



GG₂
LABTORIALS
MINI Handbook

Expand your environmental science skills with hands-on experiments using everyday cosmetics

GG₂ LABTORIALS MINI Handbook

Expand your environmental science skills with hands-on experiments using everyday cosmetics,

Created within the project GreenGate₂.

Authors: Anja Bubik & Katrin Školnik Škrabe

Editor: Anja Bubik

Issued by: Faculty of Environmental Protection

Responsible person: Gašper Gantar

E-book

URL: ...

Design: Petr Miloš/GreenScan

ISBN: ...

Velenje, 2025

© Faculty of Environmental Protection, 2025

All rights reserved

The GreenGate₂ project (reg. n. 2023-2-CZ01-KA220-YOU-000174554) was implemented in the period 2024–2026 as part of the Erasmus+ programme with financial support from the European Union.

Disclaimer

Co-funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Education and Culture Executive Agency (EACEA). Neither the European Union nor EACEA can be held responsible for them.

Table of Contents

Welcome to GG ₂ LABTORIALS.....	2
What is it about?.....	3
Five key topics.....	4
General safety precautions.....	6
Experiment 1 MAKING NATURAL SOAP AND BODY SCRUB.....	7
Experiment 2 DECODING CHEMICALS ON COSMETIC LABELS.....	18
Experiment 3 THE SCIENCE OF CLEANING WATER.....	28
Experiment 4 LEPIDIUM TEST.....	38
Experiment 5 ALLIUM TEST.....	48

Welcome to GG₂ LABTORIALS



Have you ever wondered what's really in your cosmetics?

How might these ingredients influence the environment and your health?

In this handy guidebook, we're going to dive into the **fascinating world of science** to explore how different cosmetics might affect our environment and lives.

It's crucial to understand how the chemicals in cosmetic products impact our environment. Every product we use eventually ends up in nature, affecting plants, animals, and entire ecosystems. By learning about these effects, you can make more informed choices that help protect our planet.

But this isn't just any lab — it's a hands-on, exciting experiment where you get to be a scientist! You'll see the results with your own eyes and understand how these findings can apply to real life. Imagine being able to choose safer, healthier products based on your own scientific discoveries.



Get ready to roll up your sleeves, have some fun, and make some amazing discoveries.

Let's get started!

DON'T WORRY IF YOU'RE NOT A SCIENTIST YET

While some of these experiments are a bit more technical and complex, there are plenty of options to adapt them to different levels. Experiments are particularly suited for technical or natural science students, but you can find many adaptations in the last part of the tutorials to fit various needs and study profiles.

Let's make science fun and enlightening for everyone!



What is it about?

This MINI handbook is designed to guide students through hands-on experiments that explore the environmental and biological impacts of cosmetic products. From creating natural soaps and assessing the ecotoxicological effects of cosmetic chemicals on plants, to emphasize sustainable practices and ecological awareness.

Students will learn to identify harmful chemicals, explore methods to mitigate their effects, and develop skills in scientific observation and analysis. This handbook aims to foster curiosity, promote scientific practices, and inspire students to think critically about cosmetics' impact on health and the environment.



Are you saying I need to change my habits?



Let's just say... the planet would appreciate it.



Okay, but those experiments won't make me feel guilty, right?



Oh no, just highly aware.

To support this journey, the handbook is structured into **five** key topics, each presented in an interactive, varied, and practical way — making learning both engaging and accessible.

Five key topics

1

MAKING NATURAL SOAP AND BODY SCRUB

DIY with natural abrasives as alternatives to microplastics

In this workshop, you will create natural peeling & soap using different abrasives like volcanic ash, grey (black) clay, bamboo powder, crushed walnut shells or even sugar. After making the soaps and body scrubs, you will test and evaluate their texture, exfoliating properties, and overall effectiveness. You will also discuss the benefits of natural ingredients, and their environmental impact compared to synthetic alternatives.

120 min.

2

DECODING CHEMICALS ON COSMETIC LABELS

Making Healthy and Responsible Choices

Through a combination of fieldwork, mobile app usage, and database research, you will learn to read and interpret labels. You will bring your own cosmetic products, analyse their ingredients using a mobile app, and verify the data in scientific databases. Then you will compare results, discuss the potential health and environmental impacts, and gain a deeper understanding of cosmetic formulations.

90 min.

3

THE SCIENCE OF CLEANING WATER

Cleaning Up Cosmetic Wastewater with Filtration

In this workshop, you will explore methods for filtering and removing non-soluble chemicals from cosmetic wastewater. Through experiments using various leave-on and rinse-off cosmetics, you will learn about water purification, environmental protection, and sustainable practices.

90 min.

4

LEPIDIUM TEST

Can Cosmetic Chemicals Affect the Garden Cress Germination?

In this workshop, you will explore the impact of cosmetic chemicals on the germination of garden cress. You will expose seeds to different concentrations of cosmetic substances, monitor their germination, growth, and potential stress signs, and then compare the results with a control group to analyse the ecological effects.

45 min. + 45 min.

5

ALLIUM TEST

Investigating the Impact of Cosmetic Chemicals on Onion Growth and its DNA

In this workshop, you will investigate how cosmetic chemicals affect onion root growth and cell division. You will expose onion roots to different chemicals, measure their growth, and analyse their impact on mitosis using a microscope. The experiment will conclude with data analysis and a discussion on the biological and environmental implications.

90 min. + 90 min. + 90 min.

General safety precautions



The following general safety precautions apply to all experiments in this handbook.

Please review them carefully before conducting any lab work. Some experiments may also have additional specific precautions listed within their respective tutorials.

-  **Follow Instructions:** Always listen to your teacher/educator/assistant and follow the lab instructions carefully. If you're unsure about any step, ask for clarification before proceeding.
-  **Know Safety Equipment:** Familiarize yourself with the location and proper use of safety equipment like eyewash stations and fire extinguishers.
-  **Be Careful with Glassware:** Exercise caution when handling and washing glassware to avoid breakage and injury.
-  **Safety Gear:** Always wear a lab coat, safety goggles, and gloves. Ensure you have closed-toe shoes and tie back long hair.
-  **Handle Chemicals Safely:** Handle chemicals and equipment with care. Never taste or sniff chemicals. Always label containers or tubes.
-  **Check Pictograms:** Before using any chemical, review the safety pictograms on the label to understand the hazards associated with it.
-  **Handle Solvents Carefully:** Use solvents in a fume hood to avoid inhaling fumes and ensure proper ventilation.
-  **Dispose of Waste Properly:** Follow proper procedures for disposing of chemical and biological waste. Do not pour chemicals down the drain unless instructed.
-  **Report Accidents:** Immediately inform your teacher/educator/assistant of any accidents, spills, or injuries, no matter how minor they seem.

MAKING NATURAL SOAP AND BODY SCRUB

DIY with natural abrasives as alternatives to microplastics

BACKGROUND

Cosmetic products are personal care products we use to take care of our skin and body. They help us clean, protect, moisturize, and sometimes improve the way we look. One important group of cosmetics are exfoliating products, like scrubs and soaps, which remove dead skin cells. This keeps our skin feeling soft, looking healthy, and allows new skin cells to grow. It also helps other skincare products — like lotions or creams — work better.

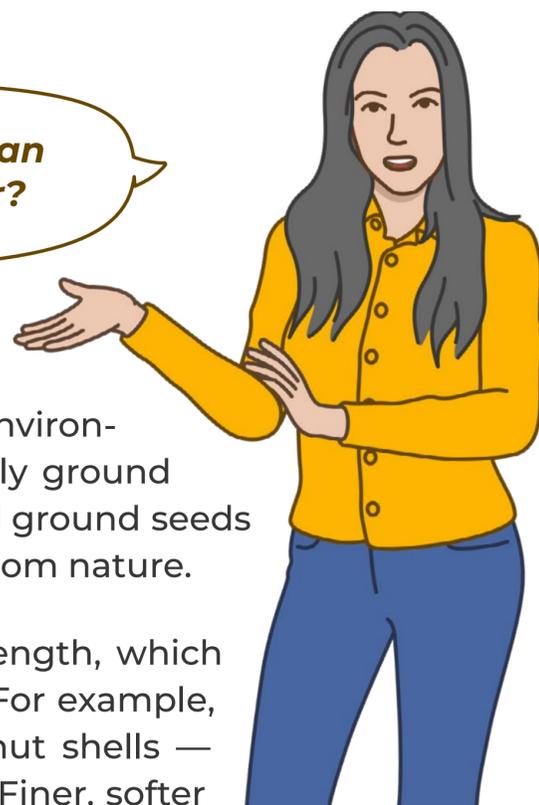
To **exfoliate** the skin, many products use small, gritty particles called abrasives. These **abrasives** scrub away old, dead skin from the surface. In the past, many cosmetics used plastic microbeads for this purpose. But microplastics are harmful to the environment and have been banned in many countries.

