AQUAtox 2000

International School Network on Water Toxicity

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International School Network on Water Toxicity

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Disclaimer

The International Development Research Centre (IDRC) emphasizes that:

- the experimental kit will be sent to the participating schools;
- these schools designate the teachers who will supervise students during the experiments; and
- it is the schools' responsibility to ensure that the designated teachers are competent and follow all the safety instructions required.

Therefore, IDRC assumes no liability for any misuse of the experimental kit by school staff or students, for any harm to school staff, students or others resulting from participation in the experiments, or for any other accident whatsoever resulting from misuse of the experimental kit.

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A Teacher's Guide

1: Introduction

Welcome to AQUAtox 2000: International School Network on Water Toxicity!

You are now part of an important network linking your school with other teachers, scientists, and student researchers around the world. In the months ahead you will be working together on the vital issue of water quality and learning simple methods for testing water quality. You will also have a chance to win a trip to Ottawa to visit the Canada and the World exhibition!

IDRC CRDI CIID CANADA

AQUAtox 2000 is an initiative of Canada's International Development Research Centre (IDRC). The Centre is a public corporation created by the Parliament of Canada to help researchers and communities in the developing world find solutions to their social, economic, and environmental problems. IDRC connects people, institutions, and ideas to ensure that the results of the research it supports and the knowledge that research generates are shared equitably among all its partners, North and South. IDRC operates from its head office in Ottawa and seven regional offices located in Africa, Asia, and Latin America.

Why Is Water Quality So Important?

Access to safe drinking water is a basic human need that remains unmet for millions of people worldwide. According to the World Health Organization (WHO), more than 1.4 billion people around the world consume water that is unsafe because of contamination with potentially harmful microorganisms or toxic substances.

For many years, IDRC has been committed to helping develop methods of testing water quality that local communities can use to monitor the safety and cleanliness of their own sources of water. The latest chapter in these efforts was created by the IDRC program Ecosystem Approaches to Human Health (or Ecohealth for short). The Ecohealth team has launched a three-year project known as "Integrated Approaches to Safe Drinking Water." AQUAtox 2000 is one part of this larger project. It is designed to help people in communities in both the North and the South to learn about equitable and sustainable ways to protect our water resources.

What You Can Do for Water Quality through AQUAtox 2000

IDRC created the AQUAtox 2000 network so that young researchers can test for water pollution in their communities, around the world. Through "hands-on," in-school experiments students see before their eyes the practical contributions of scientific research to a more sustainable future. The network links young students and schools from different countries with scientific experts working in water quality laboratories in the North and the South.

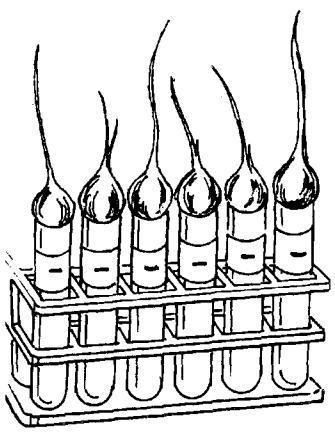
Beginning in January 1999, groups of students will use simple and inexpensive tests to measure chemical toxicity and microbiological pollution in water samples taken directly from their local environment. The network will use the Internet to exchange information among participants. Alternative communication methods will allow rural and peri-urban schools without easy access to the Internet to also participate.

Our Project Strategy and Objectives

Through applied environmental studies, the project encourages the protection of the environment. Specific objectives are:

- to help school children understand – through practical scientific experiments – how important it is to protect water resources in their own communities and the world;
- to involve students in thinking about environmental protection, sustainable development, and health and social implications in their communities and the world;
- to develop and implement an electronic network of young researchers providing an international forum for dialogue about environmental issues and priorities; and
- to give science teachers an opportunity to carry out with their students a project that is practical, crosses scientific disciplines, and relates to the health of humans and ecosystems.

AQUAtox 2000 involves students from the senior primary and junior secondary education levels. Students and their teachers will be linked via the Internet with an international network of water quality laboratories from Argentina, Canada, Chile, Colombia, Costa Rica, India, Mexico and the Ukraine. This network of laboratories is called WaterTox, and students and teachers will be able to share experiences and ask questions of the scientists in the network through the web site of AQUAtox 2000. Together with Environment Canada's National Water Research Institute (NWRI), the WaterTox network has been investigating for the last two years a set of simple and inexpensive bioassays (please see the glossary section for a definition of this and other technical terms) for assessing water toxicity. The organisms used in the tests include different plants and aquatic invertebrates.

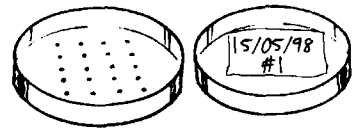


Three of these bioassays are suitable for use in a school setting. They are:

Onion Bulb Germination

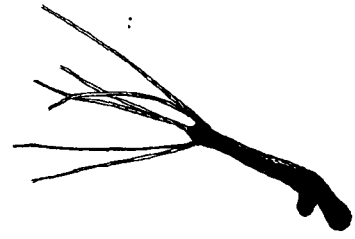
This test relies on germinating the bulb of the common onion, *Allium cepa*. A series of six bulbs (the size of small pearl onions) are placed over the mouths of test tubes containing the sample to be measured. One series is prepared with pure bottled water as a control. The bulbs are withdrawn from the test tube mouth after 72 hours, and the length of their roots is measured with a ruler. The average root length from the test sample is compared with that of the control sample. A change in the normal growth of the roots is used as an index of toxicity.

Germination of Lettuce Seeds



The bottoms of petri dishes are covered with absorbent paper (such as paper towels, filter paper, etc.) and moistened with the water sample to be tested or with pure bottled water as a control. Into each container, 20 lettuce seeds are deposited. The seeds will have germinated at the end of 48 to 72 hours. The containers are opened, and the root growing from each seed is measured using a metric ruler. The average length of the roots is calculated and compared to that of the control seeds. A change in the normal growth of the roots is used as an index of toxicity. This test is similar to the onion bulb test, but the two tests will produce different results for several toxic compounds.

Toxic Effect on Fresh-Water Hydra



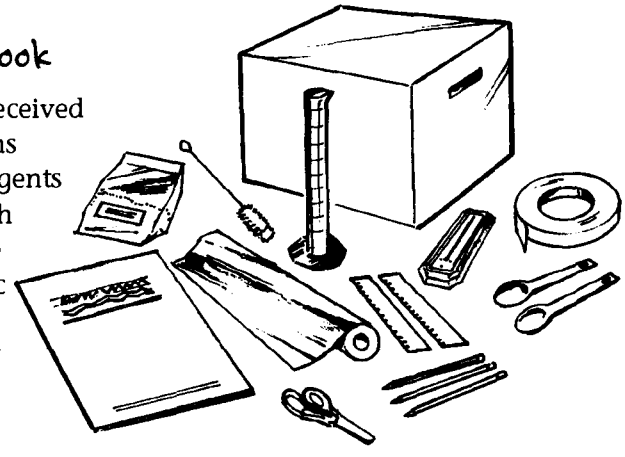
This bioassay uses the reaction of the freshwater hydra (*Hydra sp*) to toxic compounds as an index of water pollution. Hydra cultures are easy to maintain, making them an especially useful microorganism. The hydras are placed in groups of three in small plastic containers containing the water to be tested, and are observed every day for four days. The hydra takes on highly specific shapes in the presence of toxic substances, providing an easy-to-assess index of toxicity.

The three toxicity bioassays will be complemented with the following test for measuring microbiological contamination:

Hydrogen Sulphide (H_2S)

Microbiological contamination of faecal origin can be assessed by using "sentinel bacteria" (also called "indicator bacteria") that are normally present in the intestines of humans and animals. These bacteria produce hydrogen sulphide (the gas that smells like rotten eggs) as a by-product of their digestive process. In order to check for the presence of these sentinel bacteria in water, we put them in contact with a strip of absorbent paper impregnated with a nutritive substance plus an indicator that turns black upon contact with hydrogen sulphide. The test is performed in a sterilized glass bottle (for example, a test tube or an old fruit-drink bottle with a screw-on cap). The water sample (10 ml) is introduced into the bottle, and is then incubated at 27-37°C for up to three days in a home-made incubator in order to allow the bacteria (if there are any) to grow and to tint the water black.

The Experimental Kit and the Activity Book



As a participant in AQUAtox 2000, your school has received one experimental kit, free of charge. The kit contains instructions, an activity book, and the necessary reagents and supplies to carry out 20 sets of experiments with each of the four bioassays (hydrogen sulphide, lettuce seed germination, onion bulb germination, and toxic effect on fresh-water hydra). The Activity Book contains all the necessary technical information to carry out each of the bioassays. As you can see from the

table of contents, the Activity Book takes students and teachers step-by-step through the bioassays. It also covers other topics, including water and your health, the scientific process, how to collect water samples, and how to use the Internet to report your test results.

Basic Requirements for Participation in AQUAtox 2000

All participating schools in the project must perform at least two sets of experiments with the lettuce seed and hydrogen sulphide tests. The onion and hydra tests will be optional. Participants are also required to post their test results on the AQUAtox 2000 web site. Schools without Internet access can make arrangements to post test results through a local institution that has Internet access (alternatively, results can be mailed or faxed to IDRC, which will then post the results on the Internet). The project begins in January 1999 and will end in January 2000. All experiments are designed to be run in 3-month time-blocks. Schools can carry out the experiments and post their results in any one of four trimesters.

We hope that the flexibility in the choice and timing of experiments will allow schools and teachers to tailor the project to local environmental conditions and budgetary constraints. Participation in more than one trimester is welcomed.

Win a Trip to Ottawa!

All participating schools will have the chance to win a trip to Ottawa to attend the Canada and the World activities in March 2000. A total of five draws will be held to determine the winners. Winning schools will be able to send one teacher and two students to Ottawa. Complete details and eligibility rules of the competition will be posted on the AQUAtox 2000 web site.

For more Information

Contact Silvia Caicedo (SCaicedo@idrc.ca), or write to: Aquatox 2000 Coordinator, Ecosystem Approaches to Human Health Program Initiative, International Development Research Centre, P.O. Box 8500, Ottawa, Canada, K1G 3H9. You may also reach us by sending a fax at (613) 567-7748.

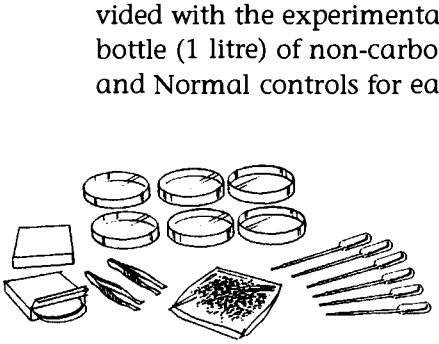
Please visit the AQUAtox 2000 Internet site at:
http://www.idrc.ca/aquatox

2: Notes to the Teacher

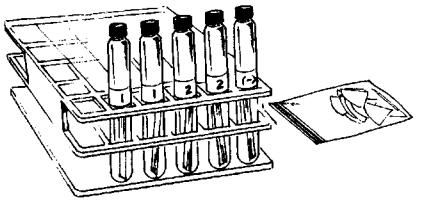
Kit Contents

The experimental kit that you received should contain the necessary reagents and supplies (with the exception of onion bulbs and bottled water) to carry out 20 sets of experiments with each of the four bioassays. Please take a few minutes to examine the illustrated kit contents and verify that all items are included. What follows is a list of other materials each school will need to provide to perform the various tests.

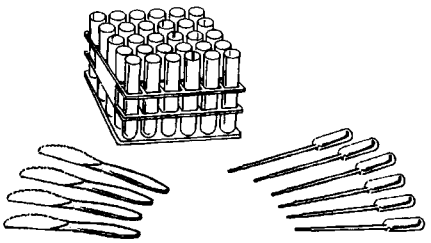
- Hydrogen Sulphide (H_2S): school to provide one small bottle (500 ml) of non-carbonated water or freshly boiled and cooled water for each set of experiments.
- Germination of Lettuce Seeds: school to provide one bottle (1 litre) of non-carbonated water for the preparation of Positive and Normal controls for each set of experiments.
- Onion Bulb Germination: school to provide 36 onion bulbs, and one bottle (1 litre) of non-carbonated water for the preparation of Positive and Normal controls for each set of experiments.
- Toxic Effect on Fresh-Water Hydra: the fresh-water hydra will be provided to the school only after the responsible teacher agrees, in writing, to grow and maintain the hydra for the purpose of the school experiments. This will require some additional effort from the teacher and some additional equipment to be provided by the school. An application form to receive the hydra and instructions on how to grow and maintain them are provided with the experimental kit. The school will also need to provide one bottle (1 litre) of non-carbonated water for the preparation of Positive and Normal controls for each set of experiments.



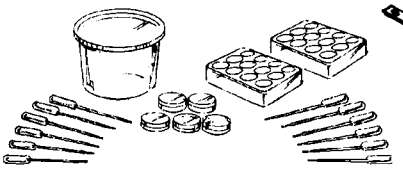
Lettuce Test



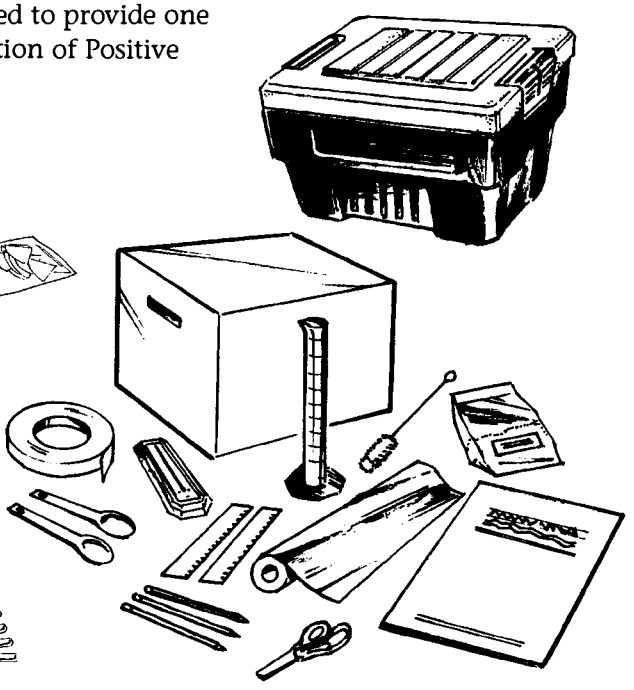
H_2S Test



Onion Test



Hydra Test



General Materials

Special Requirements

Onions

To perform one set of experiments you will need to provide 36 small onion bulbs of about 1 to 2 cm in diameter. It is better to use onion bulbs that have been stored for 2 to 3 months after having been dug out of the ground. The bulbs may be kept for more than a year under dry conditions at temperatures between 10 to 20°C. Some bulbs will likely dry up and some may be destroyed by mold. For this reason, you should store about twice the number of bulbs that you expect to use in the experiments. It is wise to plan ahead.

Hydra

Note: Read this section only if you are planning to grow the hydra in your school. If you do not intend to do the hydra test, or you are able to get enough organisms from a laboratory near you to conduct the experiments, then you can proceed to the next section (timetable instructions).

Receiving the Hydra and Setting Up

Before we supply you with hydra, please ensure that you are set up to receive them. All solutions and equipment should be prepared beforehand as described in the sections below. The hydra will come in a plastic container. Upon receiving the animals, follow the steps below.

