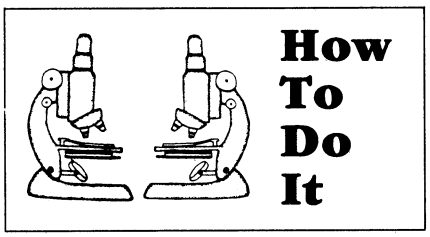

Using the Allium Test to Detect Environmental Pollutants

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Contamination of the environment by carcinogenic and other toxic chemicals is an ongoing concern of public health professionals and scientists, particularly ecologists, toxicologists and geneticists. A simple but reliable test, first introduced 50 years ago, may be used both by professional researchers and biology students to identify toxic environmental contaminants. This test, called the Allium test, was initially used by Levan (1938) to study the effects of colchicine on chromosomes from *Allium cepa*, the common onion. A recently developed modification of the Allium test by Fiskesjo (1985; 1988) of the Institute of Genetics, University of Lund, Sweden, permits monitoring of an array of environmental pollutants in water sources.

The Allium test is easily conducted with inexpensive and readily available equipment. It can be adapted for use at both the high school and college level. The test uses macro and micro observations of onion root tips to demonstrate mutagenic and other toxic effects of various contaminants. Results are obtained in less than a week and may often be extrapolated to human cell systems. In a recent report compiled for the Environmental Protection Agency, the Allium test was described as excellent for assay of chromosome aberrations following exposure to certain chemicals. It was recommended that this test be routinely used for that purpose (Grant 1982). This paper describes the procedures used to conduct several versions of this test, a versatile tool for the laboratory component of a variety of biology courses.

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Materials & General Methods

Each series of tests requires a minimum of 20 (10 experimental, 10 control) fresh, unsprouted, mold-free, young onion bulbs of approximately equal size. Small white bulbs between 1.5 and 2.0 cm in diameter, weighing 10 to 20 grams are recommended, but we have used a variety of sizes with success. Commercial onion bulbs which may have been exposed to a growth inhibitor to prevent sprouting during storage should be avoided because they may produce poor and uneven root growth. However, even these may be used late in the winter when the growth inhibitor is less effective.

One test tube or small jar is required for each bulb. The base of the onion should just fit over the top of the tube. Before placing the bulbs on the tubes or jars, remove the loose outer scales and cut off a 2 mm slice from the base to expose the root primordia. Too thick a slice will remove the primordia completely so that roots will not grow.

Potentially toxic solutions may be obtained from streams adjacent to chemical and/or pharmaceutical factories. Precautions should be taken to avoid skin contact with the material. Alternatively, water-soluble chemicals may be used from available supplies. Sodium chloride solutions yield satisfactory results (Figures 1 and 2). Several dilutions of the chemical should be tested. For lipid-soluble chemicals, use the specific lipid solvent as a control. When acidic or alkaline solutions are tested, a buffer control may be necessary. All school and college chemical stockrooms contain an array of chemicals for experimentation.

The bulbs are placed, with their bases in the test solution, in dark or in dim light at room temperature. While not absolutely essential, changing the solutions daily will help eliminate bacterial or fungal contamination, thus contributing to the accuracy of the test. Daily observations of water levels should be made for five days at which time the experiment is usually complete.

Another form of the Allium test involves starting root growth in pure water. Specific treatments are begun when the roots are between 1–2 cm in length. This was, in fact, the original version of the test (Fiskesjo 1985). An advantage of this method is that nonsprouting bulbs can be replaced. Accordingly, several additional bulbs should be added to each series.

Irrespective of the method used, liquid levels in the tubes should be checked periodically and the appropriate liquid replaced as needed.

Macroscopic Procedures

The simplest format of the Allium test is to determine mean root length in a root bundle for each bulb by measuring root lengths of experimental and control bulbs on day 5. In procedures where different concentrations of chemicals are used as the test solutions, the mean root length for each bulb could be plotted as a percent of the control on the ordinate against treatment concentrations on the abscissa. From this curve, the effect concentration (EC) may be determined. For example, EC_{10} and EC_{50} represent the effect concentration causing 10 percent or 50 percent, respectively, of growth reduction compared to controls. EC_{50} could be used as a standard, analogous to LD_{50} in animal toxicology studies.