Did you know microplastics can end up in your drinking water?

As an alternative, there are many natural abrasives that work just as well — and are much better for the environment. These include sugar, salt, coffee grounds, finely ground walnut shells, volcanic ash, bamboo powder, clay, and ground seeds or plant fibers. They are all biodegradable and come from nature.

Each of these materials has its own texture and strength, which makes them suitable for different parts of the body. For example, larger and rougher particles—like raw sugar or walnut shells — are better for rough areas like feet, knees, or elbows. Finer, softer particles — like bamboo powder, clay, or volcanic ash — are gentler and better for the face or sensitive skin. Choosing the right particle size helps avoid irritation and keeps skin healthy.

In this experiment, students will make natural soaps and body scrubs using different types and sizes of natural abrasives. They will test how each one feels on the skin, compare how well they exfoliate, and discuss which ones work best for different parts of the body.





AIM OF THE EXPERIMENT

To understand how different natural abrasives affect the texture, exfoliation properties, and suitability of handmade soaps (A) and scrubs (B) for various parts of the body.

LEARNING OBJECTIVES

By the end of this experiment, students will be able to:

General Learning Objectives

- ✓ Understand the importance of replacing microplastics in personal care products.
- ✓ Recognize the role of abrasives in personal care and cosmetic products.
- ✓ Appreciate the environmental benefits of natural ingredients in cosmetics.

Specific Learning Objectives

- ✓ Identify different types of natural abrasives and describe their characteristics.
- ✓ Follow step-by-step procedures to create natural soap and body scrub products.
- ✓ Compare the physical properties (texture, exfoliation level) of products with different abrasives.
- ✓ Evaluate which types of abrasives are most suitable for different parts of the body.
- ✓ Assess the ecological and health risks associated with microplastic use in cosmetics.
- ✓ Reflect on the role of consumer behaviour and industry responsibility in sustainable product development.

TIME

120 min

Additional time: ~1h after 24 h; ~1h after 6–8 weeks (if soap curing is included)

MATERIALS NEEDED



Equipment

- | | |
|--|--|
| <input type="checkbox"/> Table scale (up to 100g) | <input type="checkbox"/> Glass stirring rod |
| <input type="checkbox"/> Hot plate | <input type="checkbox"/> Cold water bath (optional) |
| <input type="checkbox"/> Heat-resistant thermometer | <input type="checkbox"/> Stick blender |
| <input type="checkbox"/> Mixing bowls = Heat-resistant glass beakers (100 mL – 500 mL) | <input type="checkbox"/> Spoons or spatulas |
| <input type="checkbox"/> Weighting cup (up to 100g) | <input type="checkbox"/> Silicone moulds for soap |
| <input type="checkbox"/> 100 mL evaporator | <input type="checkbox"/> Jars or containers for scrubs |
| <input type="checkbox"/> Mixing jars, cups | <input type="checkbox"/> Labels and markers |
| | <input type="checkbox"/> Paper towels |

Materials

- Sodium Hydroxide (NaOH) pellets
- Distilled Water
- Olive oil (Other oils like coconut or almond can be used, but keep in mind that the saponification ratio varies — you must adjust the amount of NaOH based on the type of oil.)
- Coconut oil
- Natural abrasives such as:
 - Sugar
 - Finely ground walnut shells
 - Volcanic ash
 - Bamboo powder
 - Gray-black clay
- Essential oils (optional)

Sugar isn't just for tea...

it's amazing for soft skin too!



SAFETY PRECAUTIONS



Before conducting this experiment, ensure you have read and understood the **General Safety Precautions** section of this handbook.

When handling Sodium Hydroxide (NaOH):

- ⚠ Always add NaOH to water, never the other way around (to prevent splashing or violent reaction).
- ⚠ Use gloves, goggles, and a lab coat.
- ⚠ Work in a well-ventilated area or under a fume hood.
- ⚠ Handle hot containers and mixtures with care.
- ⚠ Do not ingest any materials.
- ⚠ Clean spills immediately to prevent slipping.

GHS Safety Pictograms for Sodium Hydroxide (NaOH):

Corrosive – causes severe skin burns and eye damage

Irritant – may cause respiratory irritation (if inhaled as dust or fumes)

First Aid

Skin Contact: Rinse with water for 15 minutes; remove contaminated clothing.

Eye Contact: Rinse thoroughly for 15–20 minutes; seek medical help immediately.

Inhalation: Move to fresh air; seek medical help if symptoms occur.

Ingestion: Rinse mouth; do not induce vomiting; seek medical help immediately.

When using natural soap or scrub

It is recommended to use the soap/scrub immediately or as soon as possible, as it may harden or change texture over time without preservatives.

EXPERIMENT SETUP



Step 1 → Prepare the Work Area

Ensure your workspace is clean and free from distractions. Set out all necessary materials. Wear your safety gear.

Step 2 → Prepare the Experiment

Sodium Hydroxide (NaOH) Solution

This step should be supervised closely. For safety reasons, educators may prepare this solution in advance for younger or less experienced students.

⚠ IMPORTANT: Follow Safety Precautions described above. Don't forget: always **add NaOH to water**, never water to NaOH, to avoid violent exothermic reactions (splashing, boiling, etc.).

- Work in the fume hood. If you do not have access to a fume hood, ensure good ventilation. Always wear appropriate personal protective equipment.
- Use a digital table scale and weight separately:
 - 10g of distilled water into a heat-resistant beaker
 - 5g of NaOH pellets into a weighing cup
- Use cold or room-temperature water to reduce the heat from the exothermic reaction.
- Optionally, place the beaker with water into a cold-water bath (e.g., a larger container filled with cold water or ice water) to help dissipate heat during mixing.
- Slowly and carefully add small portions of NaOH to the water (NOT the other way around) while stirring continuously with a glass stirring rod.
- Stir gently to avoid splashing and hold the beaker with your other hand or fix it in place to prevent it from tipping over.
- Wait for each portion to dissolve before adding more.
- The solution will heat up, this is normal.
- Once all NaOH has dissolved, allow the solution to cool to room temperature.

- ➔ Transfer the cooled solution to a suitable, labeled storage container.
- ➔ Label the container clearly (include concentration, date, and hazard info).

Note: The quantities listed above are suitable for making approximately 40g of soap. If you wish to prepare a larger batch, you can scale the solution accordingly.

Step 3 → Conduct the Experiment

A Making NATURAL SOAP (40 g)

1. Weigh separately 25g of olive oil and 7g of coconut oil.
2. Combine both oils in a heat-resistant container (e.g., evaporating dish or glass jar).
3. Melt the oils using a hot water bath.
4. Stir thoroughly using a glass rod until oils are dissolved and mixed.
5. Allow the oil mixture to cool to approximately 40°C.
6. Transfer the cooled oils in a beaker or container suitable for blending.
7. Slowly add the cooled NaOH solution into the melted oils while stirring with a glass rod.
8. The mixture will turn darker, opaque, and thicker, this is normal.
9. Blend the mixture with a stick blender until it reaches a “pudding-like” (approx. 5 minutes).
10. Be careful not to splash—keep the blender fully submerged and mix gently at first to avoid the soap mixture spraying out.
11. Divide the soap mixture into three small bowls.
12. Add approx. ½ teaspoon of a different abrasive into each bowl (e.g., volcanic ash, clay, walnut shells).
13. You can adjust the amount of abrasive depending on the desired exfoliating strength.
14. Mix each thoroughly.
15. Pour into soap moulds and allow to cool and harden.

After 24 h hours

16. Remove soaps from the moulds carefully.
17. Wear gloves during unmoulding to protect your hands from any uncured soap.
18. Place them in a well-ventilated area to continue curing.
19. The longer the soap is left to cure in a cool, dry place, the better it becomes—it hardens more, lasts longer, and is gentler on the skin.

After 4–8 weeks

20. The soap is fully cured and ready to use.
21. Label your samples and record observations (texture, colour, smell, exfoliation feel).



Bro, this scrub lowkey feels like sandpaper.



That's 'cause you picked walnut shells, not fairy dust.