1. Transfer enough hydra medium into a circular glass bowl (20 cm diameter) until it is ⅔ full.
2. Open the plastic container and with your index finger carefully detach the hydra from the sides and bottom of the container.
3. Transfer the hydra and hydra medium from the container into the circular glass bowl ⅔ filled with hydra medium.
4. Rinse the plastic container with hydra medium (20-50 ml) to detach the remaining hydra and transfer them to the glass bowl.
5. Repeat the previous step if necessary.
6. Keep the glass bowl at room temperature.
7. If for some reason it is not possible to transfer the contents of the plastic container into the glass bowl upon reception, store the container at room temperature for no more than 24 hours and follow steps 1 through 6 as soon as possible.

The hydra are maintained in a circular (20 cm diameter) glass culture bowl ⅔ filled with hydra medium. They will have to be kept at a temperature between 20 and 24°C with light for about 16 hours a day and darkness for 8 hours a day.

The hydra must also be fed daily (except for weekends) with freshly-hatched, iodine-disinfected *Artemia* (small brine shrimp) that you have to hatch.

Note: It is better to keep the organisms at 20-24°C. If local conditions do not allow for this, the hydra can be kept at a higher temperature. But this temperature difference could influence the results of experiments.

Materials and Equipment You Need To Provide

- 4 circular (20 cm diameter) glass bowls
- 3 circular (10 cm diameter) glass bowls
- 50 cm of plastic tubing (5 mm diameter) to be used with an air pump
- Two sieves: 38 mm and 125 mm mesh
- Iodine tablets (tetraglycine hydroperiodide)
- Air pump (for brine shrimp hatching procedure)

Hydra Medium

This medium provides the hydra with a healthy environment in which to live and reproduce. Bottled, non-carbonated water can be used for the purpose of the school experiments. You will need about one litre per day of this type of water.

Preparation of the Brine Shrimp Medium

Note: This preparation should be done **before** you receive the hydra if you are not able to do the test within 48 hours of receiving the *hydra* or if you wish to grow your own hydra.

Hydra feeds on small brine shrimp. You have to prepare this food for the hydra by hatching the shrimp, which you then feed to the hydra on a regular basis (once a day, Tuesday through Friday). Each hydra eats one to three shrimp per day.

To prepare the brine shrimp solution you need:

• Artemia cysts (small brine shrimp)	1 teaspoon
• Salt (Sodium chloride, NaCl)	2 level teaspoons (about 10 g)
• Bottled (non-carbonated) water	1 litre
• Jar or container	1-litre capacity

Note: Dissolve the salt in the water. The salt solution can be stored at room temperature in a clean and capped bottle until you are ready to begin hatching the shrimp. You will need about **5 litres** of bottled, non-carbonated water **per week** for hatching the shrimp.

CULTURING Hydra

Hatching Brine Shrimp and Feeding Hydra

1. Early in the work day, dispense 700-800 ml of brine shrimp solution into a 1-litre container.
2. Add one teaspoonful of cysts to the surface and continuously aerate the solution by introducing a Pasteur pipette attached to the plastic tubing that is connected to the air pump.
3. Cover the container with a cloth to keep flies and other insects out of the solution.
4. **After 24 hours**, use a pipette to draw up the hatched shrimp and expel them into a sieve (125 mm mesh) until sufficient *Artemia* have been harvested for feeding (each hydra normally eats 1-3 shrimp per feeding period).
5. Disinfecting the hatched shrimp: place the sieve for 10-15 minutes into a small glass bowl containing 150-200 ml of hydra medium into which half an iodine tablet has been dissolved. This exposure to iodine will ensure the shrimp are disinfected before they are fed to the hydra.
6. Remove any unhatched cysts with a pipette.
7. After the disinfection period, remove the sieve from the bowl, let it drain, then rinse the sieve and shrimp for 5 minutes by placing them into a second small glass bowl containing 150-200 ml of fresh hydra medium. Repeat this rinsing process with another 150-200 ml of fresh hydra medium to ensure the shrimp are thoroughly rinsed before they are fed to the hydra.
8. Transfer these disinfected and rinsed shrimp with a pipette into the hydra culture bowl with a zig-zag motion to disperse them evenly.

Note: Hatching can take place without aeration but the process will take longer (36 to 48 hrs. instead of 24 hrs.). Without aeration, you must use a shallow container whose surface area is large enough to provide adequate oxygen to the shrimp.

Rinsing the Hydra

Approximately 1-2 hours after feeding, carefully empty and discard the old hydra medium from the culture bowl. Normally, the hydra attach themselves to the sides and bottom of the bowl, so the medium can be slowly poured out and the hydra will remain attached to the bowl. Replace the discarded hydra medium with fresh hydra medium. Repeat this step at the end of each day.

Be certain to wash your hands before this procedure. Once a week (at the last rinse of the week before the weekend), free the hydra from the bottom of the bowl by stirring the water with a counter-clockwise movement and using your fingertips to detach them. Maintain this movement for 15-20 seconds, until the animals are gathered in the centre of the bowl. With a clean pipette, the hydra are then drawn up and deposited into a sieve (35 mm mesh) with fresh hydra medium to remove uneaten *Artemia* and other small debris resulting from the feeding process. Place the hydra into a new bowl containing fresh hydra medium.

Note: When using the sieve with 35 mm mesh, *Artemia* debris will pass through the mesh but the hydra will be trapped onto the mesh. Inverting the sieve over a clean glass bowl and rinsing it with hydra medium will allow you to have clean hydra.

If hydra are not fed during the day, it is not necessary to change the hydra medium that day.

Monitoring Hydra Growth Rate

Before conducting assays with hydra, it is important to verify that the culture conditions allow the organisms to reproduce at the same rate as in their natural habitat. It is important to monitor their growth periodically, and to maintain good culture conditions. To check the growth rate, place five hydra of similar size (each possessing a young bud) in a small culture bowl containing hydra medium. Using a magnifying glass, observe the organisms and count the number of hydranths (heads). Record the total number of hydranths each day for five to six days.

What is a Hydranth?

The head of a hydra is a hydranth. A bud is also a hydranth. Thus, a hydra with no bud has one hydranth. A hydra with one bud has two hydranths. A bud that is starting to form is also counted as a hydranth. Feed the hydra once daily and rinse them, taking care not to lose any hydra or buds. Keep a daily record of the total number of hydranths over a period of 5 to 6 days.

Calculate how long it takes for the hydra population to double. A **healthy population should double by the second or third day.** Therefore, if you start with five hydra at day 0, you should have between 15 and 20 hydra after five days. If the animals have not increased in number there may be a laboratory problem linked to animal culture and maintenance. You may need to take corrective measures such as changing the hydra medium or bottled water more often, or checking that the pH of the medium is between 6 and 7.

Application Form for Hydra Test

The fresh-water hydra will be provided to your school **only after** the responsible teacher completes and **returns** to IDRC a **signed copy** of the form below.

It is the responsibility of the school to supply all the equipment and supplies needed for the purpose of growing and maintaining a hydra culture.

1. Name of school: _____

2. Mailing address where hydra should be sent:

   _____

   _____

   Tel.: _____

3. I have read the instructions on how to grow and maintain a hydra culture. Please send me a culture of this organism for use in conducting the school experiments.

   Teacher (Name **and** mailing address if different from 2.)

   _____

   _____

   Telephone: _____ FAX: _____

   E-mail: _____ Date: _____

   (signature) _____

Return form to:

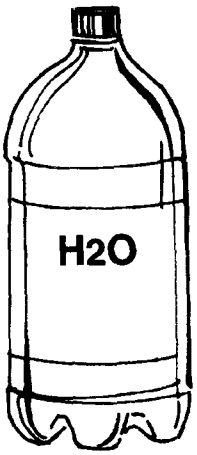
AQUAtox 2000 Coordinator
Ecosystem Approaches to Human Health Program Initiative
International Development Research Centre
P.O. Box 8500
Ottawa, Canada, K1G 3H9
or fax: (613) 567-7748.

TimeTable Instructions

Different tests have different preparation and time requirements. Teachers may find it easier to carry out the tests either sequentially or concurrently, with different groups of students working on different tests. Suggested timetable:

Test Activity	Approximate Time Needed	F	Sat	Sun	M	T	W	Th	F
Preparing: Stock & control solutions	Teacher : 1 hr.	1 hr.							
Lettuce Seed:									
– sampling	varies	1 hr.							
– testing	1 hr.	1 hr.							
– observing & measuring results	1 hr. in last day								
H_2S test:									
– preparation	Teacher: 30min.	30'							
– sampling	varies				1 hr.				
– testing	30 min.				30'				
– observing & measuring results	10 min. first 2 days; 30 min. last day					10'	10'	20'	
– cleaning materials	Teacher: 30 min.							30'	
Onion test:									
– sampling	varies				1 hr.				
– testing	1 hr. start & 10'/day refilling tubes	1 hr.			1 hr.	10'	10'		
– observing & measuring results	1 hr. last day .							1 h	
Hydra test:									
– preparation	Teacher: 1 hr. 30 min.	15'			15'	15'	15'	15'	15'
– sampling	varies				1 hr.				
– testing	1 hr.					1 hr.			
– observing & measuring results	30 min. last day								30'

3: Getting Started Instructions



Why Bottled Water?

Use bottled non-carbonated water of the same brand to prepare the solutions you need for the experiments. You want a "good quality" water with no toxins or microorganisms in it that could interfere with the tests. By using the same brand, you ensure that the quality and mineral content of the water is the same. This gives you a good basis for comparing results between samples and also reduces the possibility of error.

You will need several bottles of the same brand, non-carbonated, bottled water. For one set of experiments with the four tests, you may need about seven litres. Always keep water bottles capped when not in use.

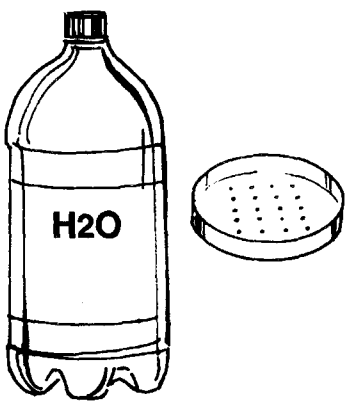
Normal Controls

For each experiment, it is necessary to know how the lettuce seeds, onion bulbs, hydrogen sulphide strips or the hydra will grow or react if there is no toxic contamination in the water. Normal, or negative, controls are special test water samples that produce no toxic reaction because the water is good. When you see how the seeds, bulbs, hydrogen sulphide strips or hydra react under these controlled or "pure" conditions, you can then compare how the same organisms (seeds, bulbs, etc.) respond under other conditions.

Including normal controls in your experiments also allows you to check for error. If you get toxic test results with them, you must conclude that you are doing something wrong. This also means the rest of the results in that experiment cannot be trusted. You may need to start over.

Hints:

- Always keep the water in the bottles as clean as possible.
- Always put the cap back on the bottle as soon as possible.
- Never pour any water back into the bottles. You do not want to take any chances of contaminating the water in the original bottles.
- Never put anything but the appropriate pipette into this water.



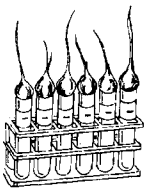
Specific Types of Normal Controls

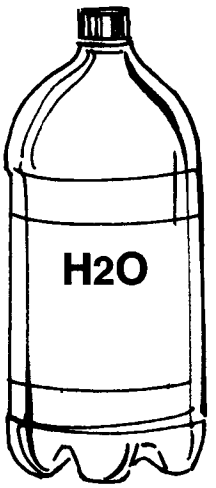
For the lettuce seeds test:

- Use bottled, non-carbonated water.
- Pour a little more water than you need into a clean container and use this water to do the tests.
- Always recap the bottles when not in use.

For the onion bulbs test:

- Use bottled, non-carbonated water.
- Fill six test tubes directly from the bottle and label them with an "N" (for "normal" control). Recap the bottle.
- To "top up" the test tubes containing the onion bulbs, pour a little water into a small container (such as an empty test tube) and use this water to refill the six normal control test tubes during the experiment. Throw away any water left over in the small container.



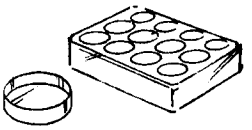


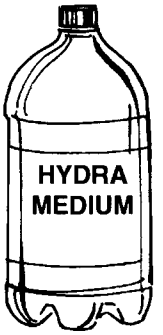
For the hydrogen sulphide test:

- Use bottled, non-carbonated water or water that has been freshly boiled (for at least one minute) and allowed to cool to room temperature. The water – bottled or boiled – goes into the normal control test tube just before you put the samples into the incubator.

For the hydra test:

- The hydra medium is the normal control; for the purpose of school experiments, it is also non-carbonated, bottled water.





Positive Controls

The positive control solutions are designed to give results similar to those that would be obtained with contaminated water. The positive control solutions that you will prepare for the lettuce seed, onion bulb, and hydra tests are quite safe. They are not toxic to people. You will simply be making a series of salt water solutions using ordinary table salt (NaCl). These solutions should allow you to observe how the seeds, bulbs, or hydra react to polluted water. All three forms of life need fresh water to grow and are highly sensitive to the salt content of water. Since each of the three forms of life have different levels of tolerance to salt, each will require slightly different concentrations (amounts) of salt to produce effects (for example, a shortening of the roots by half, or survival of half the number of hydra) that are easy to observe in the test water.

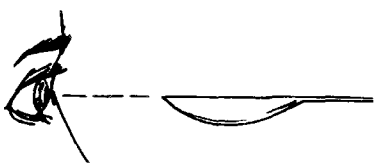
Note: For safety reasons there is no positive control for the hydrogen sulphide test. It will be obvious that the water in your sample test tubes is contaminated if the treated paper strips (tan colour) turn black.

To prepare the positive controls for the other three tests you begin with **the same stock solution**, then dilute the solution in some cases.

A. Preparation of Stock Solution

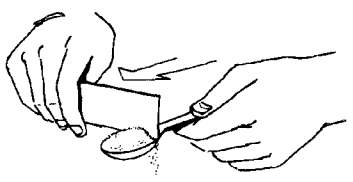
Requirements

- 1 teaspoon
- 1 graduated cylinder (100 ml)
- 1 clean 1.5 litre container with non-metal cap (to prevent rusting of the cap from the salt solution)
- 11 grams of salt (NaCl); this is equivalent to 2 level teaspoons



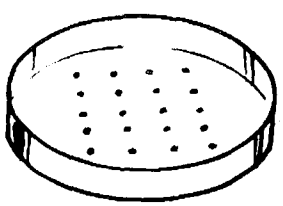
Procedure:

- Add two level teaspoons of salt (NaCl) to the 1.5 litre container.
- Add 1 litre of bottled non-carbonated water to the container.
- Using the graduated cylinder, add another 100 ml of non-carbonated, bottled water to the 1.5 litre container
- Cap, shake well, and label "NaCl Stock 10 g/L." Store away from direct sunlight and extreme heat.



Note: Salt readily absorbs moisture. To ensure that the right amount of salt is added to the stock solution, always keep the bag of salt provided in the kit tightly closed when not in use.

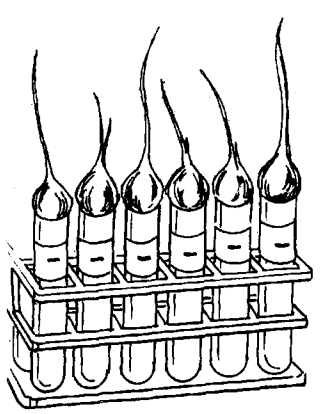
B. Lettuce Seed Positive Control Solution



Procedure:

- Use a clean 500 ml (minimum) container with a non-metallic cap.
- Using the graduated cylinder add 250 ml of the prepared NaCl 10 g/L stock solution to the 500 ml container.
- Add 250 ml of non-carbonated bottled water to the 500 ml container.
- Cap, shake well, and label "Lettuce (+) 5 g/L." Write in the date.

