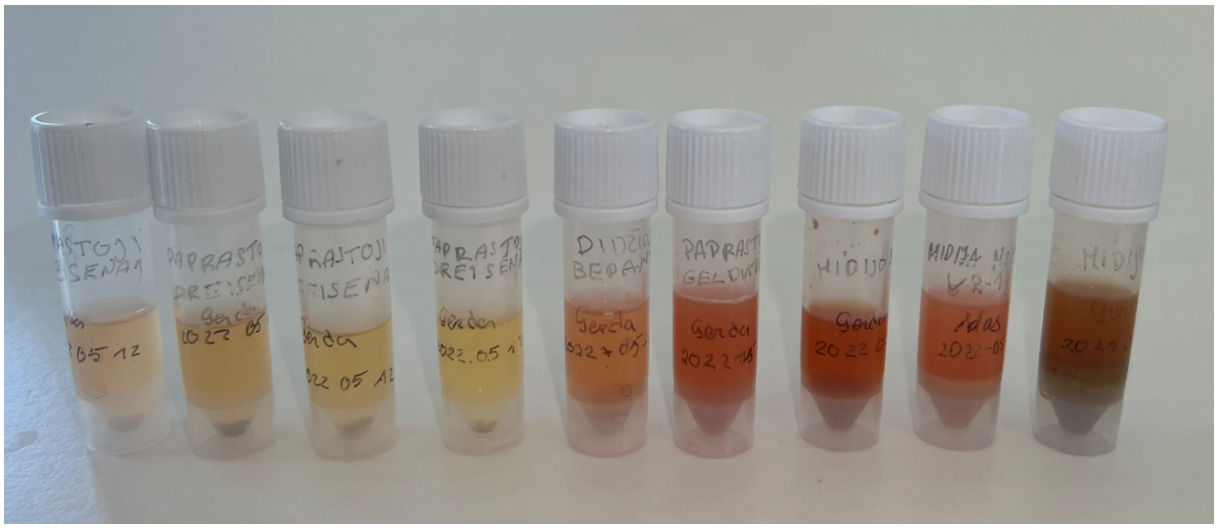






Samples in themostatic bath

Samples taken from thermostatic bath



Heating the mantle of molluscs exposed to organic acids produces one of two chromophores depending on the gender of the mollusc: the reaction with male molluscs produces a bright yellow color and in female molluscs, due to the reaction with malondialdehyde, a pink color is formed.

7. Spectrophotometric analysis. Samples were filtrated throught 0.45 μm pore size dimeter syringe filters and transfered to the cuvettes.

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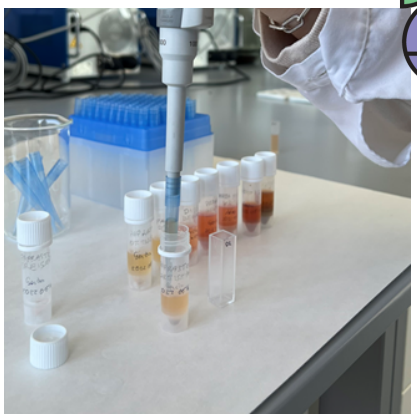
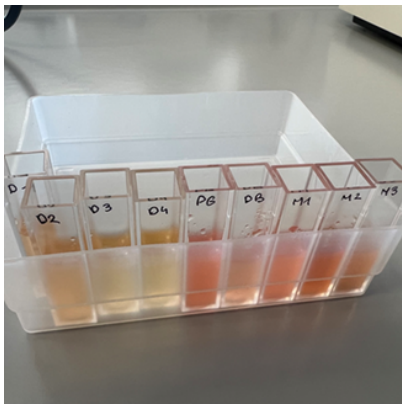
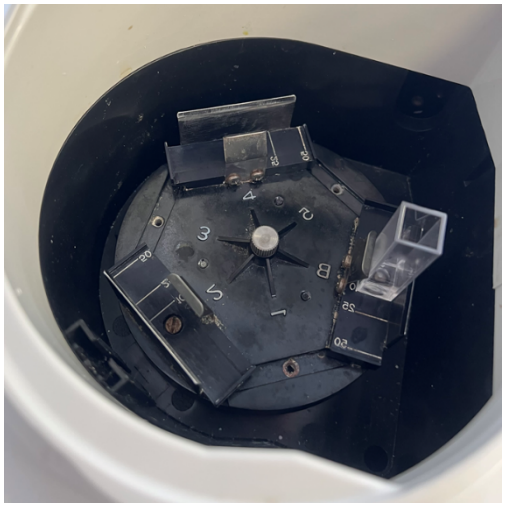
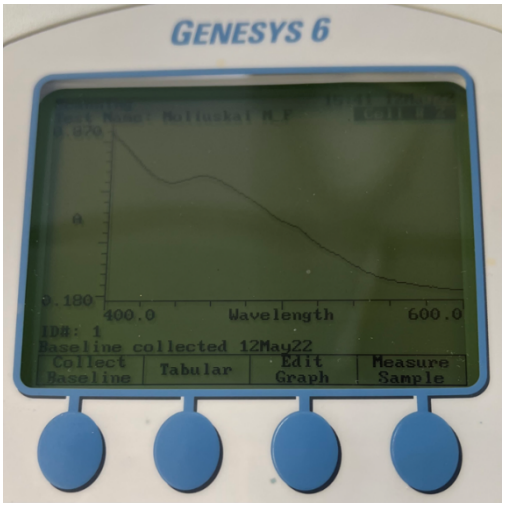




In order to accurately measure color changes, the samples are examined with a Genesys6 Termo scientifics spectrophotometer, selecting the wavelength of the light spectrum from 400 nm to 600 nm. The absorption maxima of the solutions are recorded. The male molluscs produces a bright yellow color (λmax 453 and 490 nm), which is likely to be due to 2-deoxyribose in DNA and oxidizable lipids, and in female molluscs, due to the reaction with malondialdehyde, a pink color (λmax 532 nm) is formed. The absorption maxima were recorded.





8. IT and mathematics. The obtained results of the experiment are used to calculate the distribution of mollusc species in water bodies and to develop/apply modeling programs.

9. Art and colors. We checked the colours created by mixing yellow and red. The colour varies from the portion of yellow and red that you add; for example if you add red more and just a little yellow, then the colour would come up to tomato red. The more yellow you add to red, it would change to the orange tone. If you add more yellow and a tint of red, you would see a school bus yellow colour. Therefore, the colour range basically depends on the proportion of the colour mixture.

10. Stating of conclusions.

11. Work presentation. Pupils not only made experiments, they also did integrated tasks in different school subjects: physics, biology, mathematics, chemistry, art. Visual and graphic presentation of the fulfilled tasks was done using art and IT tools.

10. Learning objectives/competencies:

In this STEM project were included these competencies: scientific concepts, scientific thinking, inquiry practice, information literacy competencies, and attitudes and accountability.

11. Evaluation/Assessment guidelines:

Group work assessment methods were applied. In different school subjects formal assessment marks were written to pupils according to their contributions in the project. Cumulative scores were applied to some pupilss (pupils who worked in several groups). Color chemistry was integrated with art and changes of the color according concentration was determined.

12. Lessons learned:

Pupils expanded their social, health, cognitive and communicational competencies. Pupils improved their knowledge about molluscs, their organs, ecological factors and ecology. Got acquainted with the basics of physics (color spectrum) and deepened the knowledge about the chemical reactions. Colour

13. Additional information/Links:

14. Contact person:

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