B**Making NATURAL BODY SCRUB (10 g)**

1. In three separate containers, mix:
 - 2 teaspoons of coconut oil (10 g)
 - 1 teaspoon of a single natural abrasive (sugar, bamboo powder or ground walnut shells)

You can adjust the amount of abrasive depending on the desired exfoliating strength.

2. Add a few drops of essential oil (optional).
3. Mix well and label each container.



Chill, Dr. Coconut Oil.



I've made one scrub and now I think I'm a skincare guru.

Step 4 → Monitor and Record Data



- Observe and describe the texture of each abrasive before and after mixing.
- Compare the feel and consistency of each soap and scrub.
- Gently test each product on a small area of the forearm.
- Record how each feels on the skin and note any differences in exfoliation power.

Create a table to record abrasive type, product type (soap or scrub), texture, exfoliation strength (gentle/moderate/strong), and suggested body area for use (face, hands, feet, etc.).

RESULTS

Use a table or chart to compare the properties of each product. Compare your product with those of your classmates or other groups that used a different abrasive.

	<i>Abrasive</i>	<i>Texture</i>	<i>Exfoliation Level</i>	<i>Suitable Body Area</i>	<i>Special Notes</i>
Soap 1					
Soap 2					
Soap 3					
Scrub 1					
Scrub 2					
Scrub 3					

ANSWER KEY QUESTIONS



Answer the questions either orally or in writing. Emphasize collaboration and critical thinking throughout the process!

1. Which abrasive was most effective and why?
2. Which product was the most skin-friendly or pleasant to use?
3. How would the choice of oil influence the final texture or effectiveness?
4. Can you identify real-world products that use similar natural abrasives?

Let's think critically:

5. How does this experiment connect to real-world applications or further research?
6. What further research or testing could be done to improve these DIY formulations?
7. Could these types of natural products be marketed sustainably?



Natural stuff just feels more real, y'know?



Like skincare that actually has good intentions.

FOR EDUCATORS



Additional Activities/Extensions (Optional):

- Challenge students to redesign the experiment with a new variable (e.g., oil type, abrasive ratio).
- Organize a presentation or poster session where groups compare their formulations.
- Research and try a new natural exfoliant found in the literature such as poppy or strawberry seeds.

Adapting the Experiment for **Secondary School Students:**

- Use only one type of soap and one scrub to simplify.
- Pre-measure all ingredients.
- Focus on basic sensory testing (feel, smell).

Adapting the Experiment for **Primary School Students:**

- Focus on sensory exploration: texture, smell, and safe play; present as a “science of touch” activity.
- Use pre-measured ingredients and clear visual instructions.
- Work only with scrubs (no lye or soap base).

DECODING CHEMICALS ON COSMETIC LABELS

Making Healthy and Responsible Choices

BACKGROUND

Cosmetic products contain a variety of chemical ingredients, each serving a specific purpose — from preserving freshness to creating texture, scent, or desired effects on skin and hair. A single product can contain anywhere from 10 to over 50 different substances. Many of these names — like Methylchloroisothiazolinone, Butylphenyl Methylpropional, or Cyclopentasiloxane — may sound complex, unfamiliar, or even intimidating. It's no surprise that consumers, especially young people, often feel overwhelmed and unsure of what they're really applying to their skin, hair, or body.

At the heart of every cosmetic product is the **INCI list** — the International Nomenclature of Cosmetic Ingredients. This is a standardized system used globally to list all ingredients on product packaging. The INCI list follows specific rules:

- ➔ Ingredients are written in Latin (for plant extracts) or English (for chemical substances).
- ➔ They are listed in descending order of concentration (highest amounts first).
- ➔ It provides a transparent and universal way to understand what's really inside a product.

Understanding how to read an INCI list allows consumers to identify key functional ingredients (e.g., active agents, preservatives, emulsifiers), spot potential allergens or harmful substances, compare products from different brands, and make choices that align with personal health, ethical, or environmental values. By learning to navigate these lists with the help of science-based databases and user-friendly mobile applications, we can gain practical life skills — becoming more informed, responsible, and health-conscious consumers in a world saturated with marketing claims and complicated labels.

In this workshop, students will learn how to critically analyse cosmetic products using digital tools, understand the role of chemical ingredients, and make informed decisions about product safety and sustainability.



AIM OF THE EXPERIMENT

To explore how mobile applications and scientific databases can be used to analyse the ingredients in cosmetic products and assess their functions, safety, and environmental impact.

LEARNING OBJECTIVES

By the end of this experiment, students will be able to:

General Learning Objectives

- ✓ Read and interpret labels on cosmetic product declarations.
- ✓ Utilize mobile applications for ingredient research and safety assessment.
- ✓ Use scientific databases to analyse and evaluate cosmetic ingredients.

Specific Learning Objectives

- ✓ Identify and explain common chemicals found in cosmetic products.
- ✓ Distinguish between active ingredients, preservatives, emulsifiers, and fragrances in cosmetics.
- ✓ Compare the safety profiles of similar ingredients across different products and brands.
- ✓ Evaluate the potential effects of these ingredients on human health and the environment.
- ✓ Interpret ingredient ratings and potential risks as provided by different databases.
- ✓ Draw conclusions about the safety and sustainability of different cosmetic products based on ingredient analysis.

TIME

90 min

MATERIALS NEEDED



Equipment

- Various cosmetic products (shampoos, lotions, creams, makeup, etc.)
- Mobile device with internet access
- Mobile app GreenScan for ingredient analysis and product evaluation
- Access to scientific databases (e.g., PubChem is freely available)

SAFETY PRECAUTIONS

-  Before conducting this experiment, ensure you have read and understood the **General Safety Precautions** section of this handbook.



Additionally, be aware of the following specific safety precautions:

-  Follow ethical guidelines when using mobile applications and researching scientific databases.

EXPERIMENT SETUP



Step 1 → Prepare the Work Area

- Assign students into small groups (3–4 students).
- Make sure each group has a mobile device with internet access and a computer or tablet.
- Download the selected mobile application for ingredient analysis – **GreenScan app**: <https://green-gate.eu/greenscan-app/>
- Ensure that each group has access to the **PubChem database**: <https://pubchem.ncbi.nlm.nih.gov/>

Step 2 → Prepare the Experiment

Before the session:

- Each student brings 3 different cosmetic products from home in their original packaging (e.g., skin care items, oral care products, decorative cosmetics, etc.)
- If needed, you can also visit a shop together to purchase products as a group.

At the start of the session:

- Each group collects and arranges all products on the table.
- Each group should have approximately 9–12 different cosmetic products to analyse.

Step 3 → Conduct the Experiment

Follow the instructions A, B and C and write down all observations and data in the spreadsheet attached after the experiment procedure.

A

Conduct the Product Review

1. Sort the cosmetic items into categories: skin care, oral hygiene, hair care, decorative, etc.
2. Carefully read the ingredient list on each product.

3. Count how many ingredients each product contains.
4. Identify and mark ingredients that are difficult to pronounce or unknown.
5. As a group, decide, does the product seem healthy/safe to use based on the label? Assign a rating “**My FLAG**” from 1 to 5 for each product:

1 = Red = Poor (not safe, would avoid)

2 = Orange = Questionable (use with caution)

3 = Yellow = Fair (acceptable, but not ideal)

4 = Light Green = Good (mostly safe, reliable choice)

5 = Green = Excellent (safe, good choice)



Guys, I'm trying to pronounce this one: 'Methylchloroisothiazolinone'. Did I just summon a demon?



Nah, that's just your conditioner trying to preserve itself for the next 300 years.

B

Conduct the Product Review

1. Scan the product barcode using the GreenScan app.
2. If the product is not in the database of the GreenScan app, scan the barcode and enter it yourself (and move to another product).
3. Check if the ingredient list shown in the app matches the actual label.
4. Look at the app's ingredient classification: How does the app rate the overall product? What colour or score does the app assign to it?
5. Focus on ingredients marked in red or flagged as hazardous in the app.
6. Write down the names of 2–3 of the most concerning chemicals found.
7. Assign a rating “**GreenScan FLAG**” based on the category in the app.





Hey, I just scanned my shampoo with GreenScan, and it said: 'RED FLAG'!



Dude, your hair's been surviving a chemical war zone, and you didn't even know?

C

Research Using Scientific Database

1. Search the ingredients on PubChem.
2. Search for each of the red-flag ingredients.
3. Record the following information:
 - Chemical function
 - Health risks
 - Environmental impact
 - Toxicity level (if available)
4. Assign a rating “**Database FLAG**” from 1 to 5 for each product:

1 = Red = Poor (not safe, would avoid)

5 = Green = Excellent (safe, good choice)

Step 4 → Monitor and Record Data

Write all your findings clearly and accurately in the spreadsheet below.