Note: This positive control is good for one or two weeks, **but** it is best to make a new positive control for each experiment.



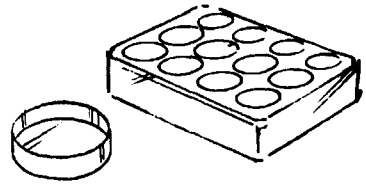
C. Onion Bulb Positive Control Solution

Procedure:

- Use a clean 500 ml (minimum) container with a non-metallic cap.
- Transfer 500 ml of the prepared NaCl 10 g/L stock solution to the 500 ml container.
- Cap and label "Onion (+) 10 g/L." Write in the date.

Note: This positive control is good for one or two weeks, **but** it is best to make a new positive control for each experiment.

D. Hydra Positive Control Solution



Procedure:

- Use a clean 500 ml (minimum) container with a non-metallic cap.
- Using the graduated cylinder add 100 ml of the prepared NaCl 10 g/L stock solution to the 500 ml container.
- Add 400 ml of non-carbonated bottled water to the 500 ml container.
- Cap, shake well, and label "Hydra (+) 2 g/L." Write in the date.

Note: This positive control is good for one or two weeks, **but** it is best to make a new positive control for each experiment.

Notes and Hints on Positive Controls:

- The stock solution is good until it is used up.
- **Never** pour any stock solution left over from an experiment back into its original bottle.
- **Always** keep stock solution capped when not in use.
- Avoid at all times **any possibility** of contamination.

Hygiene and Safety

Why It Is Important to Maintain a High Level of Hygiene

1. You may be working with microorganisms that are capable of causing diseases (pathogenic microorganisms)
2. If these pathogenic microorganisms **are** present they may multiply in large numbers during the incubation period in the bottles and test tubes.
3. The following precautions are **essential**, not only to avoid contamination of the samples and control solutions, but also to prevent students conducting the tests from becoming infected by the water samples.

Note: The most common form of infection is through "hand to mouth" or "hand to eye" contact. **Be very careful.**

Precautions should be taken to ensure that if a test indicates the sample is contaminated, this contamination is in the sample and that it **is not** due to poor laboratory procedures.

Cleaning the Work Area

It is essential to clean **and** disinfect the work surfaces **before and after** each use.

Preparation of Cleaning Solution

(It is recommended that the teacher prepares this solution.)

- Add 5 ml (1 teaspoon) of bleach (home disinfectant with 5.25% chlorine) to a clean container.
- Add 0.5 litres (2 cups) of water. Cap and shake well.
- The solution must be applied and left on the surface of the work area for at least 30 seconds to ensure that the surface is disinfected.

Note: If you wish to make more cleaning solution, simply double or triple the portions as required.

Hygiene and Safety Precautions:

- Keep fingernails short and tie back long hair.
- **Always** wash your hands with soap and warm water **before** and **after** the experiment.
- Turn your head away from your work area if you cough or sneeze.
- Keep your work area clean and dry at all times.
- Inform your teacher of any accidents or spills.
- **Never** touch your eyes or mouth while working on the tests.
- **Never** work on tests if you feel sick.
- **Never** eat or drink in the work area.

In Case of Accidents or Spills

*Students should **immediately** inform the teacher.*

TEACHER: **carefully** pick up all broken equipment and put it in the proper container.

If a contaminated (or possibly contaminated) sample is spilled, **disinfect** the area with a strong chlorine solution (use one part bleach to five parts water) **immediately**.

Do not touch your eyes or mouth! **Wash your hands** immediately after cleaning the spill. If you are hurt, see a nurse, doctor, or local health worker as soon as you can.

Washing of Labware

1. All equipment **must** be washed before reuse to prevent contamination in the next set of experiments.
2. After washing the equipment with soap and water, rinse it well with tap water or bottled water.
3. Store the equipment in a safe place away from dust and out of direct sunlight.
4. Be **very careful** when disposing of the contents of the tubes from the hydrogen sulphide tests. Carefully follow the directions indicated in the experimental procedure.

Notes on Microorganisms

Additional Background Information for the Teacher

In this section we will use the longer, scientific names of organisms. For instance, *E. coli* may be a common term to some people because of news stories. It is actually the abbreviated name for the bacterium, which, according to the scientific classification system, belongs to the *Enterobacteriaceae* family, the *Escherichia* genus, and the *Coli* species, or *E. coli* for short.

About one in every thousand bacteria found in an adult's intestines is *E. coli.* But in a newborn baby's intestines this type of bacteria is more abundant, as are other bacteria like lactobacilli and enterococci. *E. coli* form part of a group of rod-shaped bacteria known as coliform organisms. They are important in water quality testing because simple means to detect their presence in the water have been developed over the years.

Generally it is healthy to have coliforms and other kinds of bacteria in our intestines. They help us digest our food and keep us healthy. For example, the bacteria *E. coli* in our intestines are a source of Vitamin K and B-complex vitamins. We are never without these bacteria, which is good because they keep us healthy. There are about as many bacteria in your intestine as there are people on the entire earth, 5.5 to 6 billion of them. They also reproduce very fast in our intestines. Each person discharges from 100 to 400 billion coliforms every day. Coliform bacteria are therefore very numerous and are also easier to detect than pathogenic (disease-causing) microorganisms. Their presence in water is taken as an **indication** that the water has been contaminated with human or animal waste.

In other words, if tests show that coliform bacteria are in the water then there is a pretty good possibility that there are also microorganisms in that water that cause disease. The test that you will perform with the hydrogen sulphide (H_2S) indicator strips will show if H_2S is produced. Some common bacteria living in the intestines of people and animals produce H_2S as they grow. So if your test shows that H_2S is present (the indicator paper strip turns black), then coliforms are likely present in your sample. If this happens, you should **not** drink water from that source unless it is boiled or disinfected in another way.

Testing for the Presence of Bacteria With the Experimental Kit:

Many members of the *Enterobacteriacae,* including several types of coliform bacteria, produce hydrogen sulphide (H_2S) while they grow. Under ideal conditions (plenty of food and warm temperature) they will grow and reproduce quite rapidly. Some common bacteria that will produce hydrogen sulphide are *Salmonella, Citrobacter,* and *Proteus.* The test strips (provided in your kit) contain the nutrients needed **plus** a chemical that turns black if it comes in contact with hydrogen sulphide. The ideal temperature is provided by the incubator (also included in your kit). You will have to experiment a little with this simple incubator to get the right temperature range (26-39°C).

Set up your equipment, put a thermometer in the bottom and keep an eye on it. Adjust the light bulb and the opening of the lid so that the temperature remains in the ideal range (see illustrations on the set-up and use of the incubator on the following page).

Note:

- The H_2S test usually will indicate if bacteria are present before the third day of incubation, **but** you may want to allow the experiment to go on for five days if the temperature is below 30°C, since the bacteria grow more slowly at lower temperatures (36-37°C is the ideal temperature range).
- The test is designed to promote the growth of coliform bacteria inside the test tubes. Most coliforms are non-pathogenic, but there is always a possibility that some disease-causing microorganisms will develop along with them inside the tubes. **Be Safe!** Don't allow students to open the test tubes once they are incubated and **follow proper disposal procedures** to discard the contents of the tubes as described in this manual.

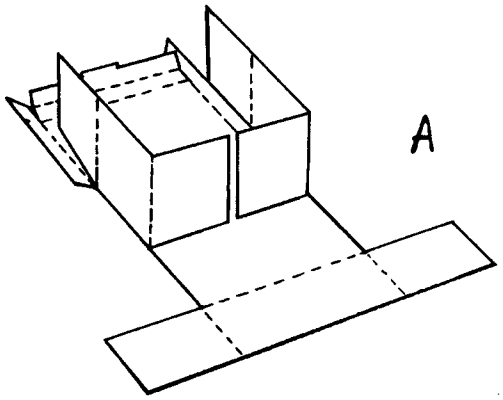
Setting Up the Incubator

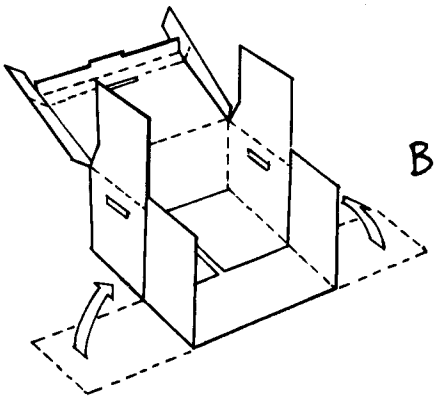
The incubator is included in the package. However, due to the different modalities of electrical systems and voltages in each country, it was decided not to include the light bulb fixture. This should be provided by each school. The device is necessary only if the lowest ambient temperature where the incubator is located is below 27°C. If the temperature exceeds 27°C, the incubation can be carried out without the electric device.

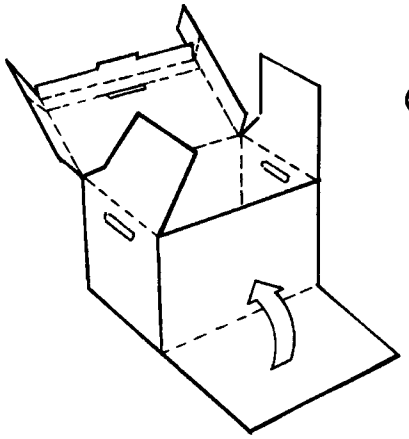
To install the light bulb, you need sufficient electric wire (cord) to reach the wall outlet. The wire should have a socket for a light bulb of 15–30 watts on one end and a plug on the other.

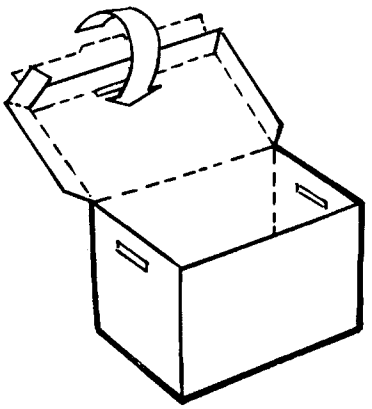
The teacher must ensure that this electrical wiring meets the approved norms and does not present a risk to the students during normal usage.

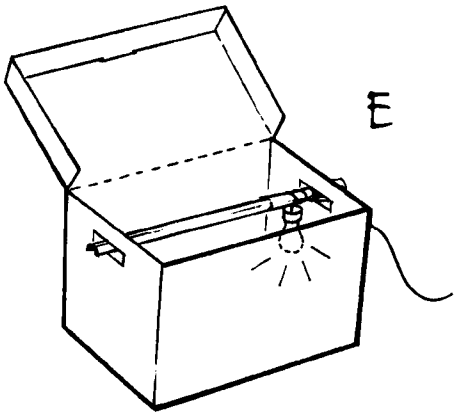
Setting Up the Incubator — Illustrations

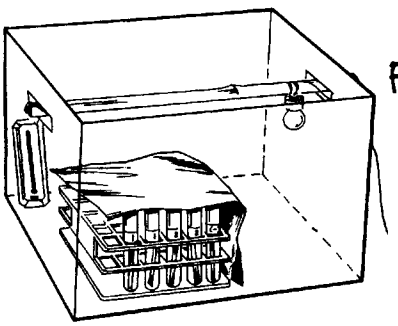


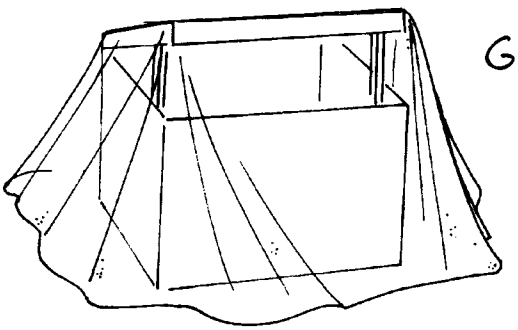












AQUAtox 2000

Activity Book

A Student's Guide

A. Water and Your Health

Why Is Water Important?

Water is essential for all life. A few very simple organisms can survive without air, but none can live without water. The quality of water can affect the life of plants, animals, and people because all depend on water for survival.

Water and health go hand in hand. We need water to drink, keep clean, grow food, and also for manufacturing a great number of things. Clean, fresh water is essential for our health and in our day-to-day living. For every 100 litres of fresh water consumed in the world, 70 are used to irrigate farmland, 23 are used by industries, and only 7 are used by people for household purposes.

Amazing Properties of Water

Water has many unusual properties that make it the basic ingredient for life.

- **Water** alone occurs on earth in three different forms at the same time. We find it in nature as a **solid** (ice or snow), as a **liquid** (rain or river water), and as **gas** (humidity or water vapour). This allows water to move around the world (in rivers, oceans, clouds or rain) or be stored in ice caps.
- **Water expands when it freezes** while most other substances contract (become smaller). It is also lighter as a solid (ice). Most other substances are heavier in solid than in liquid form, but a block of ice floats! This property has allowed the movement of people and animals for thousands of years over frozen ice passages between land masses.
- **Water can absorb a great amount of heat** without much rise in temperature. This is another way of saying that "a watched pot never boils" (or takes a long time to do so). Again, this useful property benefits all living organisms on earth. Because of it, the oceans serve as a form of temperature control and help prevent extreme changes in climate between the summer and winter months. Speaking of temperature control, about 83% of your blood is made of water. It is this capacity of water to absorb heat that keeps your body temperature constant as your blood circulates continually inside your body.
- Another unusual property of water is its ability to stick to itself (the scientific name for this is "surface tension"). **Water has the highest surface tension of all liquids** found in nature (with the exception of mercury). This means that when water drips from a faucet or when it condenses in the sky, its individual particles will stick together to form water drops.

It also means that water falling on the ground will spread out in a thin film or layer over the soil. If the soil is dry, the water will then move (flow) into the soil and will become available to the roots of plants. If the soil is already wet, the water will tend to flow overland into streams, rivers, and lakes.

- Perhaps the **most important property of water is its ability to dissolve so many substances**. Pour some table salt into a glass of water and the salt will dissolve and become evenly distributed throughout the water as tiny, invisible particles. The function of water as a solvent is essential to all forms of life. Because of it, water can carry and distribute nutrients (food) to plants, animals, and to the different parts of our body through our blood.

DID YOU KNOW?

Contaminated water and poor sanitation cause 30,000 deaths around the world daily, or the equivalent of 100 jumbo jets crashing every day.

The Water Cycle

Ancient people revered water. They believed that the flow of streams and rivers was a gift from the gods. Water was thought to come from deep inside mountains or the centre of the earth. It was not until the late 1700s when scientists (the famous astronomer Halley among them) began to realize that water followed a natural cycle moving continuously between the earth and the atmosphere.

This cyclic movement is called the "water cycle." Water evaporates from wet ground, from the leaves of growing plants and trees, and from lakes and streams, leaving any salts behind. It is carried in the air as water vapour. Clouds then form and the moisture falls as rain. The rain feeds the rivers and lakes. Rivers carry water back to the ocean, and the whole cycle begins again. Water moves from oceans and land to the atmosphere, then falls back again to the oceans and land in its continuous cycle. No water is lost from the earth. In a way, we could say that we are drinking the same water that once quenched the thirst of dinosaurs!

Water Quality and Pollution

As water recycles itself throughout the world, it dissolves minerals (natural salts) and also carries with it small soil and dust particles. Water naturally picks up many things along its path. Therefore, its quality will vary from place to place, with the season, and with the kinds of rocks and soil through which it moves. For the most part, it is natural causes that affect water quality. But added to nature's influence are the activities of human beings throughout the world that also affect the quality of water.

Pollution means different things to different people. A simple definition of water pollution can be "any damage in water quality that makes it harmful to living things or unsuitable for beneficial use." If we add things to water that make it unhealthy for people, plants, and animals, we are polluting the water.