Although these relative values are reliable, other measurements would improve accuracy. Each root could be removed from each bulb and measured and/or weighed separately. The disadvantage of this procedure would be termination of the experiment.

Other macroscopic parameters include measurement of shoot growth, assessment of root tip hardness, changes of root tip, swelling of root tip and bending of roots or root tips.

Data from an experiment using a 1 percent sodium chloride solution are presented in Table 1. Statistically significant differences in both number of roots and average length of roots are always found under these experimental conditions.

Microscopic Procedures

1. Fixation & staining

For standardization purposes, root tips are removed on day 2, irrespective of length. One root tip from each bulb is used to prepare each slide. If necessary, root tips may be stored in 70 percent ethanol.

Preparations may be fixed immediately or pretreated prior to fixation with colchicine. Colchicine arrests chromosomes in metaphase, permitting scoring of chromosome interchanges and deletions. To do this, expose specimens to a 0.1 percent aqueous solution of colchicine for one–two hours at 20°C.

Prior to staining, pour off the 70 percent ethanol and replace it with 5N HCl for 15 minutes at 20°C. Then pour off the acid and wash roots three times in distilled water for one minute each wash. Add enough water to cover the roots. Remove water by means of a Pasteur pipet. The roots are now ready for staining with Feulgen reagent.

2. Preparation of Feulgen reagent

The Feulgen reaction is an aldehyde-specific reaction based on formation of a purple-colored compound when aldehydes react with basic fuchsin-sulfuric acid. DNA gives this reaction after removal of its purine bases by acid hydrolysis. Accordingly, the Feulgen staining technique is used to stain nuclei and provides greater contrast than orcein. On the other hand, the orcein technique is rapid and effective and is especially advantageous when preparing many slides (Fiskesjo 1985). (For orcein staining, squash root tips in 2 percent orcein in 45 percent acetic acid after fixation.)

Feulgen reagent is prepared as follows:

1. Place 1 liter of water in a 3-liter Erlenmeyer flask and bring to a boil.
2. To the boiling water, slowly add 4 grams of basic fuchsin, shake well and allow suspension to cool to 50°C.
3. Filter through Buchner funnel under vacuum through Whatman #1 filter paper.
4. Add 120 ml of 1N HCl, then add 12 grams of potassium metabisulfite ($K_2S_2O_5$) and allow the solution to bleach in the dark for 24 hours.
5. Add 3 grams of decoloring charcoal (carbon) to the filtrate and shake well for one minute.
6. Filter rapidly through Whatman #1 filter paper under vacuum. Do not change filter paper.

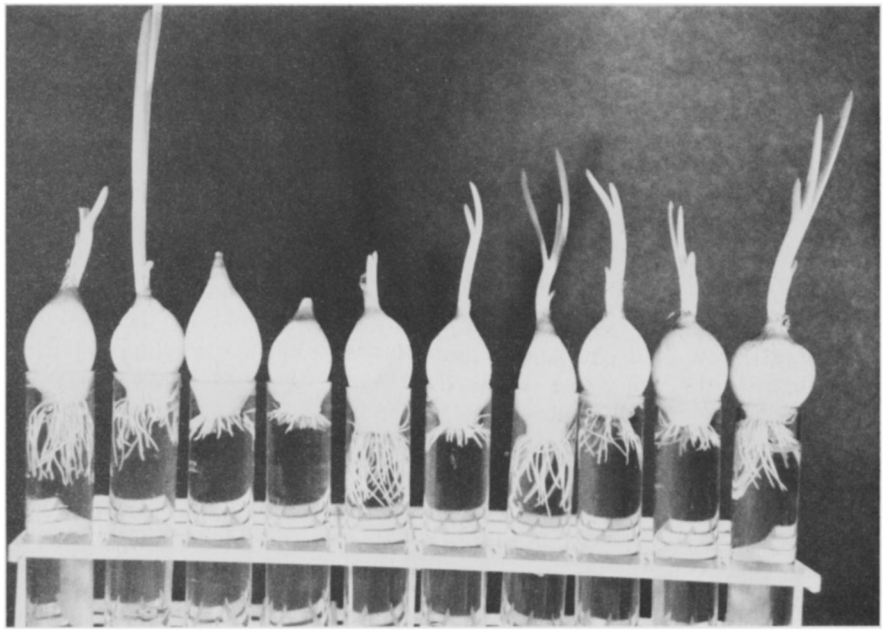

Figure 1. Onion root and shoot growth in distilled water on day 5.