RESULTS

Consider your initial score, app rating, and database findings. Put all observations in the spreadsheet and compare the results.

Colour the product based on your own feeling (My FLAG), on the GreenScan app (GreenScan FLAG) and the database data (Database FLAG).



Based on your three-step evaluation process assign a final product flag as a group (“**OVERALL FLAG**”):

Green Flag = Safe, good choice, low-risk ingredients

Yellow Flag = Moderate, mixed ingredients, some concerns

Red Flag = Unsafe, contains hazardous or toxic substances

	NAME of the product	TYPE of the Product	No. of Ingredients	My FLAG	Green-Scan FLAG	Data-base FLAG	OVERALL Flag
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							



ANSWER KEY QUESTIONS

Work together as a group to discuss your results and reflect on your findings. Answer the questions either orally or in writing. Emphasize collaboration and critical thinking throughout the process!



1. Why is it important for cosmetic products to list all ingredients clearly on the label?
2. How can ingredient labelling help protect consumer health and safety?
3. What challenges do consumers face when trying to understand these labels?
4. What trends did you notice in the data regarding common cosmetic ingredients?
5. Why do you think certain chemicals are more frequently used in cosmetic products?
6. How do ingredient ratings vary between different databases or mobile apps?
7. What are the real-world applications of understanding cosmetic ingredients?
8. How can consumers use this knowledge to make informed purchasing decisions?

Let's think critically

9. What patterns or trends did you observe in the ingredients used across different products?
10. What surprised you most about the data you found? (e.g., a natural-looking product having many red-flag ingredients?)
11. What are some real-world applications of being able to analyse cosmetic ingredients?
12. How can this knowledge empower consumers when choosing products?
13. If you were to create your own cosmetic product, what standards or guidelines would you follow for ingredient safety and sustainability?

FOR EDUCATORS

Additional Activities/Extensions (Optional):



- ➔ **Analyse:** compare chemical compositions of different cosmetic products; identify recurring ingredients; create basic statistics such as frequency charts or tables; categorize ingredients by function; discuss trends and patterns
- ➔ **Debate:** Harmful or Helpful? debate about controversial cosmetic ingredients (e.g., parabens, sulphates).
- ➔ **DIY Safer Product Labels:** redesign cosmetic labels to include safety icons.
- ➔ **Greenwashing Investigation:** research brands that market themselves as “natural” or “eco-friendly” and verify the accuracy of those claims using databases.

Adapting the Experiment for **Secondary School Students:**

- ➔ **Create Ingredient Flashcards:** Each group designs cards for 10 common ingredients. with info like function, risk level, and environmental impact—use them for class review games.

Adapting the Experiment for **Primary School Students:**

- ➔ **Posters:** Create visual posters categorizing common cosmetic ingredients (preservatives, emulsifiers, etc.) with examples and functions.
- ➔ **Interview a Professional:** Invite a cosmetic chemist, pharmacist, or dermatologist to share insights, or have children prepare questions and research expert opinions online.

THE SCIENCE OF CLEANING WATER

Cleaning Up Cosmetic Wastewater with Filtration

BACKGROUND

Cosmetic wastewater contains various contaminants, including surfactants, oils, dyes, preservatives, fragrances and microplastics. These pollutants can persist in aquatic environments, disrupting ecosystems, harming wildlife, and even entering human water supplies. Many of these compounds are resistant to natural degradation and can bioaccumulate in aquatic organisms.

As more personal care products are used daily and washed down the drain, understanding how to treat and clean this wastewater becomes essential. Filtration is one of the foundational steps in water treatment, helping remove suspended solids and improve water clarity and quality.

Filtration is a mechanical or physical process that separates solids from liquids by passing the mixture through a porous medium. In environmental water treatment, gravity filtration and vacuum filtration are commonly used to remove suspended particles. **Gravity filtration** relies on the natural force of gravity to move water through the filter, while **vacuum filtration** uses suction to accelerate the process, often resulting in more efficient and thorough removal of fine particles.

The **Urban Wastewater Treatment Directive (UWWTD)** is a key piece of EU legislation that regulates how municipalities treat wastewater. A recent revision of the directive aims to address emerging pollutants, such as pharmaceuticals and cosmetic residues, which were not previously covered (check the G-book₂ for details).



Wait, this glittery scrub and perfume water actually ends up in rivers and lakes?

Oh yes! Whatever goes down your drain doesn't just disappear — it keeps moving.



So, fish are swimming in sparkles and smelling like a perfume shop?

Unfortunately, yes. And trust me, glittery gills are not a trend in the wild.



In this experiment, students will explore how filtration methods—such as gravity and vacuum filtration—can reduce contaminants found in cosmetic wastewater, helping to improve water quality and support environmental protection.

AIM OF THE EXPERIMENT

To determine the effectiveness of filtration in removing solid contaminants from cosmetic wastewater and analyse changes in pH and turbidity that cosmetics cause in water.



LEARNING OBJECTIVES

By the end of this experiment, students will be able to:

General Learning Objectives

- ✓ Identify and explain the role of filtration in water treatment.
- ✓ Understand how different cosmetic products (rinse-off vs. leave-on) behave in water treatment.

Specific Learning Objectives

- ✓ Compare gravity vs. vacuum filtration in removing cosmetic effluent chemicals.
- ✓ Measure pH and turbidity to observe changes in water quality.
- ✓ Use a sedimentation funnel to determine the total solid particles (TSP) before and after filtration.

TIME

90 min

MATERIALS NEEDED



Chemicals

- 3 rinse-off products: e.g., shampoo, face wash & liquid soap
- 3 leave-on products: e.g., foundation, lotion & sunscreen.

Equipment:

Gravity Filtration System:

- Plastic bottle
- Filtration layers: gravel, coarse sand, fine sand, activated charcoal, cotton or filter paper

Vacuum Filtration System:

- Lab vacuum pump (manual or electric)
- Büchner funnel and flask
- Filter paper (0.45 μm)
- Rubber tubing

Sedimentation & Analysis Tools:

- Sedimentation funnel (e.g., Imhoff cone or any conical sedimentation funnel)
- Graduated cylinder (100 mL or larger)
- Beakers (for sample preparation & collecting samples before & after filtration)
- pH paper or pH meter
- Turbidity meter (or visual clarity assessment)

SAFETY PRECAUTIONS

Before conducting this experiment, ensure you have read and understood the **General Safety Precautions** section of this handbook.

-  There are no additional safety precautions specific to this experiment.



EXPERIMENT SETUP



Step 1 → Prepare the Work Area

- Ensure your workspace is clean and free from distractions.
- Set out all necessary materials.
- Wear your safety gear.

Step 2 → Prepare the Experiment

Prepare the cosmetic wastewater samples:

- Label two beakers: "*Rinse-off products*" and "*Leave-on products*".
- Prepare 2 samples of cosmetic wastewaters:
 - Mix shampoo, face wash, and soap in approx. 500 mL of tap water (rinsed-off sample).
 - Mix lotion, foundation, and sunscreen in approx. 500 mL of tap water (leave-on sample).
- Stir both samples thoroughly to simulate wastewater.



Do people really wash all this down the drain?



Yep. Every shower is like a mini pollution party.

Assemble the gravity filtration system with the following layers:

- ➔ Cut the bottle into funnel shape.
- ➔ Place cotton or filter paper at the bottom (first barrier).
- ➔ Add layers in this order (from bottom to top):
 - activated charcoal (removes chemicals, odours)
 - fine sand (traps small particles)
 - coarse sand (removes medium particles)
 - gravel (supports upper layers and prevents clogging)

Assemble the vacuum filtration system:

- ➔ Place filter paper (0.45 µm or similar) in a Büchner funnel.
- ➔ Attach the funnel to a vacuum flask.
- ➔ Connect the flask to a manual or electric lab vacuum pump using rubber tubing.
- ➔ Check for airtight seals.

Step 3 → Conduct the Experiment**Initial Testing:**

- ➔ Measure and record the initial pH and turbidity of both samples using a pH meter/paper and a turbidity meter (or visual scale).
- ➔ Measure Total Solid Particles (TSP) using a sedimentation funnel or graduated cylinder (let solids settle and record volume).

Filtration Process:

- ➔ Divide each sample into two subsamples.
- ➔ Pour one subsample through the gravity filtration system; collect filtered water in clean beakers.
- ➔ Repeat the process using the vacuum filtration setup with new filter paper using another subsample.