Types of Pollution

Pollution comes in many forms. It may appear as germs (microbial contamination) that cause disease, as poisonous chemicals (chemical pollution), as too many minerals and soil particles, or even as changes in water temperature (physical pollution). Water pollution usually occurs when something outside the natural water cycle disrupts the balance of life.

Microbial Pollution Microbiologically unsafe water is one of the leading causes of disease and death in many communities around the world. Diseases are transmitted through ingestion of water that has been polluted with excrement from people or animals. The ingestion can be direct, as through drinking water; or indirect, as through ingesting foods or beverages that have been contaminated with polluted water; or accidentally swallowing water during swimming or bathing. Some infectious organisms can also penetrate the skin of people when they bath in polluted waters. Many excreted microorganisms are able to survive for extended periods of time outside the human body, particularly in water and occasionally in soil. Communities with poor sanitation are likely to have problems with microbial contamination of water sources in the form of pathogenic (disease-causing) bacteria, viruses, protozoa, and helminths (see the glossary at the end of this manual for an explanation of these terms).

Chemical Pollution Pesticides and fertilizers in agriculture, waste from mines flowing into streams, and untreated or partially treated waste waters pollute rivers, streams, lakes, and oceans with chemicals. The waste we bury underground often also pollute ground waters. When this happens, the inaccessibility of the pollution source makes it difficult to clean up. Air pollution affects the quality of rainwater, and in turn, the quality of the water where that rain falls. Acid rain, for example, can destroy the natural chemical balance of a water source. Emissions of gases from industrial plants and from car exhausts are the main culprits. Metals naturally present in water in minute amounts are not harmful to people or animals. However, acid rain can increase the amount of metals such as mercury, aluminum, lead and others, changing the natural, healthy composition of the water. Water that is too high in metal content is unhealthy for us to drink. There are also hundreds of new chemicals being developed every year that find their way into water sources. Because these chemicals are new, we may not know what long-term risks they may pose to our health!

Physical Pollution Changes to our environment may also cause pollutants to enter the water system. For example, poor agricultural practices and deforestation are a common problem worldwide. They cause continuous erosion of the soil. Heavy rains transport the soil, often contaminated with pesticides and other chemicals, into streams and rivers. Over consumption of water can also affect the quality of the water in many rivers, lakes, and even ground water. As water levels in these rivers and lakes falls, concentrations of contaminants will tend to increase since there is less water to dilute them. Over-pumping of ground water in coastal zones can attract salt water further inland to replenish the missing water, thereby contaminating this fresh water source with salt.

DID YOU KNOW?

Almost 80% of the Earth's surface is covered in water. Of this, 94% is salt water, 3% is found deep underground, and 2% is glacial ice, leaving less than 1% as fresh water available for us to use.

DID YOU KNOW?

Water accounts for about 70% of your body weight and 90% of your body volume. If we lost as little as 12% of it we would die.

Natural Contaminants In some parts of the world, certain naturally occurring chemicals are found in concentrated amounts and have the potential to cause harm to people. These include metals such as arsenic and lead, or radioactive elements such as radium and uranium.

What Can We Do?

We must think in terms of sustainable development, which we can define as using and managing resources and the environment while preserving a healthy and viable ecological base in today's world and for the future. Protecting water sources from pollution and maintaining the high quality of water supplies play a fundamental role in efforts to protect the health of people, ensure a good quality of life and provide for sustainable development. This process starts with investigating water quality in your community and thinking about ways to prevent pollution. Remember, what happens in one corner of the earth affects us all. Without the cooperation of all the world's people, our environment is in danger. But by cooperating, we can make our planet a better place to live!

DID YOU KNOW?

Water never actually "disappears." Fresh water circulates constantly among oceans, rivers, glaciers, soil, atmosphere and all living things. We can say that we are drinking the same water that once quenched the thirst of dinosaurs!

It is important to learn about the situation in your community. You should find out where the local water supply is coming from, how safe it is, what it is used for, how it is being polluted and what you can do to prevent pollution. The community has the right to be well informed, but also has the obligation to participate actively in decisions concerning the quality and surveillance of its drinking water and its environment. In becoming informed, you can motivate your community to explore corrective measures to protect your health and the environment.

Unfortunately, pollution in water is sometimes difficult to detect. Water that is odourless and clear is not necessarily free of pollutants. You can use the four tests provided in the experimental kit to check for forms of pollution that you cannot see with your own eyes. These tests, called bioassays, are easy to do and simple to interpret. Bioassays are based on the way living organisms react to a given treatment. You can tell simply by the reaction of the organisms if the water is toxic. The first three bioassays – the onion bulb test, the lettuce seed test and the fresh-water hydra test – measure the chemical pollution in water. For water contaminated with microbes, a fourth test, the hydrogen sulphide (H_2S) test, is used.

By using these four simple tests, you can begin to learn about the quality of the water in your area. By contacting students, teachers and scientists over the Internet, you can share research results, learn about other communities around the world, and find out the state of the world's water supplies.

B. The Scientific Process

Everyone – young or old – has questions about the natural world around them. We learn much by first asking questions and then trying to find answers. Science is a way of answering these questions, or solving problems. Although people choose different ways of dealing with a question or problem, there are common steps that most of us go through to find an answer or solve a problem.

Scientists follow certain basic steps that taken together are called the scientific method. These steps are highly logical. When we solve a problem we tend to follow these steps almost without realizing it. The following are the formal steps used in research to solve a problem.

The Scientific Method

Steps one through six below are carried out **in preparation** for an experiment. You should begin with a title for your investigation. Note the date and be sure to add your name and those of others if you have partners.

1. Determine the problem you wish to answer (the "Purpose")

   There should be a single purpose, or a single question you wish to answer. Often other questions will come up while you work on this problem. These other questions then become other experiments or investigations.

   It is best to write down the purpose of your work so that everyone involved is aware that you are all working on the same problem. It also reminds you what your purpose is as the investigation goes on.

2. Make a list of all the things you will need in order to do your investigation ("Apparatus and Materials")

   In order to do this properly, you will probably have to do some research. Look in your text books, the library, and on the Internet. A good source of information is someone with experience solving a similar problem, perhaps your teacher, classmates, or parents. Grandparents are excellent sources of information. The purpose of this research is to determine the things you will need to do your experiment.

3. While you are doing your research, you are collecting information about your topic.

   Take some notes along the way. Based upon the information you have gathered, you now should be able to make a guess about the answer to your question. You may have an idea about the answer. Write down your best guess. This guess is called a **hypothesis**.

   The hypothesis can be quite simple or it can be detailed. You may want to include how you intend to make your observations, how you will make any measurements, and how you will use these measurements to arrive at a conclusion.

DID YOU KNOW?

Water can dissolve more substances than any other known liquid. Water transports dissolved minerals that are needed by our body, but it may also carry chemical substances and organisms that may cause us harm. For this reason it is important to test its quality regularly.

Keep in mind that the hypothesis is only your best guess. It is satisfying if your guess is correct, but often it will be mistaken. Making a mistake is fine, because you can then make a new prediction.

4. Now is the time to formally write down the precautions that you should take.

   After listing your apparatus and materials, and predicting how you will test your hypothesis, start making a list of **precautions** or "things to watch out for." As you prepare the next step, you will probably add to this list of precautions. No precaution should be taken for granted. This is a reminder for you and others who may be doing the same experiment to be aware of certain things.

5. You are now ready to plan the steps you will take to answer your question.

   This plan is called a "procedure". It describes the steps you need to take in the order you will take them. You may choose to write the procedure in paragraph form, but it is easier to follow the steps if they are in point form. Just write down the steps in the order you will take them. This stage is of **great importance** in preparing the experiment.

6. As you go through the steps in the procedure, you can think of certain measurements and observations that you will need to make. Write these down.

   Write down every measurement you plan in your experiment as well as things you intend to look for. Observations are important since they can furnish clues to understanding what is happening in your experiment. Think about how you will organize the measurements and observations you will make and figure out the calculations you will want to make. Different types of information can be recorded and presented in various ways. Preparing tables to fill in as you carry out the experiment is a good way to make sure you do not lose any information or forget to make a measurement. Tables are also very useful when it comes to do your analysis. They allow you to spot any trends or sudden changes in your results. They also make it easy to do any calculations, since all the information is together, ordered in a logical and clear manner.

   Note: In the experiments on water quality you will try to find out how living things (plants and animals) are affected by pollution. For this step you will compare your "samples" to "controls." Controls are special samples you prepare to check how the things you are testing will behave, grow, or change under conditions that you create ahead of time and can control. You compare the results of your field samples to the special control samples. You will use both "positive" and "normal" controls. With normal controls using clean water there should be zero toxic influence from external sources. In the case of the positive control, you will know precisely the source and the level of the toxic influence because you will have introduced the toxicity yourself.

DID YOU KNOW?

We can survive without food for several weeks but cannot live without water for more than four days.

DID YOU KNOW?

About 83% of your blood is water. Water is what keeps your body at a constant temperature.

In other words, in addition to doing experiments with the water samples you collect from the field, you will also prepare two special samples or controls (one with good, clean water and another with "toxic" water), so you can compare results between them. There is no cause for alarm about the positive control because you will simply use a mixture of table salt and water to simulate toxicity.

DID YOU KNOW?

Water, unlike most liquids, increases in volume (by about 10%) when it turns to ice. In this solid form, it floats rather than sinks.

7. You are now ready to actually begin doing the experiment.

   a. Gather your equipment and materials – all the things you need.
   b. Prepare your equipment and tables to record your measurements and observations.
   c. Prepare the controls. Your teacher will explain how.
   d. Collect your samples. But first read "Collecting Water Samples" in the next section.
   e. Follow the detailed procedure for each of the bioassays.
   f. Make and record your observations and measurements.
   g. Do your calculations.

8. Finally you are at the most exciting part of your experiment. You now get to check your hypothesis.

   Based on your observations and calculations you may now make a **conclusion**. This is a statement that answers your original question. It is the whole purpose of the experiment.

   Was your hypothesis correct? If it was not correct, you may have to repeat the experiment **or** you may have to make a new prediction (hypothesis) based on your results. This decision is left to your judgement. Often, the results of one experiment lead to more questions and more experiments. This probing is how we learn more about how things work.

DID YOU KNOW?

All living things, from the smallest insect to the tallest tree, need water to live.

9. Write some comments to explain your results.

   Other people will be reading about your work and may wish to do the same experiment. So you should add some **comments** that explain your results and help guide others to carry out a successful experiment. **Suggested comments:** If you were to do the experiment again, what would you do to make it better?

A Sample Report

(please use a separate sheet of paper)

Name(s): _____ Date: _____

Title of Experiment: *(Brief and to the point)*

Purpose: *(Only one reason explaining why you are doing this experiment)*

Apparatus and Materials: *(Essentially, a grocery list of things you need.)*

Hypothesis: *(Your best guess about what you think will happen.)*

Precautions: *(Things to watch out for.)*

Procedure: *(The steps you will take in their proper order.)*

Observations and Results: *(What you have seen and measured.)*

Conclusion: *(Was your hypothesis correct? Why? Why not?)*

Comments or Discussion: *(How you would make this experiment better.)*

C: Collecting Water Samples

You will be doing simple experiments to test chemical and bacterial pollution in water samples taken directly from your local environment. Plan with your teacher what types of water sources you can test, then go together to collect your samples. Make sure your teacher accompanies you and remember:

YOU MUST COLLECT THE SAMPLES IN A SAFE MANNER!

- Stay away from fast moving water.
- Stay away from sources that you are quite sure are heavily contaminated, such as factory outlets.
- Stay away from sewers, treatment ponds, and hazardous waste sites.
- If you are **uncertain about the safety** of a site, choose another site that you know is safe.

Materials and Equipment

The equipment that you need will depend on where you plan to collect your samples. Certain items will be handy no matter where you collect the samples. They are:

- a small clean bucket with a strong rope securely attached to the handle
- paper towels or a clean cloth to dry things off
- a small sheet of plastic on which to lay your equipment
- two 1-litre clean sample bottles with screw caps (used bottles that have been washed with soap and thoroughly rinsed with bottled or boiled water)
- two small clean containers for measuring the pH of your water samples (small used glass bottles that have been washed with soap and thoroughly rinsed with bottled or boiled water)
- one pH measuring kit
- masking tape and a pen or pencil for labelling your samples

A general rule:

Begin with clean containers, **then rinse** the containers with the water from the source that you are about to sample. Finally, collect your sample, seal the container, and label it immediately.

Note:

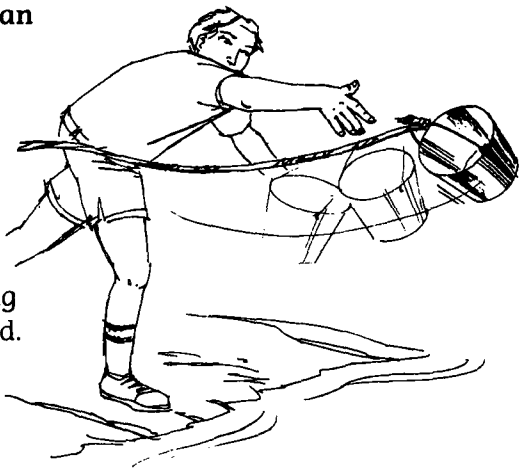
- The hydrogen sulphide test (H_2S) **must** begin immediately at the site once the sampling is done. In this case, the water samples are taken directly from the bucket or the water tap. Carefully follow the instructions for collecting water samples as described in the testing procedure for this test. Do not freeze your water sample nor leave it exposed to the sun.
- To do the lettuce seed, onion bulb, and hydra tests, take the samples in their properly labelled sample bottles back to the school. Then begin the experiment within three hours of sample collection. If this is not possible, you can keep the samples for about 24 hours if you keep them cold (at about 4°C). Keep the samples in a refrigerator or store on melting ice.

DID YOU KNOW?

We use water for many things. Of 100 litres of water, 70 are used to irrigate farmland, 23 for industrial use, and only 7 for home use.

How to Collect Samples from Different Sources

Collecting Samples from a Lake or Pond

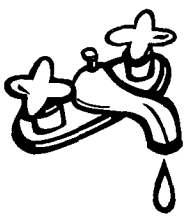


Stay on shore. Tie a rope to a **clean** bucket and throw the bucket out as far as possible into the water. Pull the bucket back quickly enough so it does not fill up and sink (half to two-thirds full is fine). The water should come from the surface of the body of water. The idea is to avoid stirring up the bottom of the lake or pond. Rinse the bucket with this first collection of water, then empty the bucket along the shore. Now throw the bucket out again and bring in your sample.

Fill your sample bottle from the bucket following the "bottling technique" described below.

Collecting a Sample from a Well

Lower a **clean** bucket into the well. Rinse the bucket with water from the well and **throw the water away from the well, not back in the well.** Lower the bucket again to retrieve a fresh sample and follow the "bottling technique" described below. If you are sampling from a well with a hand-pump, pump water out for several strokes and then fill your sample bottle directly from the spout. **Do not** allow the mouth of the sample bottle to touch the pump's spout.



Collecting a Sample from a Tap

If you are sampling from a tap or from a well with an electric pump, allow the water to flow for about 30 seconds before collecting a fresh sample. Fill your sample bottle directly from the tap, rinse the bottle, throw the water away, then collect your sample following the "bottling technique" described below.

Measuring the pH of Samples

As you are filling your sample bottles, pour some sample water into a small clean container, rinse the container, throw the water away, then refill the container with sample water and measure the pH. Follow the instructions provided in your pH measuring kit. Record the pH of your sample in the Sample Data Sheet (examples of these data sheets are given in the Testing Procedures section).