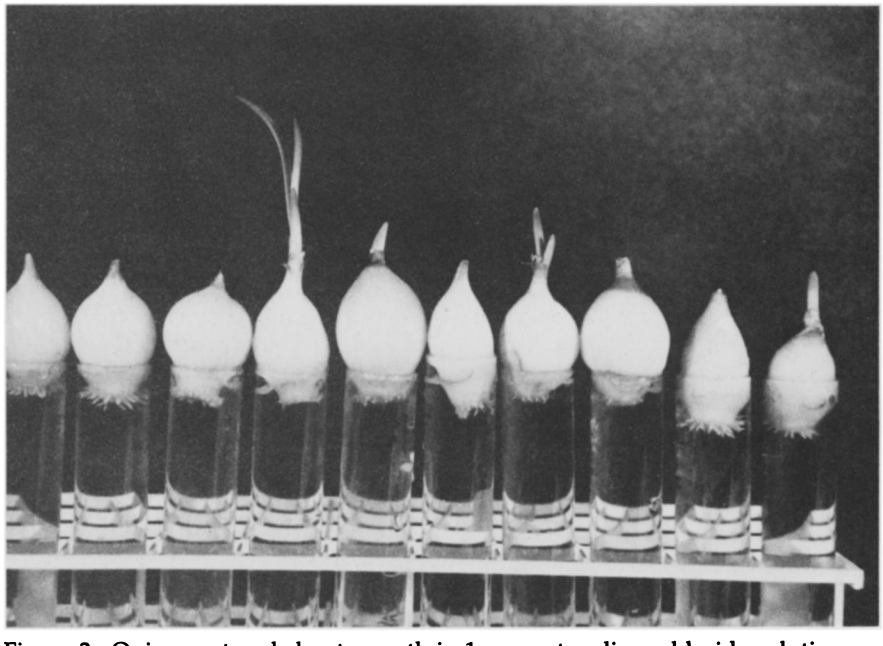

Figure 2. Onion root and shoot growth in 1 percent sodium chloride solution on day 5.

7. Store filtrate in a dark bottle in the refrigerator.

The reagent should be clear to be effective. Avoid contact with skin and clothing to prevent staining.

Add enough Feulgen reagent to cover the roots. Leave roots in the reagent for at least one hour at room temperature; they should be covered and in the dark. Reagent is removed by means of a Pasteur pipet.

3. Slide preparation

Place root on a slide using forceps and cut off 2 mm of the terminal meristem. This should be stained a pink color. Use a glass squashing rod to tap the root tip until a pink smear is produced. Then add a drop of 45 percent acetic acid and cover with a coverslip.

Place the slide between two sheets of bibulous paper and press down to blot. Examine under 430×. If preparation begins to dry out, add a drop of acetic acid to edge of coverslip and allow it to diffuse underneath. Determine the mitotic index by observing 1000 cells. The mitotic index (MI) is the number of cells undergoing mitosis per 1000 cells. MI of the experimental group is compared to MI of the controls.

Permanent preparations may be made by first attaching the cells to the slide by use of an adhesive such as Knox gelatin (5 grams per liter warm water with phenol as a preservative). Dehydrate by means of an ethanol series (15, 30, 60, 95 percent, absolute) and xylol. The coverslip is sealed with Canada balsam, applied with the wooden end of a cotton swab.