Post-Filtration Testing:

- ➔ Measure and record pH and turbidity again for each filtered sample.
- ➔ Measure TSP after filtration using the sedimentation funnel or similar method.

Step 4 → Monitor and Record Data

Enter your results into the provided data spreadsheets.



*It's like a super-sucker.
Gravity's just... chillin'.*

*Why is the vacuum one
so much faster?*



RESULTS

Fill both spreadsheets below with obtained measurements **before** and **after** filtration.



pH & Turbidity Data

Sample Type	Initial pH	Gravity Filtered pH	Vacuum Filtered pH	Initial Turbidity	Gravity Turbidity	Vacuum Turbidity
Rinse-off						
Leave-on						

Total Solid Particles (TSP) Data

Sample Type	TSP Before Filtration (mL)	TSP After Gravity Filtration (mL)	TSP After Vacuum Filtration (mL)
Rinse-off			
Leave-on			

If available, include **Before & After photos** of water samples and provide a simple note next to them:

ANSWER KEY QUESTIONS



Answer the questions either orally or in writing. Emphasize collaboration and critical thinking throughout the process!

1. What are the main differences between rinse-off and leave-on cosmetic products?
2. Which filtration method removed more solid particles—gravity or vacuum?
3. Was leave-on cosmetic wastewater harder to filter than rinsed-off products? Why?
4. How did the pH and turbidity change after filtration?
5. Why is it important to monitor water quality when treating wastewater?

Let's think critically:

6. How does this experiment relate to real-world wastewater treatment?
7. Why might vacuum filtration be more effective or efficient than gravity filtration in real wastewater treatment plants?
8. Some cosmetic ingredients are not visible (e.g., microplastics, preservatives). What challenges do these pose for water treatment systems?
9. If this experiment were scaled up to industrial levels, what environmental or economic factors should be considered?
10. Should cosmetic companies be responsible for reducing the environmental impact of their products? Why or why not?

FOR EDUCATORS



Additional Activities/Extensions (Optional):

- ➔ **Compare ingredient solubility:** Test how water-soluble vs. insoluble cosmetic ingredients behave in filtration.
- ➔ **Try alternate materials:** Replace or remove components (e.g., use cotton balls instead of sand) and observe filtration differences.

Adapting the Experiment for **Secondary School Students:**

- ➔ **Simplify data focus:** Have students record only pH changes using pH stripes before and after filtration.
- ➔ **Design investigations:** Let students modify one variable (e.g., filter thickness or contaminant type) and analyse its effect.

Adapting the Experiment for **Primary School Students:**

Simplify the experiment:

- ➔ **Visual-based observation:** Ask questions like “Is the water cleaner?” or “What changed?” instead of using tools.
- ➔ **Keep it simple and creative:** Use basic filters (e.g., cotton and gravel) and let students draw or name their water-cleaning invention.

LEPIDIDIUM TEST

Can Cosmetic Chemicals Affect the Garden Cress Germination?

BACKGROUND

Biological tests help us understand how environmental conditions might harm living things. These tests often use bioindicator organisms, like certain plants or animals, which show changes in their behaviour, health, or appearance when the environment is polluted.

Garden cress, also known as *Lepidium sativum* (*Lepidium s.*) can be used as a bioindicator. It is a fast-growing, edible herb with a peppery flavour. It is commonly used in salads and as a garnish. This plant is easy to grow and is often used in educational settings to demonstrate plant growth and germination.

Garden cress is sensitive to various pollutants, cost-effective, and easy to handle, making it an excellent indicator of toxicity through changes in germination rates. It responds to environmental changes, such as pollution, by showing alterations in its growth and development. For example, when exposed to harmful substances, *Lepidium s.* may exhibit changes in germination rates, growth patterns, or overall health, making it a useful plant for monitoring environmental conditions.



And it doesn't even need a cape!

It's like the superhero of plants – fast, sensitive, and super easy to handle.



A germination test is a method used to determine the viability of seeds. It measures the percentage of seeds that are capable of sprouting and growing under optimal conditions.

In this experiment, students will determine the potential negative impact of chemicals from our daily cosmetics on the germination of *Lepidium s.* seeds.



AIM OF THE EXPERIMENT

To expose *Lepidium sativum* seeds to cosmetic products, monitor their germination compared to a control group, and analyse the effects of these chemicals on seed viability and early growth.

LEARNING OBJECTIVES

By the end of this experiment, students will be able to:

General Learning Objectives

- ✓ Gain a comprehensive understanding of how chemicals affect living organisms and ecosystems.
- ✓ Develop skills in designing and conducting scientific experiments.
- ✓ Increase awareness of the environmental impact of everyday products, particularly cosmetics, and their potential risks to plant life.

Specific Learning Objectives

- ✓ Identify and explain the effects of different concentrations of chemicals on seed germination.
- ✓ Set up and conduct a germination test using *Lepidium s.* seeds.
- ✓ Analyse the data collected from the experiment.
- ✓ Draw valid conclusions based on the results and connect them to the scientific principles learned.
- ✓ Apply findings to environmental risk assessment.

TIME

Part I: 45 min

Part II (after 48 h): 45 min

MATERIALS NEEDED



Equipment:

- Approx. 100 Garden cress seeds (*Lepidium s.*)
- Plastic or glass Petri dishes
- Filter paper
- Plastic Pasteur pipettes (droppers)
- Beakers (100, 250 and 500 mL)
- Volumetric flasks (100 mL)
- Measuring cylinders
- Plastic tray
- Tray cover (e.g. aluminium foil)
- Dark storage area
- Millimetre ruler

Chemicals:

- Tested chemical/cosmetic product (EXAMPLE: 30% hydrogen peroxide or Shampoo)
- Tap water (negative control)
- 1% solution of 0.1 M HCl or simply just vinegar (positive control)

SAFETY PRECAUTIONS

Before conducting this experiment, ensure you have read and understood the **General Safety Precautions** section of this handbook.

-  There are no additional safety precautions specific to this experiment.



EXPERIMENT SETUP



Step 1 → Prepare the Work Area

Ensure your workspace is clean and free from distractions. Set out all necessary materials. Wear your safety gear.



Clean workspace? Does that mean I have to tidy up my desk?



Yes, and no distractions! So, no checking your phone every five minutes.

Step 2 → Prepare the Experiment

Concentrations: Prepare 3 different concentrations (c_1 – c_3) or dilutions (R_1 – R_3) of tested chemical. You can also choose the cosmetic product. Test each concentration in 2 replicates (A and B).

TESTED CHEMICAL/COSMETIC PRODUCT: _____

PICTOGRAMS: _____

Volume: Each test solution should have a final volume of 100 mL.

If using a specific chemical:

Calculate the amount of stock solution (R_0) and tap water (H_2O) needed for each test solution (R_1 – R_2).

DILUTION		Chemical Concentration		Volume of Chemical (mL)	Volume of H_2O (mL)
R_0	Undiluted (stock)	c_0		/	/
R_1	10×	c_1			
R_2	100×	c_2			
R_3	1000×	c_3			

If using a cosmetic product:

Estimate rough dilutions based on volume percentages.

DILUTION		Product Concentration	Volume of product (mL)	Volume of H₂O (mL)
R₁	25%	C ₁		
R₂	10%	C ₂		
R₃	1%	C ₃		

Control Samples: Use tap water (C_{neg}) as negative control and 1% solution of 0.1 M HCl or vinegar as a positive control (C_{pos}).

Positive and negative controls are essential for validating the results. A positive control shows the expected effect to confirm the experiment works, while a negative control shows no effect to ensure any observed changes are due to the treatment being tested.

Petri dishes: Appropriately label the Petri dishes (e.g., c₁ A) and place them on a plastic tray. Label each Petri dish both on the bottom and the cover side to prevent mixing up the samples.

Garden cress seeds (*Lepidium s.*): Collect *Lepidium s.* seeds (3 tested concentrations in 2×10 replicates of each + 2 controls), all together approx. 100 seeds.

Step 3 → Conduct the Experiment

PROCEDURE

1. Cut appropriately sized circles from filter paper to fit precisely at the bottom of the Petri dish.
2. Using a dropper, moisten the substrate of each Petri dish with different concentrations of the chemical, product or water (2 mL/Petri dish). Use two (A and B) Petri dishes/replicates for each concentration.
3. Evenly distribute 10 *Lepidium s.* seeds on the moistened substrate in each Petri dish.
4. Place the Petri dishes with the tray in a dark place.
5. Leave the seeds to germinate for 48h in total on the tray in the dark.
6. (OPTIONAL) After 24 hours, add 1 mL of the appropriate concentration solution to the Petri dishes.