Bottling Technique

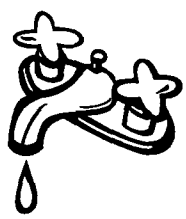
1. It is important to ensure that bottles are labelled correctly using the masking tape. You may label the bottles before collecting your samples or as you collect them. **Be sure to include**:
   - your school's name
   - your teacher's name
   - the name of the place where you took the samples
   - the date and time you collected the samples
2. Rinse the bottles **and** the cap before filling the bottle with your sample (throw away this rinse water).
3. Fill the sample bottle completely full so there is **no air left** in the bottle.
4. Cap the bottle and seal the cap with masking tape.
5. Dry the outside of the bottle. **Be sure the bottle is labelled.**
6. **Wash your hands.** If the water you are sampling is likely to be contaminated, take along soap and clean water to wash your hands as soon as you have filled and dried the sample bottles.
7. Enter in your data sheet information about the samples you have just collected. Sample data sheets are provided in the Testing Procedures section).

Note: It is a good idea to draw a simple map showing the location where you took your samples. You can indicate the exact points in your map by counting paces from particular features of the landscape. For example, "Silver River: took sample #1 from shore with a bucket and rope, at a point located 40 paces downstream from the wooden bridge, at the intersection of Farm Road and Highway 7."

DID YOU KNOW?

Water is not evenly distributed around the world. Some countries have vast supplies, but other countries have very little.

Testing Tap Water That Is Chlorinated



Chlorine in tap water has been added to kill bacteria that may cause harm to you. The presence of chlorine in the water can affect the germination of seeds or onion bulbs, as well as the growth of hydra.

The easiest way to remove chlorine from water (dechlorinate the water) is to collect the water sample in a clean container and simply let it stand uncovered overnight in a refrigerator at 4°C. The chlorine will evaporate from the water overnight. You should re-cap the sample bottle in the morning.

D: Testing Procedures

This section describes all the steps that you need to take, in the order that you will take them, for each of the four experiments. Samples of data sheets and tables for recording your observations and measurements are also provided. Read the procedures carefully before you begin your experiments.

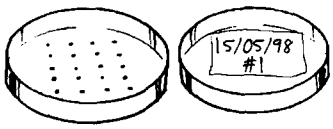
Note: The test procedures described have been simplified from their original versions specifically for the AQUAtox 2000 International School Network. If you want information on the original testing procedures please contact us through our web site or by mail at:

AQUAtox 2000 Coordinator
Ecosystem Approaches to Human Health Program Initiative
International Development Research Centre
P.O. Box 8500, Ottawa, Canada
K1G 3H9

Or contact us by fax at: **(613) 567-7748**.

The Lettuce Seed Bioassay

Assessing Water Toxicity Using Lettuce Seeds



Purpose:

To determine the toxicity of water samples using lettuce seed germination.

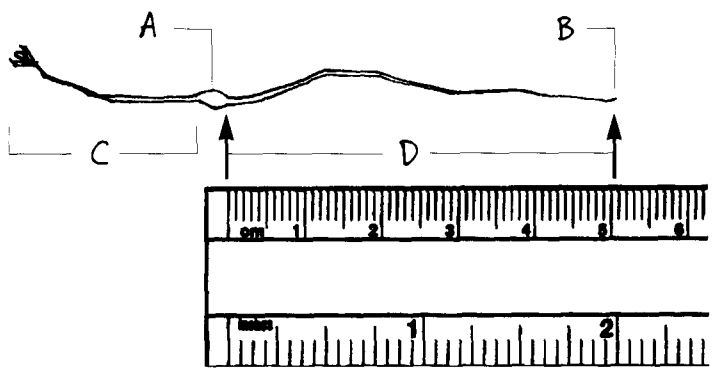
Background:

Toxic chemicals can affect the normal development of plants, especially in the early stages of development (germination and root development). By observing and measuring the length of young roots and comparing the lengths to a normal control we can learn about the possible presence of toxic chemicals in the environment. Any seed that comes in contact with water will tend to sprout, but this sprouting can be affected by the amount of pollution in the water. If the water is very polluted, only a few seeds – or none at all – will succeed in sprouting. Polluted water can also affect the development of the root.

This test will look at both aspects of seed growth: 1) the effect the water being tested has on the number of seeds that sprout (germination), and 2) the length to which the roots grow (root development). Both observations will be compared to "normal control" and "positive control" tests. As you learned earlier, a normal control is an example of seed germination and development from **clean** water. In this case, the seeds should grow normally. A positive control test means that something is causing the seeds **not to grow** as they should. Water that has been purposely polluted or salted gives a positive result.

DID YOU KNOW?

Human beings need only about 5 litres of water each day for cooking and drinking. Another 25 to 30 litres are needed for bathing, and washing clothes and kitchen utensils.



A: root node, B: tip of root, C: seedling, D: root

Apparatus and Materials

- 80 lettuce seeds (butter crunch lettuce)
- 4 petri dishes (plastic, 100 × 15 mm)
- 8 absorbent paper discs, cut to fit inside petri dishes (2 per dish)
- 4 transfer pipettes
- A minimum of 300 ml of bottled, non-carbonated water
- Stock salt solution (recipe explained in Getting Started Instructions in the Teacher's Guide) for positive control
- A 100 ml graduated cylinder
- Two 1-litre sample bottles labelled sample #1 and sample #2
- Ruler
- 4 squares of aluminum foil (large enough to wrap the petri dishes)
- Roll of masking tape
- Pair of tweezers
- Pencil

Notes and Hints:

1. You will require five days for this test, so plan ahead.
2. Ideally, the tests on the samples should be started within three hours of collecting the samples. If you must store the samples overnight, refrigerate them at 4°C or on melting ice for no more than 24 hours.
3. Use masking tape for labelling.
4. Following the instructions described in the preceding section on "Collecting Water Samples," **safely collect** two different water samples (call them sample #1 and sample #2).

Precautions:

Remember, **this water could be contaminated.** Do not put your fingers in your eyes or mouth during the experiment. Thoroughly wash your hands after completing each stage of the experiment.

DID YOU KNOW?

Canada has the largest supply of fresh water in the world. For every 10 litres of fresh water a young Canadian has access to, a young American has access to 1 litre, a young Southeast Asian has access to 0.2 litre, and a young Egyptian has access to just 0.01 litre.

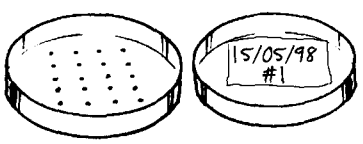
Test Procedure:

A. Preparing the Positive Control Solution

1. Use a clean 500 ml (minimum) container with a non-metallic cap.
2. Using the graduated cylinder, add 250 ml of salt (NaCl) stock solution to the 500 ml container.
3. Add 250 ml of non-carbonated, bottled water to the 500 ml container.
4. Cap, shake well, label "Lettuce (+) 5 g/L." Write in the date.

B. Performing the Test

1. Label the top and bottom of each petri dish with a piece of masking tape and pencil: label one dish (N) for "normal control", one (P) for "positive control", one (Sample #1), and one (Sample #2).
2. Cut eight pieces of absorbent paper to fit snugly in the bottom of the petri dishes and place two absorbent papers in each petri dish.
3. Using a clean pipette, add just enough non-carbonated water (about 4 ml) to wet the absorbent papers in the petri dish labelled (N).
4. Using a different clean pipette, repeat the above step for the dish marked (P) with the positive control solution.
5. Using another clean pipette, add Sample #1 water to the petri dish labelled Sample #1.
6. Using another clean pipette, add Sample #2 water to the petri dish labelled Sample #2.



Note: **Do not add too much** water or the seeds will not sprout. You only need to add about 4 ml of solution to each petri dish.

The next steps are difficult, so **patience counts**:

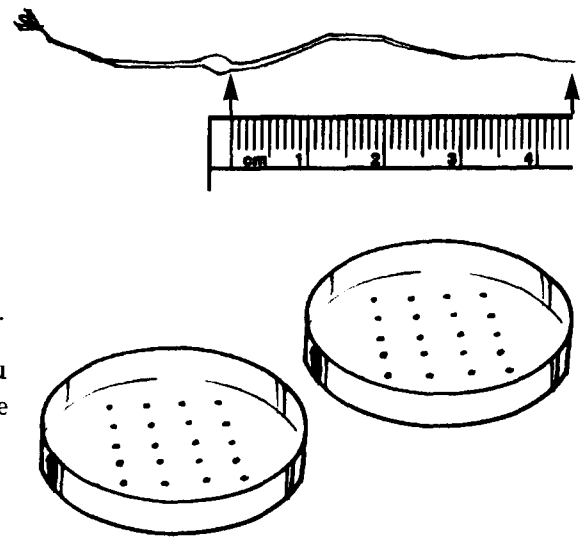
7. Place some seeds on a clean piece of paper.
8. Using tweezers, place 20 seeds (five rows of four or four rows of five seeds) in **each petri dish**; select seeds of similar size, shape, and colour.
9. Place the appropriate covers on each petri dish.
10. Carefully wrap each dish with tin foil so that the seeds are not exposed to the light (they need darkness to sprout). Make sure you **do not turn the dishes** upside down.
11. Using masking tape on the tin foil, label "Top" on each wrapped dish.
12. Place the dishes in a safe place at room temperature for five days, away from direct sunlight.

Hints:

- You may want to put the wrapped petri dishes inside a plastic bag to prevent moisture from escaping. Be careful not to shake or turn over the petri dishes. You want the seeds inside to be in contact with the wet paper.
- In your experimental kit, you received a small container with enough lettuce seeds to conduct 20 sets of experiments. Take out only the amount of seeds that you need for each experiment (about 100) and **do not** put any leftover seeds back in the container. Also make sure you close the container right after taking out the seeds you need. You want to keep the seeds for future experiments as dry as possible. Store the container in a dark and cool place (if possible, you can wrap the container with foil and keep it in a refrigerator at 4°C).

C. Making Observations and Measurements

After five days or approximately 120 hours:



1. Once again, take great care **not to turn the petri dishes** upside down.
2. Carefully unwrap each petri dish.
3. Count and record the number of sprouted seeds in each dish (be sure to record your count in the right place on your data sheet).
4. Remove each sprouted seed, measure and record the length of each root in your data sheet (see illustration).

Hint: When measuring root length, you may find it easier if you place the germinated roots against a dark background. Tape the ruler onto the background, then carefully "stretch the roots" to their maximum length.

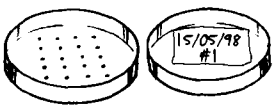
D. Clean up time

1. As per the safety precautions, wrap the seeds in paper and give them to your teacher to discard (the teacher will discard them by putting them in the garbage).
2. Carefully wash your equipment and put it away.
3. Clean your work area and ask your teacher to disinfect it with a chlorine solution.
4. Wash your hands with soap and water.

Now for the calculations and the conclusion, and then you are finished.

Great work!

Note: To interpret your results, you should compare the average size of the roots from your water samples against the normal control. The larger the difference between the normal control and the test sample, the greater the likelihood of toxic chemicals being present in the water.



SAMPLE DATA SHEET: Lettuce Seed Bioassay

Please Print

Experiment: _____

School Name and Grade: _____

Your Name(s): _____ Date tests started: _____

Teacher's Name: _____ Date tests ended: _____

Sample #1

Sample pH: _____

Type of water source (well, stream, pond, river, etc.) _____

General location (city, town, farm, etc.): _____

Location of sample (near a factory, wilderness, farm etc.): _____

Did the water appear clear? ☐ yes ☐ no

Was there sediment (small soil particles) in the water? ☐ yes ☐ no

Was there any smell? ☐ yes ☐ no If yes, how strong? _____

How long ago had it rained before you took the sample? _____days ☐ Heavy ☐ Light rain

Any other information about the sample: _____

_____

_____

Sample #2

Sample pH: _____

Type of water source (well, stream, pond, river, etc.) _____

General location (city, town, farm, etc.): _____

Location of sample (near a factory, wilderness, farm etc.): _____

Did the water appear clear? ☐ yes ☐ no

Was there sediment (small soil particles) in the water? ☐ yes ☐ no

Was there any smell? ☐ yes ☐ no If yes, how strong? _____

How long ago had it rained before you took the sample? _____days ☐ Heavy ☐ Light rain

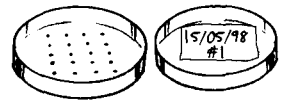
Any other information about the sample: _____

_____

_____

_____

OBSERVATIONS AND RESULTS: Lettuce Seed Bioassay



Record the individual root lengths in millimetres. You will probably find that not all 20 seeds sprouted in each petri dish. Be sure to record the measurements for the right sample in the right place.

	Normal control	Sample #1	Sample #2	Positive control
Root length (mm)	1._____	1._____	1._____	1._____
	2._____	2._____	2._____	2._____
	3._____	3._____	3._____	3._____
	4._____	4._____	4._____	4._____
	5._____	5._____	5._____	5._____
	6._____	6._____	6._____	6._____
	7._____	7._____	7._____	7._____
	8._____	8._____	8._____	8._____
	9._____	9._____	9._____	9._____
	10._____	10._____	10._____	10._____
	11._____	11._____	11._____	11._____
	12._____	12._____	12._____	12._____
	13._____	13._____	13._____	13._____
	14._____	14._____	14._____	14._____
	15._____	15._____	15._____	15._____
	16._____	16._____	16._____	16._____
	17._____	17._____	17._____	17._____
	18._____	18._____	18._____	18._____
	19._____	19._____	19._____	19._____
	20._____	20._____	20._____	20._____
Total length	mm	mm	mm	mm
# Sprouting seeds				
Average length	mm	mm	mm	mm
Percent change in root growth	—	%	%	%
Percent change in sprouting	—	%	%	%

Average Length = $\frac{\text{Total Length}}{\text{\# Sprouting Seeds}}$

Percent Change in root growth = $\frac{\text{Average length of sample} - \text{Average length of normal control} \times 100}{\text{Average length of normal control}}$

Percent Change in sprouting = $\frac{\text{\# Sprouting seeds with sample} - \text{\# Sprouting seeds with normal control} \times 100}{\text{\# Sprouting seeds with normal control}}$

Conclusions: Lettuce Seed Bioassay

Here is where you interpret your observations and the results of your experiment. Were your hypotheses correct? Why? Why not?

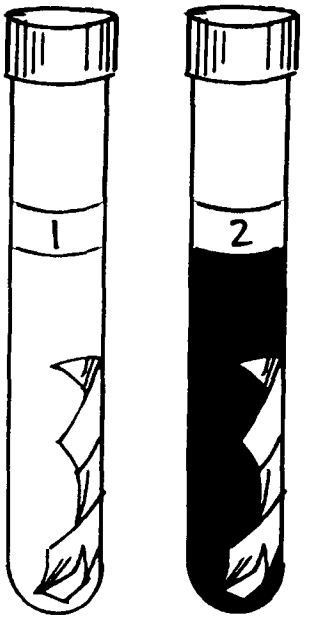
Comments:

Write down any information you think is important that would help explain your results to other people reading about your work. Did you expect to obtain the results you got? Did you have any problems doing the experiments? If you were to do the experiment again, what would you do to make it better?

The Hydrogen Sulphide (H_2S) Test

Purpose:

The hydrogen sulphide test (H_2S) paper strip bacteriological test is used to detect the presence of certain microorganisms in the water. Some of these microorganisms may be harmful to the health of people.



Background:

There are many types of microorganisms on earth. Some are helpful to humans but others can cause people to become sick. These organisms are so small (micro) that you need a good microscope to actually see them. Certain microorganisms, like coliform bacteria, are found in the intestines of warm-blooded animals, including human beings. These bacteria are excreted (left behind as waste) in large quantities in faecal material. Some of these bacteria can produce a gas, hydrogen sulphide (or H_2S for short), as they grow inside a test tube. Their presence in water is an **indication** that the water has been contaminated with human or animal waste.

