From Seed to Sprout

A Bioassay of Willow Creek

Bioassays are studies designed to determine the toxicity of a substance or the effect of a chemical compound on living cells. They are often used in the pharmaceutical industry to test new products, but are sometimes used in ecological research to study the unknown chemistry of substances from the environment, such as water from aquatic ecosystems.

Organic pollution to freshwater bodies increases concentrations of phosphorus and nitrogen, essential plant nutrients. This causes aquatic plants to grow faster. However, rapid plant growth results in greater consumption of dissolved oxygen, and this can lead to the formation of *dead zones*. Dead zones occur when the total amount of dissolved oxygen drops so low that waters no longer support aquatic life.

Bioassays can be used to identify these important organic pollution events. Plants exposed to waters from freshwater ecosystems rich with phosphorus and nitrogen will tend to grow faster than those grown with water lacking these plant nutrients, unless the concentrations of these nutrients are so high that the water lacks oxygen. Studying these differences in plant growth in combination with habitat assessments can *sometimes* help researchers and conservationists paint a preliminary picture of water quality in a given freshwater environment and identify any locations where toxic pollutants may be harming aquatic life.

This bioassay was developed specifically as a proxy measurement of water quality in Willow Creek, an urban stream located in Madison, WI. These methods are targeted for a general audience and intended for use as part of an outreach program.

Bottle Biology and the Tool Kit

The *From Seed to Sprout* bioassay was created in partnership with the University of Wisconsin—Madison *Bottle Biology* team. Bottle Biology is a program designed to get students of all ages interested in exploring science and the environment using recyclable materials. The goal is to make research a sustainable activity that any student can engage in by building tools from everyday household items that would otherwise be thrown away. This study is adapted from Bottle Biology investigations, and the tool kit used is almost identical to that described in the program's instructions for the *Brassica* Bioassay Necklace experiment. Before beginning the *From Seed to Sprout* bioassay, build the required tool kit following the modified instructions listed in the box below.

The Tool Kit

Materials:
3-foot cord or string
8 microfuge tubes*
Paper towel
6 FastPlants seeds
White glue
3.5 cm flat toothpick
Laminated millimeter measuring grid or plastic ruler
3.5 cm x 3 mm plastic cocktail straw
Deionized water* or tap water
Scissors
Forceps
Permanent marker

* Try contacting a local laboratory or university to request a donation of these materials, especially from supplies that would otherwise be thrown away.

Preparation:

1. Tie a knot in the string to make a necklace. Label six of the microfuge tubes "Site 1," "Site 2", "Site 3", "Site 4", "Site 5", and "Control," respectively. String the tubes onto the necklace.
2. Cut six germination strips from a sheet of paper towel. Each strip should be triangular and approximately 1 inch in length (2.5 cm).
3. Mix the FastPlants seeds in a few drops of white glue with the toothpick so they are covered in a thin layer of adhesive. Use the forceps to carefully stick one seed onto the center of each germination strip. Allow the glue to dry.
4. Cut six rectangular strips of millimeter measuring grid or ruler, approximately 1 inch in length (2.5 cm). Add one grid strip and one germination strip to the six labeled tubes. Make sure the seeds do not touch the interior of the tubes.
5. Fold over about 5 mm of the straw and crimp the fold. This straw can be used as a dropper by squeezing the crimped end while placing the open end in liquid. Place the straw in another microfuge tube and string the tube onto the necklace.
6. Fill the remaining tube with deionized water or tap water to use in your control. String the tube onto the necklace to complete the tool kit.

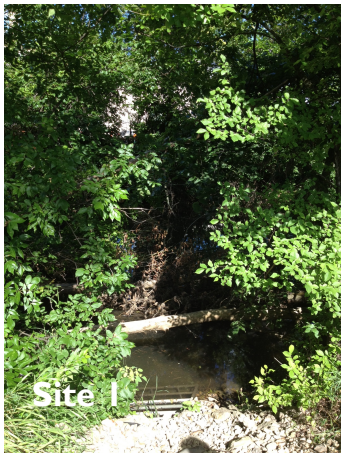
** In addition to this tool kit necklace, you will also need a Macros Kit, the collection of tools used in the *Willow Creek Critters* biosurvey. **

The Bioassay

After gathering all your equipment, follow these step-by-step instructions to complete the bioassay.

1. Identify the locations of the five sampling sites along Willow Creek using the following map and site descriptions (Figure 1).

Figure 1: Map of Willow Creek from Campus Drive to Lake Mendota. The blue line represents the creek and the pink lines show the storm sewers that drain into the urban stream. The gray boxes indicate the locations of bridges along the transect.