Looks like our garden cress is having a spa day with all these cosmetic treatments!

Step 4 → Monitor and Record Data

After 48 hours, measure the root length using a millimeter ruler.

Root Length Measurements

Concentration	C_{neg}		C_{pos}		C_1		C_2		C_3	
Replicate	A	B	A	B	A	B	A	B	A	B
Root 1 (mm)										
Root 2 (mm)										
Root 3 (mm)										
Root 4 (mm)										
Root 5 (mm)										
Root 6 (mm)										
Root 7 (mm)										
Root 8 (mm)										
Root 9 (mm)										
Root 10 (mm)										
Average Root Length (mm)										

ANSWER KEY QUESTIONS



Answer the questions either orally or in writing. Emphasize collaboration and critical thinking throughout the process!

1. Why do you think garden cress is a suitable bioindicator for environmental studies?
2. What factors can influence the germination rate of garden cress?
3. How did the different concentrations of the chemical affect the growth of *Lepidium s.* seeds?
4. Which concentration showed the most significant negative effect on seed germination?
5. How did the control groups (positive and negative) compare to the treated groups in terms of germination rates and plant health?

Let's think critically:

6. Based on the results, what recommendations can be made regarding the use of these chemicals in products that may impact plant life?
7. If you were to design a follow-up experiment, what additional variables or conditions would you include to further investigate the effects of these chemicals?
8. How can the results of a germination test with garden cress be applied to real-world environmental monitoring?

FOR EDUCATORS



Additional Activities/Extensions (Optional):

Propose a new experiment by modifying one variable – for example, select more concentrations of tested chemical/cosmetic product or add a different chemical or chemical mixture. You can also monitor the seed germination over different times (after 48, 72 and 96 hours) of exposure.

Optionally, photograph the Petri dishes daily and create a photographic collage of germination.

Adapting the Experiment for **Secondary School Students:**

- ➔ **Use Fewer Concentrations:** Reduce the number of samples to three (e.g., control, low, and high concentration) to simplify data collection and analysis.
- ➔ **Visual Observation:** Encourage students to make detailed visual observations of seed germination.

Example:

- ✔ **Objective:** Measure the effect of a household chemical (e.g., vinegar) or a cosmetic product (e.g. shampoo) on the seed germination process.
- ✔ **Data Collection:** Record root lengths and compare the average lengths across different concentrations and make a photo collage.

Adapting the Experiment for **Primary School Students:**

- ➔ **Visual Observation:** Children can learn about the germination in a simple and visually engaging way by observing seeds changes during a selected time.

Example:

- ✔ Enjoy creative activities like drawing and photographing results
- ✔ Draw and write about your observations in a journal or on a piece of paper each day. Paste the photos in your own journal or use them to create a collage.

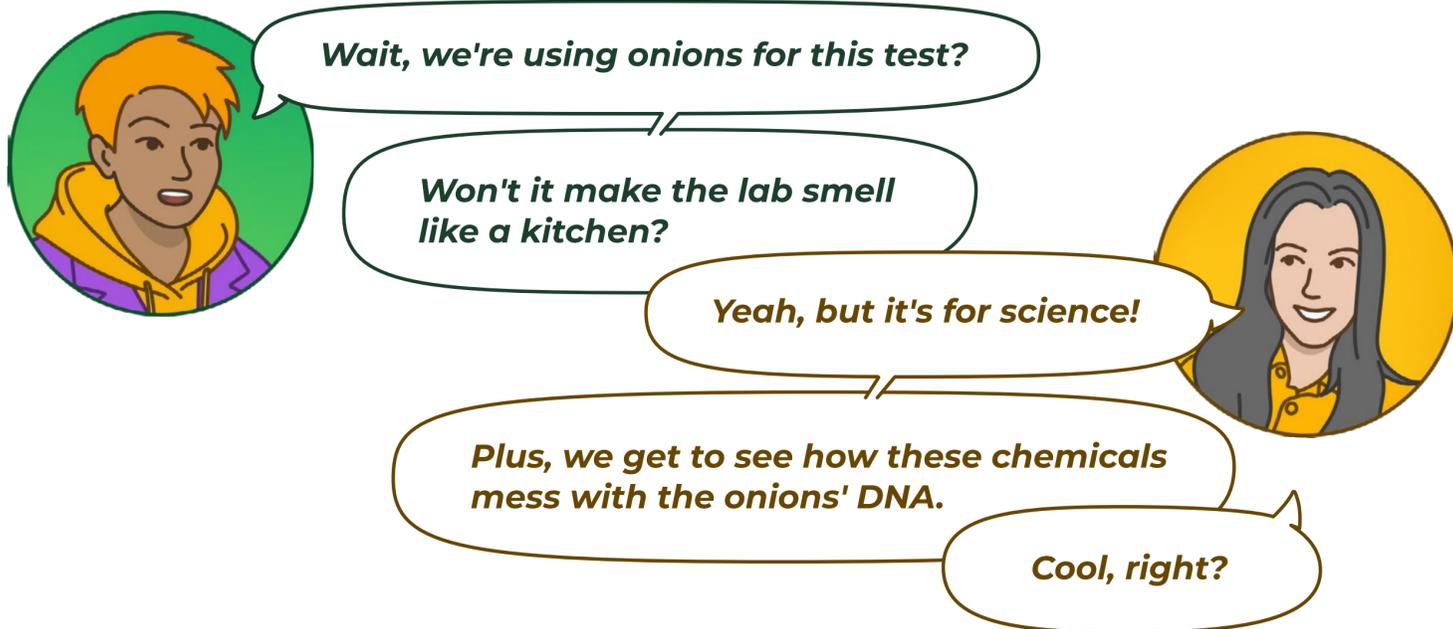
ALLIUM TEST

Investigating the Impact of Cosmetic Chemicals on Onion Growth and its DNA

BACKGROUND

The Allium test is a biological method used to assess the potential toxicity of chemicals in the environment. While chemical analyses provide quantitative information about pollutants, biological tests reveal their effects on organisms, offering insights into cytotoxicity and genotoxicity. **Cytotoxicity** refers to a substance's ability to damage or destroy cells, while **genotoxicity** affects genetic material (DNA) and may lead to cytotoxic effects.

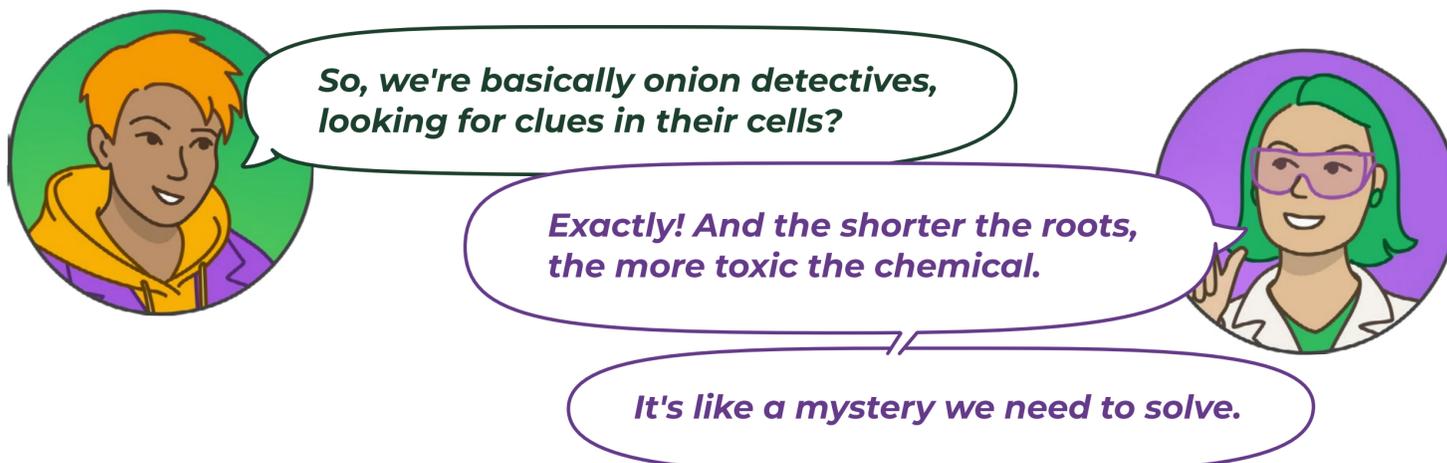
Onions, scientifically called *Allium cepa* L., can serve as a bioindicator - an organism that helps assess environmental conditions, such as pollution or toxicity, by showing measurable biological responses. The test itself is therefore called the Allium test.