This experiment is a test for H_2S. In order to check for the presence of these "sentinel" or "indicator" bacteria in water, we put them in contact with a strip of paper that has been impregnated with a nutritive substance (food for bacteria) plus a colour indicator that turns black when it comes in contact with hydrogen sulphide. If the test paper turns black, it means that H_2S was produced, which in turn means that bacteria of faecal origin are likely present in the water sample.

In your experiments with the H_2S test, you will collect water samples in sterile test tubes containing the impregnated paper strips. You will then incubate the samples (maintained at a temperature range of 26°-39°C) for three days. Under these perfect conditions of temperature and food supply, the bacteria will grow if they are present in the water samples. While they grow, they will produce (H_2S), which reacts with the colour indicator in the paper strips, making the strips turn black.

Apparatus and Materials:

- 250 ml sealed bottle of non-carbonated water
- 5 screw-cap sterile tubes with (H_2S) strips
- Empty screw-cap tube for marking the volume
- Test tube rack
- Roll of masking tape
- Incubator
- 100 ml plastic graduated cylinder
- Sample collection container (e.g. a bucket)

Hygiene and Safety Precautions:

There is always a possibility that **disease-causing organisms** are present in the samples you are testing. Therefore, it is **very important** to follow proper hygiene and safety procedures while testing the samples.

- Keep fingernails short and tie back long hair.
- **Always** wash your hands with soap and warm water **before** and **after** the experiment.
- Turn your head away from your work area if you cough or sneeze.
- Keep your work area clean and dry **at all times**.
- Inform your teacher **immediately** of any accidents or spills.
- **Never** touch your eyes or mouth while working on the tests.
- **Never** work on tests if you feel sick.
- **Never** eat or drink in the work area.

Before you can proceed with your experiment, you must sterilize the test tubes **containing new** (H_2S) strips. The teacher should do the following sterilization procedure or closely supervise it to make sure contamination does not happen. This procedure should be done a least one day before doing the tests.

DID YOU KNOW?

People in the United States and Canada are the biggest consumers of water. A person in the USA uses 350 litres of water each day. A person in Europe uses 140 litres, while a person in rural Mexico uses less than 60 litres in the same day.

Test Procedure

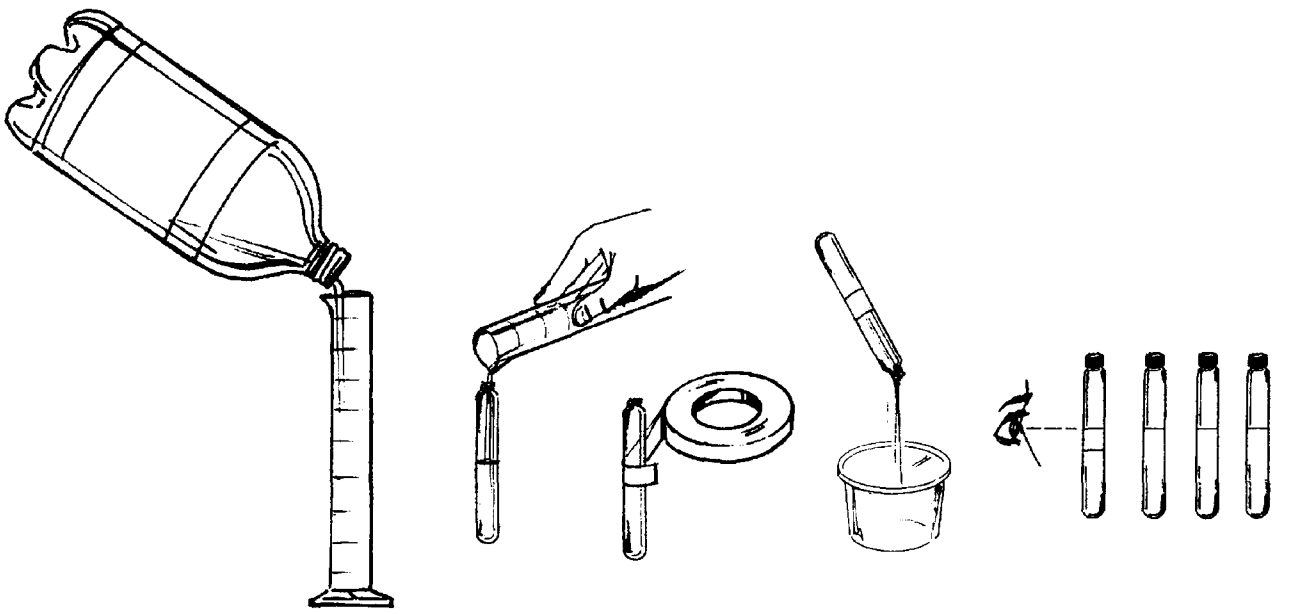
A. Sterilizing the Tubes with Paper Strips:

1. Wash the caps and tubes carefully and allow to air dry (use soap, the test tube brush, and rinse thoroughly with clean bottled or boiled water).
2. For each of the five test tubes you are preparing:
   a. Cut one paper strip provided with the kit into smaller, thin strips so they can easily fit through the mouth of the test tube.
   b. Carefully place the thin strips inside the tube.
   c. Loosely fit a cap on each tube.
   d. Cover each cap with a small piece of tin foil to protect it from the heat (this helps the cap last longer).
   e. Place the covered, capped tubes with the strips in them onto a baking sheet, then into an oven preheated to 150°C. Keep the tubes in the oven for 3-4 minutes. Be careful not to burn the paper strips.
   f. Carefully remove the tubes from the oven.
   g. Allow the test tubes and caps to cool for 10 minutes.
   h. Tighten the caps and remove the tin foil.

3. Sterilized tubes with paper strips can be stored in a dark place at room temperature for up to 12 months.

B. Preparing the Test Tubes (labelling and marking the 10 ml volume):

1. Set up and test your incubator. The temperature must stay between 26°C and 39°C (refer to the Getting Started Instructions in the Teacher's Guide).
2. Once they are sterilized, **be certain not to open** the test tubes with strips until you are ready to fill them with your water samples.
3. Using the graduated cylinder, measure 10 ml of water and add it to an **empty** (without a paper strip) test tube.
4. Place a strip of masking tape on the tube so that the **top of the tape is right at the surface** of the 10 ml level of water.
5. Empty the water; label this tube "marker tube."
6. Using the marker tube as a guide, put masking tape on the five capped tubes at the same level (see illustration below).
7. Label the tubes: (N) for "normal control"; (S1A) and (S1B) for "Sample #1A" and "Sample #1B"; and (S2A) and (S2B) for "Sample #2A" and "Sample #2B."



C. Taking the Water Samples: (this step is done in the field)

1. **Do not** open the test tubes until you are ready to pour the water sample into them.
2. **Take great care** not to contaminate the cap and tube. Do not hold the caps from the inside or put them down anywhere. To proceed, hold the test tube in one hand, unscrew the cap, and hold the tube from the outside with your other hand. Ask your teacher or a colleague to help you pour the sample into the test tube.
3. If you are collecting your sample from a body of water (river, stream or pond):
   a. Rinse your container (bucket) with the same water that you are about to sample. Throw the rinse water away **but not back** from where you will get your sample.
   b. **Carefully** collect your sample in the container (bucket) (refer to Collecting Water Samples section).
   c. Slowly pour 10 ml (to the top of the masking tape) into your sample test tubes. **Remember that two test tubes** are filled for each type of water being tested.
   d. Immediately cap your test tubes.
4. If you are collecting your sample from a tap, pump spout, or pipe:
   a. Carefully fill the test tubes directly from the tap, spout, or pipe. **But do it carefully**, because the tubes will fill to the marked line (top of masking tape) very quickly. Try not to overfill your tubes. Even if you do overfill the tubes, you can still perform the experiment.
   b. **Immediately** cap the test tube tightly.
   c. Put a piece of tape around the point where the cap meets the tube to remind yourself **not to open them again** until discarding their contents in a toilet or latrine at the end of the experiment.
5. Record the date, time, location, and description of your water samples on the data sheet.
6. Place the samples in the incubator as soon as possible when you get back to your school.

Note: When transporting your test tubes back to the school, make sure they are not exposed to direct sunlight. The sun's rays can kill the bacteria inside the tubes.

DID YOU KNOW?

Water conservation means reducing the amount of water we waste. A tap leaking one drop of water per second wastes 25 litres of water in one day. That's 10,000 litres a year!

D. Preparing the Normal ("N") Control (Back at the school):

1. Open the small bottle of non-carbonated water **just before** pouring it into the test tube.
2. Add 10 ml of bottled non-carbonated water to the tube marked (N) up to the top of the masking tape. You may also use water that has been boiled for one minute (then cooled to room temperature) as a normal control.
3. Immediately cap the tube and place it in the incubator with the four other sample tubes. Cover all five tubes with a piece of foil paper to protect them from the light of the incubator (see illustration on the following page).

DID YOU KNOW?

Every eight seconds a child dies from a water-related disease.

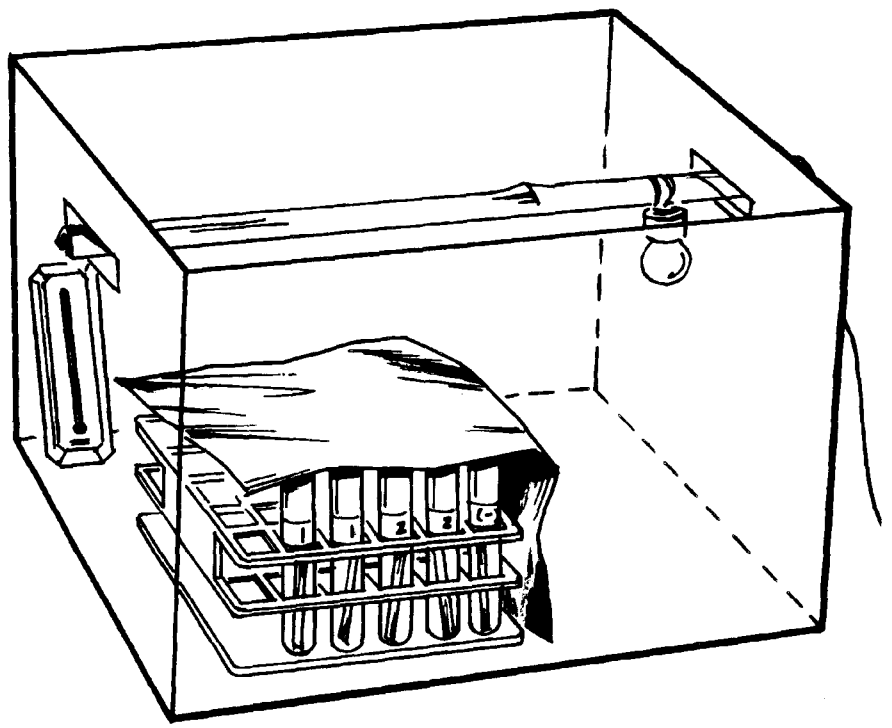
Notes and Hints:

1. The sample volume is **only 10 ml**, so be careful not to overfill the tubes. If you happen to add a little more your results will still be valid.
2. You will be doing two tests on each of two samples of the same water source (that is: two tests for sample #1 and two tests for sample #2). Samples 1 and 2 can come from different water sources.
3. For safety reasons and because it really is not necessary, a positive control is not done in the H_2S test.
4. Timing is quite important, so plan ahead. The H_2S test should be started right after the sample is taken. You should have sterilized and marked the tubes before going to the field to collect your samples.
5. **Never** use moldy or darkened H_2S paper strips to do your tests.
6. When you take out a new paper strip from its plastic bag, make sure you close the bag tightly again as soon as possible. This will help keep the remaining paper strips in the bag as dry as possible and allow them to last longer.
7. If the normal control test shows any darkening (a positive result), the tests results of your other samples are invalid. A darkening of the strips in the normal control test tube means that something in the way you are doing the tests is not working. You cannot draw any conclusions from your experiment. You must begin the experiment again, making sure you do not contaminate anything.
8. Make sure that the temperature in the incubator **does not rise above 39°C** (higher temperatures may kill the bacteria you are trying to detect.)

E. Making Observations:

1. Observe the samples each day for three days, or for five days if the temperature in your incubator is **below 30°C** (since bacteria take longer to grow at lower temperatures).
2. Record your daily observations on the data sheet.
3. Use the guide at the bottom of your data sheet to interpret your results.

Note: A **negative** result (no darkening of paper strips) is fine. It means there are no indicator bacteria present in the sampled water. A **positive** result (paper strips turn black) means there are bacteria present.



Note: The hydrogen sulphide test was developed for testing the quality of drinking water. The test is highly sensitive and detects even small concentrations of bacteria. If you use this test to check the pollution of water in rivers, ponds, or streams, you will find that it will often indicate the presence of high concentrations of bacteria. The water may not be fit to drink but it may be clean enough for swimming. Water with concentrations below 1000 coliforms per 100 ml is suitable for water-contact sports.

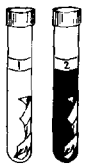
F. Procedure for Disposal of Samples (to be done by the teacher):

Wear rubber gloves and eye protection when working with strong chlorine solutions.

- Prepare disinfectant solution in a plastic container. Fill container halfway.
- Add one part bleach (5% available chlorine) to five parts water.
- Pour the test tube contents into the toilet or latrine. If some paper strips remain stuck in the tubes, leave them there. They will come out in the disinfection solution.
- Place the empty test tubes and caps in the disinfectant for 30 minutes.
- Remove the test tubes and caps and rinse with tap water.
- Wash them with the test tube brush, soap, and water.
- Rinse several times before storing.
- Discard the disinfectant in the toilet or latrine.
- Wash the container with soap and water.

DID YOU KNOW?

Safe drinking water and proper sanitation can reduce child mortality by as much as 60% in some countries.



SAMPLE DATA SHEET: The H_2S Test

Please Print

Experiment: _____

School Name and Grade: _____

Your Name(s): _____ Date tests started: _____

Teacher's Name: _____ Date tests ended: _____

Sample #1

Sample pH: _____

Type of water source (well, stream, pond, river, etc.) _____

General location (city, town, farm, etc.): _____

Location of sample (near a factory, wilderness, farm etc.): _____

Did the water appear clear? ☐ yes ☐ no

Was there sediment (small soil particles) in the water? ☐ yes ☐ no

Was there any smell? ☐ yes ☐ no If yes, how strong? _____

How long ago had it rained before you took the sample? _____days ☐ Heavy ☐ Light rain

Any other information about the sample: _____

_____

_____

Sample #2

Sample pH: _____

Type of water source (well, stream, pond, river, etc.) _____

General location (city, town, farm, etc.): _____

Location of sample (near a factory, wilderness, farm etc.): _____

Did the water appear clear? ☐ yes ☐ no

Was there sediment (small soil particles) in the water? ☐ yes ☐ no

Was there any smell? ☐ yes ☐ no If yes, how strong? _____

How long ago had it rained before you took the sample? _____days ☐ Heavy ☐ Light rain

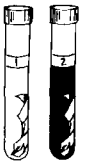
Any other information about the sample: _____

_____

_____

_____

OBSERVATIONS AND RESULTS: The H_2S Test



Start Date: _____ Time: _____

Finish Date: _____ Date: _____

Data enter by: _____

	Incubator Temp.	Test Vol. (ml)	Normal control	Sample #1A	Sample #1B	Sample #2A	Sample #2B
Day 1							
Day 2							
Day 3							
Day 4							
Day 5							
Estimation of Pollution (bacteria/100 ml)							
Observations (intensity of black colour & day)							

Notes:

1. In each sample box in the table above, write "+" for any blackening or "-" if there is no blackening.
2. Estimation of pollution:
   a. **No black colour after 72 hours**: low or no bacterial pollution; under 10 bacteria / 100 ml of water (write: "< **10 bacteria / 100 ml**" in the corresponding box).
   b. **Black colour appears between 24 and 72 hours**: likely pollution; water is **not** safe to drink unless treated; estimate more than 10 bacteria/100 ml (write: "> **10 bacteria / 100 ml**" in the corresponding box).
   c. **Black colour before or at 24 hours**: medium to high pollution; water is **not** safe to drink unless treated; estimate more than 100 bacteria/ 100 ml of water (write: "> **100 bacteria / 100 ml**" in the corresponding box).
3. Continue readings on days 4 and 5 **only** if incubation temperature is below 30°C.