Applications

The Allium test has proven useful in screening a wide variety of environmental contaminants for toxic effects (Grant 1982; Fiskesjo 1988). Introductory biology, ecology or genetics students can exploit it for this purpose for individual or group research projects. The test can be adapted by instructors to demonstrate the effect of specific chemicals on macroscopic and microscopic parameters such as root growth and chromosome abnormalities, respectively. Furthermore, variants of the test can be incorporated in the curricula of both junior high and high school biology classes.

In this context, the Allium test could be used as a focal point for discussion of adverse effects of various chemicals. It has been asserted recently that up to nine-tenths of all human cancers may be induced by environmental agents (Barfknecht & Naismith 1988). There are currently some 60,000 synthetic chemicals in daily use; between 1000 and 2000 new chemicals are introduced into the environment annually (International Commission for Protection Against Environmental Mutagens and Carcinogens 1983). These manufactured compounds appear in pharmaceuticals, pesticides, food additives, cosmetics, industrial chemicals and many other products. Among these chemicals are potential human carcinogens and other potentially harmful substances. These can be identified by screening procedures using laboratory animals. However, these tests require up to three years and cost up to one-half million dollars for each chemical screened (Weisburger & Williams 1981). Consequently, not more than one-third of the new chemicals produced each year can be scrutinized in this way.

Twenty-five years ago, Bruce Ames and his colleagues at the University of California at Berkeley developed a short-term, relatively inexpensive test that bears his name (Ames, et al. 1973). In the Ames test, mutations induced in a mutant strain of *Salmonella typhimurium* permit its growth in a histidine-free medium. Roughly 90 percent of the carcinogens so tested are mutagenic (McCann, Choi & Yamasaki 1975).

Success of the Ames test led to the development of dozens of other short-term mutagen assays, most of which test for gene mutations or for chromosome aberrations. Chemicals testing positive for gene mutations are called mutagens and those testing positive for chromosome aberrations, clastogens. As with ionizing radiation, most chemical carcinogens cause both types of abnormality. Other approaches to carcinogen screening include attention to the molecular structure of the compounds in question (Weisburger & Williams 1981).

The Allium test can be used to detect abnormal chromosomal changes and alterations in root growth (Fiskesjo 1985). It can thus be employed to assess the deleterious effects of a variety of environmental or laboratory chemicals. The rationale for extrapolating results of the Allium test to human cells is supported by a review of pesticide studies showing a high correlation between frequency of chromosomal abnormalities in plants and animals (Grant 1978). More recently, agreement between the Allium test and mammalian cell assays was shown to be approximately 70 percent for 14 chemicals (Grant 1982). Weak damage indicated by the Allium test may be indicative of severe damage in mammalian cell test systems (Fiskesjo 1981).

To conclude, the Allium test has several advantages as a laboratory procedure for detecting toxic effects of various chemicals. The bulbs are available year round and other materials required are readily obtained and inexpensive. Also, with this system a

Table 1. Number and mean length of onion roots after 4 days in distilled water or in 1 percent sodium chloride solution.

Distilled Water		*1% Sodium Chloride*	
Number	*Length**	*Number*	*Length**
35	18.2	10	1.7
35	20.0	13	1.5
35	2.6	29	1.8
26	7.8	19	2.4
36	13.3	38	1.8
33	14.2	33	2.9
46	13.4	8	1.8
35	8.4	16	1.9
35	7.8	20	2.2
39	17.0	38	2.8
$\bar{x}$35.5	12.3	$\bar{x}$22.4	2.1
s 4.7	5.5	s11.3	0.5

t = difference between means for number of roots = 3.355
p = 0.01
t = difference between means for length of roots = 5.841
* in mm
p = 0.001

large number of root tips are produced in a short period of time. Chromosomes average 10 μm in length, allowing for easy detection of aberrations. The test works exceptionally well with improvised equipment, yet it can be refined for sophisticated studies. For example, pH and temperature can be controlled, buffers can be used, solutions can be sterilized, distilled water can be substituted for tap water, roots can be weighed on an analytical balance, chromosome studies can be conducted and results can be analyzed statistically.

The Allium test's simplicity encourages its use at the junior high and high school levels. Its accuracy commends its use by undergraduates in introductory biology, ecology and genetics laboratories.

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