The Allium test is quick, cost-effective, sensitive to low chemical concentrations, versatile in assessing both cytotoxicity and genotoxicity. It is non-invasive, widely used for environmental monitoring, and provides clear observations of plant chromosomes.

The test works by exposing onion bulbs (*Allium cepa*) to different concentrations of a chemical solution. The roots of the onions are then monitored for growth over a set period, typically between 72–96 hours (3–4 days). Cytotoxicity is determined by measuring the root length — shorter roots indicate higher toxicity. In addition, genotoxicity is assessed under a microscope by examining chromosomal abnormalities during cell division (mitosis) in stained root tip cells.

In this experiment, students will detect harmful effects of environmental chemicals on both cellular growth and reproduction of *Allium cepa* L., generating valuable data for environmental risk assessment.



AIM OF THE EXPERIMENT

To assess the **cytotoxic** and **genotoxic** effects of a chemical (e.g. cosmetic ingredient) on the onion plant (*Allium cepa*) by observing root growth and chromosomal changes in root tip cells.



LEARNING OBJECTIVES

By the end of this experiment, students will be able to:

General Learning Objectives

- ✓ Understand the importance of environmental toxicology.
- ✓ Learn the difference between cytotoxicity and genotoxicity.
- ✓ Enhance laboratory skills and techniques.
- ✓ Develop scientific inquiry and critical thinking skills.

Specific Learning Objectives

- ✓ Comprehend the Allium test procedure and its significance in assessing cytotoxicity and genotoxicity.
- ✓ Measure root length to determine cytotoxicity and identify chromosomal abnormalities to assess genotoxicity.
- ✓ Evaluate the impact of tested chemicals on plant growth and genetic material.
- ✓ Understand how tested environmental toxins can affect plant growth and chromosomal integrity.



- ✔ Apply findings to environmental risk assessment.

TIME

Cytotoxicity test:

Part I: 90 min

Part II (after 96 h (4 days)): 90 min

Genotoxicity test:

Part III (after extra 24 h (1 day)): 90 min

It is possible to conduct only the **Cytotoxicity test** – the first and second parts of the experiment; especially in the case of less equipped laboratories or if the students are not directly from a science- or technology-oriented field.

Otherwise, we recommend also carrying out the third part – **Genotoxicity test**.

MATERIALS NEEDED



Equipment:

- 15 glass test tubes without the lid (adopt the final number to the number of your samples and tested concentrations)
- 6 test tube racks (suitable for at least 5 tubes)
- Plastic Pasteur pipettes (droppers)
- Automatic pipettes with tips
- Beakers (100, 250 and 500 mL)
- Volumetric flasks (100 mL)
- Tweezers
- 15 onion bulbs (*Allium cepa* L.) (or more)
- Millimetre ruler
- Microscope slides
- Coverslips
- Hot plate
- Light microscope with a magnification of 400× (or at least 100×)

Chemicals:

- Tested chemical/cosmetic product (EXAMPLE: 30% hydrogen peroxide or Shampoo)
- Tap water (negative control)
- 1% solution of 0.1 M HCl or simply just vinegar (positive control)
- Orcein-acetic stain
- 1% solution of HCl
- Fixative (ethanol and acetic acid 3:1)

SAFETY PRECAUTIONS



Before conducting this experiment, ensure you have read and understood the **General Safety Precautions** section of this handbook.

Additionally, be aware of the following specific safety precautions:

- ⚠️ **Be Cautious with Heating:** When heating substances, be careful to avoid burns. Use appropriate tools and heat sources.
- ⚠️ **Be Careful with Stain:** Work with dyes in a fume hood as they have a strong odour. Dyes are strong, so always use nitrile gloves and a protective lab coat.



Yeah, I'd rather cry from the onions, not from an accident.

Safety first! We don't want any onion-related injuries.



EXPERIMENT SETUP



Step 1 → Prepare the Work Area

Ensure your workspace is clean and free from distractions. Set out all necessary materials. Wear your safety gear.

Step 2 → Prepare the Experiment

Concentrations: Prepare 3 different concentrations (c_1 – c_3) or dilutions (R_1 – R_3) of tested chemical. You can also choose the cosmetic product. Test each concentration in 3 replicates (A, B and C).

TESTED CHEMICAL/COSMETIC PRODUCT: _____

PICTOGRAMS: _____

Volume: Each test solution should have a final volume of 100 mL.

If using a specific chemical:

Calculate the amount of stock solution (R_0) and tap water (H_2O) needed for each test solution (R_1 – R_2).

<i>DILUTION</i>		<i>Chemical Concentration</i>		<i>Volume of Chemical (mL)</i>	<i>Volume of H₂O (mL)</i>
R₀	Undiluted (stock)	c_0		/	/
R₁	10×	c_1			
R₂	100×	c_2			
R₃	1000×	c_3			

If using a cosmetic product:

Estimate rough dilutions based on volume percentages.

<i>DILUTION</i>		<i>Product Concentration</i>		<i>Volume of product (mL)</i>	<i>Volume of H₂O (mL)</i>
R₁	25%		c_1		
R₂	10%		c_2		
R₃	1%		c_3		

Control Samples: Use tap water (C_{neg}) as negative control and 1% solution of 0.1 M HCl or vinegar as a positive control (C_{pos}).

Positive and negative controls are essential for validating the results. A positive control shows the expected effect to confirm the experiment works, while a negative control shows no effect to ensure any observed changes are due to the treatment being tested.

Test tubes: Prepare glass test tubes without threads and caps.

Appropriately label the test tubes (e.g., c1 A) and place them in plastic tube racks. All three replicates of each test concentration should be placed in its own tube rack. Label also tube racks to avoid mixing samples.



And we don't want any party crashers, so label carefully!

Labelling everything? This feels like organizing a science party!



Onion *Allium cepa* L.: Choose onions (3 tested concentrations in 3 replicates each + 2 controls – all together 15 onion bulbs) of similar size to fit well on top of the test tubes, minimizing liquid evaporation.

Carefully cut off a minimal layer (up to 2 mm) of the dried root base to expose the root tissue to the test solution. Ensure the onion base remains intact.

Step 3 → Conduct the Experiment

Part I:

1. Use a dropper to carefully fill the test tubes with the prepared solutions to the top.
2. Gently place the onions on top of the test tubes, ensuring they are submerged in the test solution.
3. Add the evaporated or used solution daily with a dropper, or water in the case of controls.
4. Keep the racks with test tubes in a well-lit area, such as a bench facing a window.
5. After 4 days, measure the root lengths (Part II) and *prepare microscopic slides for cytogenetic analysis (Part III), respectively.

Part II: CYTOTOXICITY:

6. Remove the onions from the test tubes and measure the lengths of the five longest roots with a millimeter ruler.

***Part III: GENOTOXICITY:**

7. Cut 0.5 cm tips from the three longest roots for each concentration.

8. FIXATION:

- Immerse the root tips in a fixative (ethanol-acetic acid, 3:1).
- Store them in a freezer for approx. 24 h.

Fixation preserves cells and maintains similarity to living organisms.

9. STAINING:

- Prepare orcein stain (one solution per group).
- Prepare a water bath at 60°C.
- Pour 1% HCl into a beaker and heat it in the water bath.
- Take the root tip from the fixative and immerse it in heated HCl for 5 minutes.
- After 5 minutes, rinse the root tip with distilled water.
- Place the root tip on a slide, add orcein stain.
- Cover the sample with a cover slip and squash the root tip to prepare a smear (microscope slide).

Step 4 → Monitor and Record Data**CYTOTOXICITY:**

After 96 h (4 days) record the measurements in a table and calculate the average root lengths.



Measuring roots? I didn't know onions had beauty contests.



Well, the longest roots win the prize for least toxic!

Root Length Measurements

Concentration	C_{neg}		C_{pos}		C_1		C_2		C_3	
Replicate	A	B	A	B	A	B	A	B	A	B
Root 1 (mm)										
Root 2 (mm)										
Root 3 (mm)										
Root 4 (mm)										
Root 5 (mm)										
Average Root Length (mm)										

*GENOTOXICITY:

MICROSCOPY: Observe cell division and chromosomes under a light microscope. Identify at least 100 metaphase cells and assess chromosome condition. Report the percentage of all metaphase cells and cells with chromosomal damage.

1. Count and record the total number of cells observed in each sample (at least 100, the optimum 1000). Students can pool their results to reach as high number counted cell as possible.
2. Count and record the number of cells undergoing mitosis.
3. Count and record the number of cells in metaphase.