Conclusions: The H_2S Test

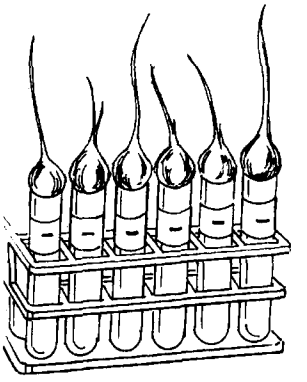
Here is where you interpret your observations and the results of your experiment. Were your hypotheses correct? Why? Why not?

Comments:

Write down any information you think is important that would help explain your results to other people reading about your work. Did you expect to obtain the results you got? Did you have any problems doing the experiments? If you were to do the experiment again, what would you do to make it better?

The Onion Bulb Bioassay

Assessing Water Toxicity Using Onion Bulbs



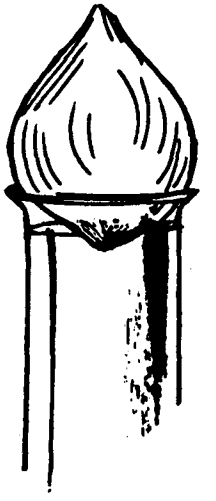
Purpose:

To determine the toxicity of water samples using onion bulbs, specifically to determine whether toxicity affects the development of the onion bulb roots.

Background:

Just as humans need healthy conditions to grow, so do plants. Toxic chemicals can affect the normal development of humans and plants, particularly in the early stages of growth. In plants, the development of the root system is highly sensitive to the presence of pollutants. By observing, measuring, and comparing the length of young roots in a non-toxic setting (the normal control), in a toxic setting (the positive control) and in a sample of water whose quality is uncertain, conclusions may be drawn about the toxicity of the water in the test sample. The test procedure is designed to provide the onions with the maximum amount of water (as much water as they require) they would consume in their natural environment. This test can be used to assess water from different types of sources: lakes, rivers, wells, or water pipes.

Apparatus and Materials:



- 24-30 onion bulbs to fit on top of the test tubes (see illustration)
- 2 litres of bottled non-carbonated water
- Stock salt solution (refer to recipe for stock solution in Getting Started Instructions in Teacher's Guide) for the positive control
- 100 ml graduated cylinder
- Clean glass or plastic container to soak onions after peeling
- Old newspaper to cover work area
- Clean cloth to wipe up spills
- Plastic knife
- 4 transfer pipettes
- Small ruler
- Roll of masking tape
- 24 test tubes
- Test tube holder

Precautions:

Remember, **this water could be contaminated.** Do not put your fingers in your eyes or mouth during the experiment. Thoroughly wash your hands after completing each stage of the experiment.

Test Procedure

A. Preparing the Positive Control Solution

Note: This salt solution should inhibit the growth of the roots in a way similar to the effect of polluted water.

1. Use a clean 500 ml (minimum) container with a non-metallic cap.
2. Transfer 500 ml of NaCl stock solution to the 500 ml container.
3. Cap and label "Onion (P) 10 g/L." Write in the date.

Note: This positive control solution may be stored for one or two weeks, but it is best to prepare a fresh positive control for each experiment.

B. Preparing the Onions for the Test

1. Choose onions of a size that will fit **on top** of the test tubes. Try to use onions of the same size for each set of samples.
2. Cover your work area with newspaper or an absorbent cloth.
3. Label six test tubes (P) for "positive" controls; six test tubes (N) for "normal" controls; six test tubes (#1); and six test tubes (#2) for samples 1 and 2, respectively.
4. Place the tubes in the test tube holder.
5. Label the pipettes: (P), (N), (#1), and (#2).
6. Fill each test tube to the very top with the corresponding solution or water sample; this is where a little water may spill over.

Note: Be sure to **save enough of each sample** to refill the test tubes each day over the next three days. You can store sufficient amounts of water samples and control solutions in additional test tubes properly labelled (N, P, #1, and #2). You will need two full test tubes for each type of sample and control. Cover the mouths of the tubes with tin foil to prevent evaporation.

Notes and Hints:

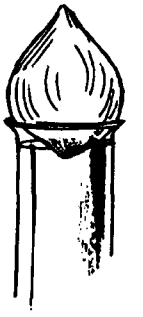
1. You will require three days for this test, so plan ahead.
2. Owing to the considerable amount of work required in this experiment, students may want to work in groups.
3. Choose the onion bulbs carefully so they fit well on the top of the test tubes. Try to use bulbs of the same size.
4. Be careful **not to remove or damage** the roots of the onions while you handle them.

DID YOU KNOW?

All 10 of the warmest years on record have occurred in the last 15 years. The 1990s have already been warmer than the 1980s, which was the warmest decade yet on record - by almost 0.1 °C.

C. Beginning the Test

1. Carefully remove the brown layers of onion skin from the onions, **taking care** not to damage the roots.
2. As you remove the outer skins of the onions, place the bulbs in a jar or bowl filled halfway with clean bottled water.
3. Once all the onions are peeled, take all the onions out of the jar and place them on a paper towel to remove excess water.
4. Place each onion, **root down**, on the top of a test tube.
5. Place the test tubes and onions in a **safe sunlit place**, but away from direct sunlight, where they can remain undisturbed **for the next three days** (you may want to put another piece of newspaper or cloth under the test tube holders again).



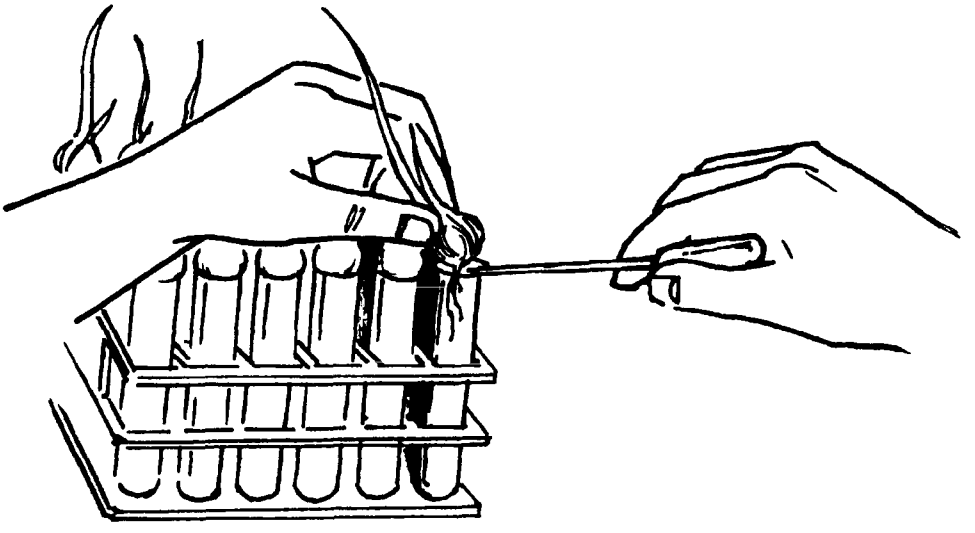
Note: **The roots must be in contact with the liquid in the test tubes at all times.**

D. "Topping up" the Test Tubes

As the onions sprout, they will begin to consume water through their roots. You need to replenish this water in each test tube every day. **Be sure to use the proper water sample and pipette to refill each test tube**. Take great care not to contaminate your solution.

At least once a day for the next two days, carefully tilt each onion bulb slightly and refill the test tube with the appropriate solution.

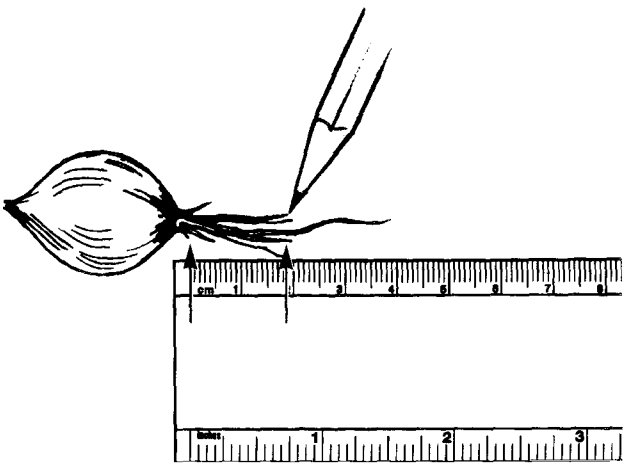
Note: If the roots are long and you remove the onion completely, you will have a difficult time getting all the roots back in the test tube. It is better to lift the onion only slightly from the tube with one hand, keeping as many roots as possible inside the tube and refill with the proper pipette.



E. Making Observations and Measurements

After three days:

1. Remove one group of six onion bulbs at a time. Discard the onion with the shortest roots. (Sometimes the test group will include a poor onion specimen, so removing the poorest one from each group makes allowances for this natural occurrence.)
2. Measure the length of the root bundle for each of the remaining five onions with a ruler. Ignore exceptionally short or long roots (see the illustration below).
3. Record the length of each root bundle on the data sheet.
4. Repeat the above procedure for the three remaining groups of onions.
5. Calculate the average length of the roots in each test group as described in the data sheets.

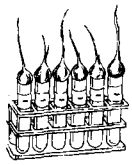


F. Cleaning Up

1. Keeping the safety precautions in mind, discard the onions safely (throw them in the garbage or pit latrine).
2. Carefully wash your equipment with soap and water and put it away.
3. Clean the work area, then ask your teacher to disinfect it with a chlorine solution.
4. Wash your hands with soap and water.

G. Calculations and Conclusion

Note: To interpret your results, you should compare the average size of the roots from your water samples against the normal control. The larger the difference between normal control and test sample, the greater the likelihood of toxic chemicals being present in the water.



SAMPLE DATA SHEET:
Onion Bulb Bioassay

Please Print

Experiment: _____

School Name and Grade: _____

Your Name(s): _____ Date tests started: _____

Teacher's Name: _____ Date tests ended: _____

Sample #1

Sample pH: _____

Type of water source (well, stream, pond, river, etc.) _____

General location (city, town, farm, etc.): _____

Location of sample (near a factory, wilderness, farm etc.): _____

Did the water appear clear? ☐ yes ☐ no

Was there sediment (small soil particles) in the water? ☐ yes ☐ no

Was there any smell? ☐ yes ☐ no If yes, how strong? _____

How long ago had it rained before you took the sample? _____days ☐ Heavy ☐ Light rain

Any other information about the sample: _____

_____

_____

Sample #2

Sample pH: _____

Type of water source (well, stream, pond, river, etc.) _____

General location (city, town, farm, etc.): _____

Location of sample (near a factory, wilderness, farm etc.): _____

Did the water appear clear? ☐ yes ☐ no

Was there sediment (small soil particles) in the water? ☐ yes ☐ no

Was there any smell? ☐ yes ☐ no If yes, how strong? _____

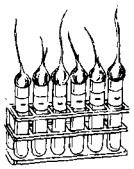
How long ago had it rained before you took the sample? _____days ☐ Heavy ☐ Light rain

Any other information about the sample: _____

_____

_____

_____



OBSERVATIONS AND RESULTS: Onion Bulb Bioassay

Start Date: _____ Time: _____

Finish Date: _____ Time: _____

	Normal control	Sample #1	Sample #2	Positive control
Root length (mm)	1._____ 2._____ 3._____ 4._____ 5._____	1._____ 2._____ 3._____ 4._____ 5._____	1._____ 2._____ 3._____ 4._____ 5._____	1._____ 2._____ 3._____ 4._____ 5._____
Total length	mm	mm	mm	mm
Average length	mm	mm	mm	mm
Percent change in root growth	—	%	%	%

Average Length = $\frac{\text{Total Length}}{\text{\# of root bundles}}$

(In this case: # of root bundles = 5)

Percent Change in root growth = $\frac{\text{Average length of sample} - \text{Average length of normal control}}{\text{Average length of normal control}} \times 100$

Conclusions: Onion Bulb Bioassay

Here is where you interpret your observations and the results of your experiment. Were your hypotheses correct? Why? Why not?

Comments:

Write down any information you think is important that would help explain your results to other people reading about your work. Did you expect to obtain the results you got? Did you have any problems doing the experiments? If you were to do the experiment again, what would you do to make it better?

The Hydra Bioassay

Acute Toxicity Assessment of Water with Fresh-Water Hydra

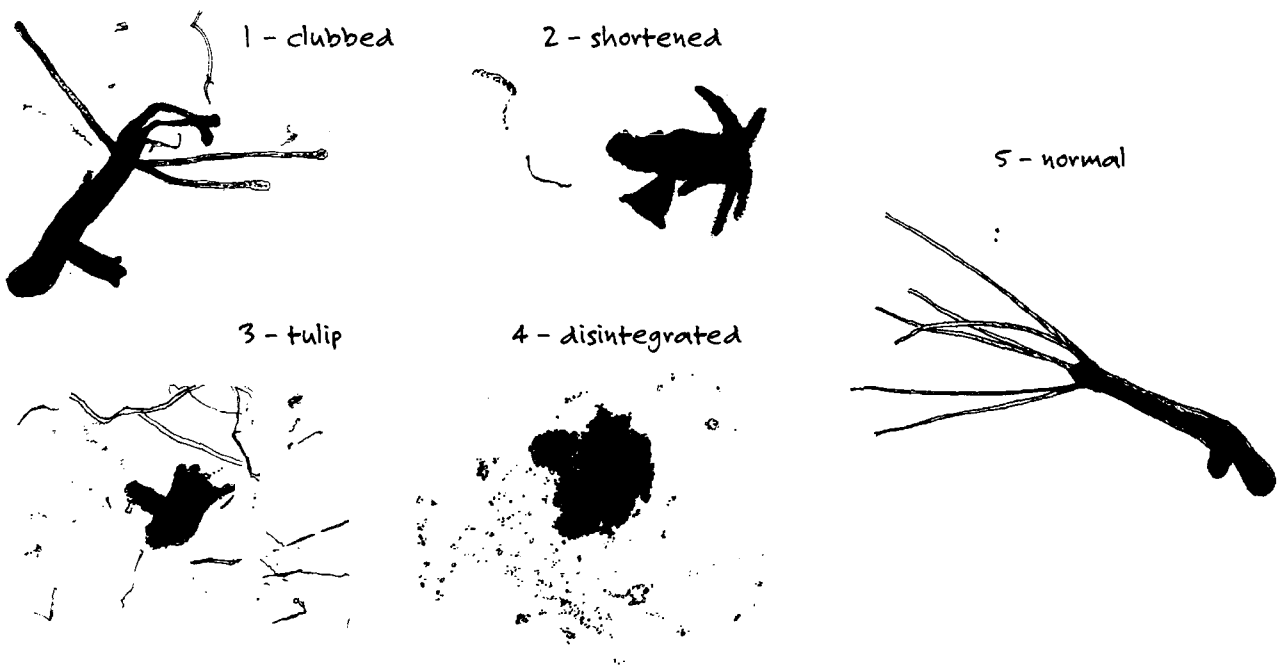
Purpose:

This experiment measures the reaction of hydra to any toxic compounds present in fresh water. The test can be used to measure the toxicity of household water, industrial wastewater, and treated or untreated surface or ground water.