Site 1: Upstream Rocks
This site is located on the east bank next to the Veterinary Hospital at the end of the rocky culvert leading into the creek. This location is shaded and the bottom sediments are rocky.

Site 2: Midstream Rocks
This sampling location is found on the east bank downstream of site one at the bottom of the rocky riparian zone just past the first bridge. The site is in full sun and bottom sediments are covered in rocks. Samples should be taken from the gap in the vegetation.

Site 3: Midstream Shore
A bench on the west bank of the creek marks this site. The bench is located downstream of site two alongside the rain gardens, just past the fourth bridge. The bottom sediments at this location are sandy and rich with organic debris, and the site is in full sun.

Site 4: Downstream Waters
This site is found farther downstream on the east bank of the creek just upstream of the Willow Creek Bridge. This sampling location is easily identified as the spot where the bank transitions from grass to dirt. The bottom sediments contain sand, gravel, and organic debris, and the site is in full sun.

Site 5: Bay Outlet
This sampling spot is located at the outlet of Willow Creek into University Bay. To reach this location, walk to the lawn area just east of the Willow Creek Bridge and find the west trail that leads to Lake Mendota. After a short distance, the trail opens up to the bay. This site is highly vegetated and completely shaded. The bottom sediments are sandy and contain lots of organic matter. A few large rocks are scattered close to the shoreline.

2. Begin sampling at site 5 and move upstream. At each site, collect samples by standing or sitting on the shoreline to minimize disturbance of surface waters.

3. It is essential to assess habitat in conjunction with ecological bioassays to give context to findings. **Before collecting a sample, make some observations and snap a few photos of the site (if possible).** Take note of the **date and time, weather,** including temperature, cloud cover, photo period, and precipitation, and **water conditions,** such as how fast or slow the current is moving, the clarity of the water, and descriptions of any organisms at or near the surface waters. Record all observations in your notebook. **These notes will help you understand your data later.**

4. To collect a sample, take the collection cup and hold it in your hand. From a sitting or standing position, submerge the container to the creek bottom and pull the cup toward you. **Be sure to scrape the bottom rocks and sediments.** Transfer collected sample into the site 5 container. Continue to collect samples using the collection cup until the water level in the site 5 container reaches the bottom lip.

5. Repeat steps 2 through 4 at each location, moving upstream, to obtain a sample from each site. **The goal is to obtain the same volume of water from each site.**

6. Using the straw pipette, collect one drop of water from the Site 5 container. Open the Site 5 microfuge tube and add the drop directly to the germination strip to wet the FastPlant seed. Repeat twice so a total of three drops of water are added to the Site 5 tube.

7. Repeat step 6 with water collected at sites 4, 3, 2, and 1, in that order.

8. Add three drops of deionized water or tap water to the control tube.

9. Hang the necklace in a window that does not directly face the sun but still receives ample UV light. This will allow the FastPlants to photosynthesize while limiting daily temperature flux.

10. Observe the FastPlant seeds twice daily for three days. Record visual observations of growth, including number of leaves and coloration, as well as daily air temperature and environmental conditions. (If you do not have a thermometer handy, use your past experience to estimate the temperature of the room.) After the seeds germinate, estimate the length of the seedlings.

11. Graph plant growth of the FastPlant sprouts by site to assess changes in water quality along Willow Creek.

Further Study

The *From Seed to Sprout* bioassay introduces students of all ages to the ecological investigations made possible with bioassays and offers students the opportunity to explore Willow Creek close-up. To add to this experience, there are many possibilities for further study.

1. Varying the seed

Try using different seeds, such as lettuce or basil, to see how the waters of Willow Creek affect the growth of various plants.

2. *Increasing organic pollution*

To see how increased organic pollution affects plant growth, add phosphates or nitrates to the surface waters collected before beginning the bioassay.

3. *Adding chlorine*

Each winter, the City of Madison lays down road salt to melt ice on busy streets. This road salt is mixed with runoff during snowmelts and spring rains and finds its way into the waters of Willow Creek. To see how road salt affects the ecology of this urban stream, add chlorine to the surface waters collected prior to starting the bioassay. Compare the plant growth seen in this altered bioassay to that under controlled conditions to determine how chlorine affects the aquatic life in Willow Creek.

4. Comparing surface waters

Try running this bioassay with water collected from different sources, such as Lake Mendota or a bubbler at your school. This will allow you to roughly compare water quality of these sources to that of Willow Creek.

Sources

For a complete list of resources used in the development of this bioassay for the Willow Creek Community Project, see the Works Cited page.