Counting cells? This is like a microscopic treasure hunt!

And the treasure is understanding how chemicals affect our environment!



Indices Calculation:

Mitotic Index: Number of cells in mitosis per 1000 cells examined.

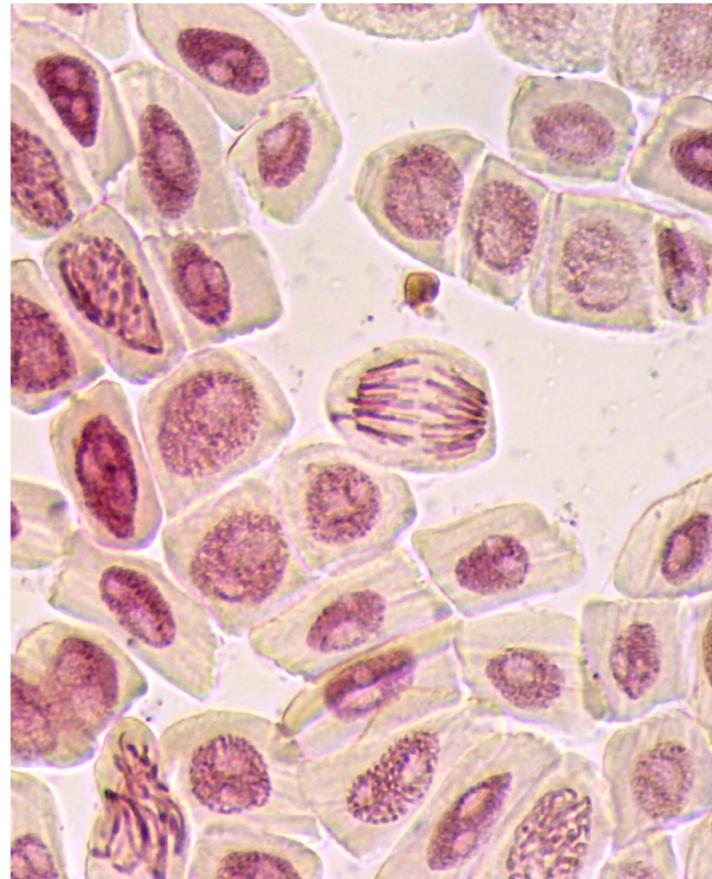
$$\frac{\text{Number of cells in mitosis}}{\text{Total number of cells}} \times 100$$

Metaphase Index: Number of metaphase cells per 1000 cells in mitosis.

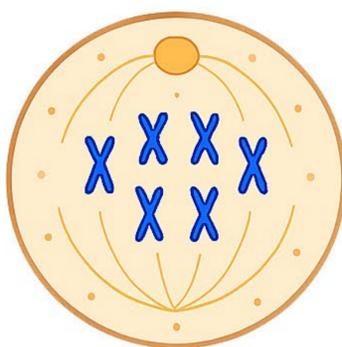
$$\frac{\text{Number of cells in metaphase}}{\text{Number of cells in mitosis}} \times 100$$



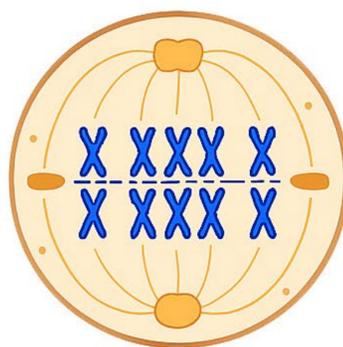
Cytotoxicity test



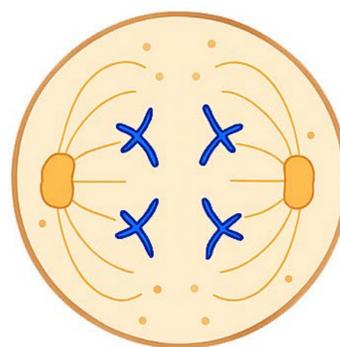
Genotoxicity test



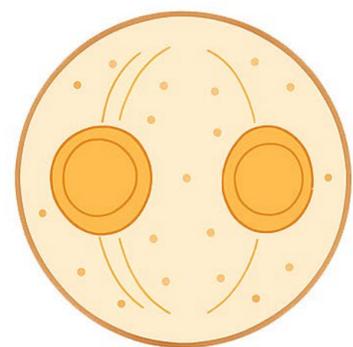
1. Prophase



2. Metaphase



3. Anaphase



4. Telophase

Cell division phases (Interphase – The cell grows and copies its DNA; **1. Prophase** – Chromosomes condense and become visible; **2. Metaphase** – Chromosomes line up in the middle; **3. Anaphase** – Chromatids are pulled apart; **4. Telophase** – Two new nuclei form; Cytokinesis – The cell splits into two)

GENOTOXICITY*Data Presentation Table for Mitotic and Metaphase Indices**

<i>Sample</i>	<i>Total Number of Cells</i>	<i>Number of Cells in Mitosis</i>	<i>Number of Cells in Metaphase</i>	<i>Mitotic Index (%)</i>	<i>Metaphase Index (%)</i>
C_{neg}					
C_{pos}					
C_1					
C_2					
C_3					

ANSWER KEY QUESTIONS



Answer the questions either orally or in writing. Emphasize collaboration and critical thinking throughout the process!

1. What is ecotoxicology and why is it important in relation to our everyday used cosmetics?
2. How can chemicals in the environment affect plant and animal life?
3. What are some common sources of environmental pollutants?
4. How do cosmetics and their chemical ingredients influence the environment?
5. Can the chemicals in cosmetics also affect human health? If so, how?
6. What differences did you observe in root lengths between the control group and the treated groups?
7. Would the exposure time affect the root growth in the Allium test in your opinion?
8. Why is it important to use a control group when conducting the Allium test?
9. Can you define all stages of mitosis?
10. What types of cell division abnormalities did you notice under the microscope in the treated samples?
11. How can observing cell division help determine the genotoxic effects of a chemical?
12. What conclusions can you draw about the genotoxicity of the tested chemical based on your observations?



Do we really need to know this?

Can't we just ask the plants and animals?

If only they could talk! But since they can't, it's up to us.



Let's think critically:

13. What do you think it means when we refer to a "chemical cocktail" in cosmetics?
14. Why is it important to consider the combined effects of multiple chemicals in a single product?
15. In your opinion, what are the potential risks of using products that contain a mixture of various chemicals?



Chemical cocktail? Sounds like a party I don't want to attend.



Yeah, mixing chemicals can be a recipe for disaster.

FOR EDUCATORS



Additional Activities/Extensions (Optional):

Propose a new experiment by modifying one variable – for example, add a different chemical or chemical mixture. You can also monitor cell division over different time scales (after 24, 48, 72, and/or 96 hours) of exposure.

Optionally, photograph the Petri dishes daily and create a photographic collage of germination.

Adapting the Experiment for **Secondary School Students:**

Simplify the experiment:

- ➔ **Focus on Root Length Measurement:** Instead of conducting cytogenetic analysis, have students measure and compare the root lengths of onions exposed to different concentrations of a household or cosmetic product.
- ➔ **Use Fewer Concentrations:** Reduce the number of samples to three (e.g., control, low, and high concentration) to simplify data collection and analysis.
- ➔ **Visual Observation:** Encourage students to make detailed visual observations of root growth and any visible changes in root morphology.

Example:

- ✓ **Objective:** Measure the effect of a household chemical (e.g., vinegar) or a cosmetic product (e.g. shampoo) on onion root growth.
- ✓ **Procedure:** Use three concentrations (0%, 10%, and 50% vinegar/shampoo solution) and measure root lengths after 96 hours.
- ✓ **Data Collection:** Record root lengths and compare the average lengths across different concentrations.

Adapting the Experiment for **Primary School Students:**

Simplify the experiment:

- ➔ **Basic Root Growth Observation:** Focus on observing and measuring root growth without involving chemical treatments.
- ➔ **Use Safe Materials:** Use safe, non-toxic substances like water, saltwater, and sugar water diluted hand soap, lotion, or conditioner to observe their effects on root growth.
- ➔ **Hands-On Activities:** Include hands-on activities like drawing and labelling parts of the onion and roots.

Example:

- ✔ **Objective:** Observe how different types of water affect onion root growth.
- ✔ **Procedure:** Use three types of water (tap water, diluted hand soap, and diluted conditioner) and measure root lengths after 96 hours.
- ✔ **Data Collection:** Record root lengths and make simple comparisons. Have students draw pictures of their observations.