Background:

The hydra is a multi-celled animal that is found in nature (usually in ponds, lakes, and streams). It needs a very healthy environment in order to grow normally. Hydra are extremely sensitive to pollution, which makes the test a good indicator of pollution. When the organism is exposed to toxic pollutants for extended periods (such as four days), its body becomes deformed, taking on certain shapes that are related to the level of pollution.

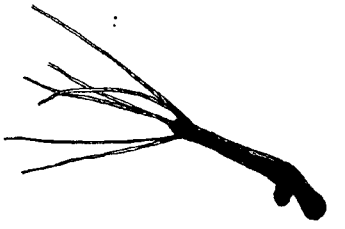
Typical shapes of affected hydra are shown in the figure below. Some of the body changes are clubbed tentacles, shortened tentacles, tulip phase, or the disintegration of the organism itself. Any of these forms would indicate the presence of toxic pollutants in a water sample. In this experiment, hydra are exposed to water samples for four days (96 hours). At the end of this period, we observe and count the hydra that have reached a tulip phase or have died.



Apparatus and Materials:

- 100 ml graduated cylinder
- 500 ml (minimum) clean bottle with cap
- 100 ml of stock solution
- 400 ml of bottled non-carbonated water
- Normal control solution (the hydra medium)
- Circular transparent bowl (about 10 cm in diameter)
- 12-well micro-plate
- 4 plastic petri dishes (35 × 10 mm)
- 8 graduated Polyethylene pipettes
- 10× magnifying glass
- Hydra in a hydra medium (supplied only after applying to IDRC for them)
- 2 samples of water to be tested (See section on Collecting Water Samples)

Precautions:
Remember, **this water could be contaminated.** Do not put your fingers in your eyes or mouth during the experiment. Thoroughly wash your hands after completing each stage of the experiment.



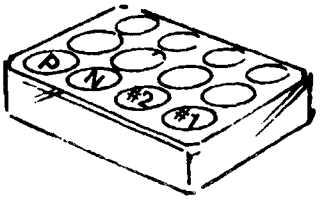
Notes and Hints:
This test takes more planning than the other tests. Your teacher may have decided to grow the hydra for your experiments. In certain countries, an environmental laboratory will be growing this organism, and it may be possible for your teacher to obtain hydra directly from the lab just prior to your experiments. In this case, it is **most important** that you are ready to do the tests within 24 hours from the moment the hydra arrive.

Test Procedure

Note: Steps A and B are to be completed **before receiving the hydra.**

A. Preparing the Positive Control Solution:

1. With masking tape, label a clean 500 ml (min.) bottle "hydra (P) 2 g/L."
2. Note the date of preparation on the label also.
3. Using the graduated cylinder, add 100 ml of stock solution to the labelled 500 ml bottle.
4. Add 400 ml of bottled non-carbonated water to the labelled bottle.
5. Close tightly and shake well.
6. You can store this control solution for up to two weeks, although it is better to prepare a fresh solution before each new experiment.



B. Setting up the Equipment:

1. Use masking tape to label the 12-well micro-plate cover and bottom as follows: one row of three wells labelled (P) for "positive control"; a second row of three wells labelled (N) for "Normal control"; the next row of three wells labelled (#1) for the first water sample; and a fourth row of three wells labelled (#2) for the second water sample.
2. Label the outside of the four petri dishes and covers: one with (P), one with (N), one with (#1) and the last with (#2). These petri dishes will be used as intermediate transfer wells for the hydra. They are used to rinse the organisms **before** putting them in the 12 micro-wells.
3. Label each graduated pipette to match the petri dishes N, #1 and #2).

C. When the Hydra Arrive:

The hydra should be kept at a temperature between 20 and 24°C under standard conditions of daytime and nighttime (about 16 hours of light and eight hours of darkness). The tests must begin within 24 hours of receiving the hydra.

Note: If you keep the hydra longer than 24 hours you must feed them unless you plan to perform the test shortly thereafter. Follow the procedure for maintaining hydra in the Teacher's Guide section of this manual. **The hydra should not be fed for 24 hours prior to doing the test**. When you are ready to do the test, continue with the procedure that follows.

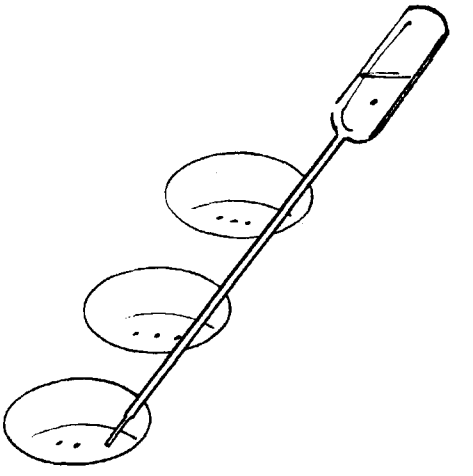
1. Using a clean transfer pipette, transfer four ml of (P) control solution into each of the three wells labelled (P), and transfer another four ml into the petri dish labelled (P).
2. Using the appropriate pipette, do the same for the wells and petri dish marked (N); repeat for the ones labelled #1 and #2.
3. Add some fresh hydra medium (bottled, non-carbonated water) to a small transparent bowl until it is about ⅔ full.
4. **Wash your hands before this step.** Gather the hydra in the centre of the culture bowl (the one where you are keeping hydra that have not been fed for 24 hours) by stirring the liquid medium with your index finger.

Note: By swirling the medium with your finger for 15-20 seconds in one direction, the hydra will be drawn into the centre of the container.

5. Using a clean pipette, draw up about 50 hydra and transfer them to the small transparent bowl containing the fresh hydra medium.
6. With a clean pipette, draw up 15 clean hydra from this last bowl and place them into each of the four petri dishes (15 hydra per dish).

Note: It is best to select hydra without buds or with only slightly developed buds. Healthy hydra should have extended tentacles about the same length as their body column.

7. Using the pipette labelled (P), transfer three healthy hydra from the petri dish labelled (P) and place them in one of the three micro-wells marked with the same letter; transfer another three hydra in the same manner to each of the two remaining micro-wells.
8. Using the appropriate pipette, do the same for the other wells: (N), (#1) and (#2). At the end of this task, every well in the micro-plate should have three healthy hydra that were transferred from their corresponding petri dish.
9. Using the 10× magnifying glass, observe and record the shapes of the hydra immediately after placing them in the test wells (this moment can be considered the start time, or 0 hours).
10. Cover the micro-plate with its lid and place it in a safe sunlit place, but away from direct sunlight, where it can remain undisturbed for the next four days (the hydra are **not** fed during the duration of the experiment).



D. Making Observations and Measurements

After four days or about 96 hours:

Using the 10× magnifying glass, observe and record the shapes of the hydra in your data sheets.

Note: Pay attention to possible changes in body shape: slight effect (clubbed tentacles), moderate effect (shortened tentacles), severe effect (tulip stage or died). Sketches of your observations will be helpful.

Interpreting changes in the shape of hydra in the normal control: Remember that the normal control uses **clean** water. None of the hydra in your normal control solution should die. **But** in some cases you should allow for up to one death among nine hydra. If more than one out of nine organisms have shortened tentacles or reach the tulip stage, your results for the other samples are probably invalid. This means that your hydra population was not healthy to begin with, so you cannot conclude anything about the other samples that you tested.

Interpreting changes in the shape of hydra in your water samples: The higher the number of hydra that are in a tulip stage or dead after 96 hours, the greater the likelihood that the sample of water is toxic. If five or more out of nine hydra in a particular row die, it means the mortality rate is greater than 50%. You can conclude that the sample is probably contaminated. The amount of time it takes for the hydra to show any bad (positive) effects will also give you some idea of the strength of the toxic elements in the sample.

Note: The positive control solution that you prepared should result in **all** the hydra being dead after 96 hours.

SAMPLE DATA SHEET:
Hydra Bioassay

Please Print

Experiment: _____

School Name and Grade: _____

Your Name(s): _____ Date tests started: _____

Teacher's Name: _____ Date tests ended: _____

Sample #1

Sample pH: _____

Type of water source (well, stream, pond, river, etc.) _____

General location (city, town, farm, etc.): _____

Location of sample (near a factory, wilderness, farm etc.): _____

Did the water appear clear? ☐ yes ☐ no

Was there sediment (small soil particles) in the water? ☐ yes ☐ no

Was there any smell? ☐ yes ☐ no If yes, how strong? _____

How long ago had it rained before you took the sample? _____days ☐ Heavy ☐ Light rain

Any other information about the sample: _____

_____

_____

Sample #2

Sample pH: _____

Type of water source (well, stream, pond, river, etc.) _____

General location (city, town, farm, etc.): _____

Location of sample (near a factory, wilderness, farm etc.): _____

Did the water appear clear? ☐ yes ☐ no

Was there sediment (small soil particles) in the water? ☐ yes ☐ no

Was there any smell? ☐ yes ☐ no If yes, how strong? _____

How long ago had it rained before you took the sample? _____days ☐ Heavy ☐ Light rain

Any other information about the sample: _____

_____

_____

_____

OBSERVATIONS AND RESULTS:
Hydra Bioassay

Start Date: _____ Time: _____

Finish Date: _____ Time: _____

Number of hydra organisms in each stage (body shape):

STAGE:	N at start time	Total # of hydra	N (96 h)	S (96 h)	T & D (96 h)	% M
Normal control						%
Positive control						%
Water sample # 1						%
Water sample # 2						%

Symbols: N = no change in shape; S = clubbed and/or shortened tentacles; T = tulip stage; D = Dead (disintegration); %M = percent mortality after 96 hours

Note: To calculate percent mortality, use the total number of hydra at 96 hours, including the newly reproduced hydra (if any).

$$\text{Percent Mortality} = \frac{\text{Total number of hydra in tulip or dead stages at 96 h}}{\text{Total number of hydra at 96 h in set of 3 wells}} \times 100$$

$$\text{or} \quad \%M = \frac{T + D}{N + S + (T + D)} \times 100$$

Conclusions: Hydra Bioassay

Here is where you interpret your observations and the results of your experiment. Were your hypotheses correct? Why? Why not?

Comments:

Write down any information you think is important that would help explain your results to other people reading about your work. Did you expect to obtain the results you got? Did you have any problems doing the experiments? If you were to do the experiment again, what would you do to make it better?

E: Using the Internet for AQUAtox 2000

What is the Internet?

The Internet – also called the Net – is not truly a "thing" but rather a concept (an abstract idea). The Net is the way that computers all over the world can talk to each other electronically. Special wires and machines are used to do this. When you access the Internet, you are actually talking to an individual computer somewhere, which can then talk to another computer, and so on around the entire globe!

While seated at your computer, you can talk to other people or computers and visit websites in countries all over the world. All you do is dial up and connect to the Net, launch the appropriate Internet software, and before you know it, you are able to exchange information and messages with people from around the world.

Why the Internet?

The Net is an important tool for the AQUAtox 2000 project. The web site acts as a home base where students, teachers, and scientists can access, post, and exchange information about water, health, and the state of the environment worldwide. Everyone involved in the AQUAtox 2000 project can communicate on a regular basis. It is a fast, efficient, and fun way to communicate. As a participant, you will be able to share the results of your experiments with other students around the world and discuss how water affects our quality of life.

Our web site address: http://www.idrc.ca/aquatox

When you first reach the site, you will arrive at the AQUAtox 2000 Home page. You will have the option to proceed in one of three languages: **Spanish**, **English**, or **French**. There is also a "search engine" that looks up and retrieves specific information. This way, if you have a question in mind, you can find the information you need quickly.

There are five sections featured on the Home page:

The Experimental Kit

This kit includes a copy of the manual with a step-by-step description on how to do the water tests and information that will assist you with the project. There is also a link to the basic documents that describe the original versions of the tests.

The Resources Page

Test your knowledge about water facts and figures by answering an interactive quiz. You will also find links to other Internet sites that deal with education and the environment, other sources of related information, and a bank of images (photos) and drawings sent by participating schools.

The Bulletin Board

Exchange information with other students, teachers, and scientists involved with AQUAtox 2000 by posting and reading messages. You can share knowledge and experiences about what's happening in the different schools around the world!

Teachers will find also a "teachers corner" where they will be able to exchange teaching tips and more theoretical materials.

The Results Section

In this section, students can enter the results of their experiments. They can also access a map of the world that features each participating school and its country or region of the world. By pointing and clicking on the school of interest, you can get information about that particular school and the teachers and students involved. Here, you can also link to the "results page" where you can find out how the experiments are coming along in different parts of the world.

The What's New Page

Here you will find several items: **announcements** that will notify all participants in AQUAtox 2000 about new events and activities taking place; the **essay of the month,** which will feature noteworthy student essays; and **special contributions** where those who submit materials (essays, pictures, drawings, etc.) to the web site will be acknowledged.

F: Glossary

Acid Rain: rainwater that, having been contaminated with chemicals introduced into the atmosphere through industrial and automobile emissions, has had its acidity increased from that of clean rainwater.

Bacteria: a type of microbe. They are unicellular (made of a single cell). Some, like *Salmonella* or *Shigella,* can cause disease in humans and animals.

Bioassay: the testing of a substance (such as water or industrial effluents) with living organisms to determine its quality and potential harm to the health of humans and the environment.

Chemical contamination: can adversely affect normal reproduction and growth. It can lead to the development of cancers, poisoning, or damage to our genetic material.

Fertilizer: a chemical or mixture of chemicals that aids the growth and development of plants.

Global warming: atmospheric pollution has increased the normal greenhouse effect by adding more gases that trap heat to the atmosphere. This warming is causing the earth's temperature to rise.

Greenhouse effect: the warming of the earth's atmosphere caused by a build-up of carbon dioxide or other gas emissions. Many scientists believe that this build-up allows the sun's rays to heat the earth while it prevents heat from escaping the atmosphere. It is similar to the way a greenhouse works – except that a greenhouse uses glass instead of gas.

Ground water: the supply of fresh water found beneath the earth's surface.

Helminth: a worm, especially a parasitic intestinal (stomach) worm.

Normal control solution: also called a *negative control,* is a sample of "good," non-toxic water that is tested along with other water samples. The *negative control* solution gives us an example (or reference point) of test results using clean water.

Microbes: are minute living beings that can only be seen with the help of a microscope. They are also called **microorganisms**. Some can cause disease in other organisms or in humans, animals, and plants. They are responsible for a variety of waterborne diseases such as cholera, typhoid fever, bacillary dysentery, infectious hepatitis, and many others.

Pesticide: a chemical or mixture of chemicals used for destroying insects or other organisms harmful to cultivated plants or to animals.

Pollution: anything added to the environment that causes harm to living things.

Positive control: a test solution that is prepared to produce results similar to those that would be obtained with contaminated water.

Protozoa: are also unicellular microscopic organisms, but are bigger than bacteria and have a more complex body. Some can live in a wide variety of animals and people and are excreted in their faeces. Amoebas or *cryptosporidia* are a common cause of diarrhea.

Toxicity: the potential or capacity of a substance to cause harmful effects in living organisms.

Viruses: are tiny organisms smaller than bacteria that reproduce by infecting living cells.

Water Cycle: less than 1% of all the water on the planet is fresh. This fresh water is continually recycled by nature through evaporation, condensation, and rain. Water circulates constantly among oceans, rivers, soil, atmosphere, and all living things.