Observing the Effects of Pollution in Microcosms Lab II-3

EQUIPMENT AND MATERIALS

You'll need the following items to complete this lab session. (The standard kit for this book, available from *www.thehomescientist.com*, includes the items listed in the first group.)

MATERIALS FROM KIT

- Goggles
- Coverslips
- Centrifuge tubes, 50 mL
- Hydrochloric acid
- Fertilizer concentrate A
- Lead(II) acetate
- Methylcellulose
- pH test paper
- Pipettes
- Slides, flat
- Yeast, live (baker's or brewer's)

MATERIALS YOU PROVIDE

- Gloves
- Foam cup
- Pond sediment/vegetation (from Lab II-1)
- Tablespoon
- Water, spring or boiled tap

BACKGROUND

Any externally applied change to the environment of a community forces changes to occur within the community until a new equilibrium is reached. For example, after a major flood or a forest fire, dramatic changes occur to the community and the populations within that community. Eventually, a new equilibrium is reached, in which the community and populations may or may not be similar to the original equilibrium. Species formerly present may be absent in the new equilibrium, for example, and species not originally present in the original community may be present in large numbers in the new community.

One of the most common causes of such equilibrium changes is pollution, whether of natural or human origin. Floods, hurricanes, forest fires, volcanic activity, and other natural phenomena often cause dramatic changes to the environment, as does pollution of human origin, such as acid rain, drainage from mine tailings, and so on. Nor are all equilibrium changes that occur from natural causes abiotic in origin. For example, algae blooms may cause gigantic die-offs of fish and other aquatic creatures, in some cases severe enough to result in long-term changes to the environment.

In this lab session we'll make up six small microcosms in 50 mL centrifuge tubes. (We call them *nanocosms*, although that's not an official term.) We'll make these microcosms as similar as possible initially, retain one as a control, and then subject the others to various environmental pollutants, as follows:

- Tube A is the control microcosm.
- Tube B treated with fertilizer to simulate phosphate pollution.
- Tube C treated with lead(II) acetate to simulate heavy-metal pollution.
- Tube D treated with hydrochloric acid to simulate acid rain pollution.
- Tube E treated by adding live yeast (foreign microorganisms can shift equilibrium in a microcosm).
- Tube F exposed to direct sunlight (yes, excessive light can be considered a pollutant).

We'll observe succession over a period of several days in each of these microcosms and attempt to draw conclusions about the effects of various pollutants upon the various organisms present.

PROCEDURE II-3-1: BUILD POLLUTED MICROCOSMS

1. Put on your gloves and goggles.
2. Label six 50 mL centrifuge tubes A through F.
3. Obtain about 500 mL of spring water. You can substitute tap water if you boil it for a few minutes and then allow it to cool. (Water treatment chemicals kill the same microorganisms we're trying to grow in our microcosms.)
4. Transfer eight rounded tablespoons of the dried sediment and vegetation you obtained in lab session II-1 to the foam cup. Add sufficient spring water to make a runny mud in the cup.
5. Transfer sufficient mud to each of the centrifuge tubes to bring the sediment level in the bottom of the tube to the 15 mL line. Try to make sure that the sediment in each tube is of similar makeup and that the amount of vegetation is about the same.
6. To tubes A, E, and F, add sufficient spring water to bring the water level to the 40 mL line. Cap the tubes, invert them several times, and then place them aside.
7. To tube B, add sufficient spring water to bring the water level to the 35 mL line. Add 1 mL of the fertilizer concentrate A, which contains phosphates, and then

sufficient spring water to bring the water level to the 40 mL line. Cap the tube, invert it several times, and then place it aside.

8. To tube C, add sufficient spring water to bring the water level to the 35 mL line. Add 1 mL of 0.1 M lead(II) acetate solution, and then sufficient spring water to bring the water level to the 40 mL line. Cap the tube, invert it several times, and then place it aside.

9. To tube D, add sufficient spring water to bring the water level to the 40 mL line. Cap the tube, invert it several times, and allow the sediment to settle. Add one drop of 6 M hydrochloric acid, mix the solution and use the pH test paper to test the pH. Continue adding 6 M hydrochloric acid dropwise, mixing the solution, and retesting the pH until the pH of the solution reaches about 5.0. (The amount of acid you'll need to add varies according to the makeup of your sediment specimen.)

10. To tube E, add a breadcrumb size piece of live yeast. Cap the tube, invert it several times, and then place it aside.

11. Incubate tubes A through E at room temperature in a location where they'll be exposed to daylight but not direct sunlight. (For better consistency you can use a plant-grow light if you have one available.) Incubate tube F in direct sunlight outdoors or on a windowsill. If outdoor temperatures are extremely hot or cold, do the incubation indoors. A tube exposed to direct sunlight will obviously be warmed by it, but our goal is to avoid temperature extremes as much as possible.

The pH of tube D may change over time as the acid reacts with components in the sediment. Our goal is to reach a reasonably stable acid pH in tube D. On the day you build this microcosm, retest the pH every hour or two and then again the morning of the second day. If necessary, adjust the pH by adding more acid dropwise until the pH stabilizes.

PROCEDURE II-3-2: OBSERVE SUCCESSION IN POLLUTED MICROCOSMS

WARNING

Once again, remember that you don't know exactly what's growing in those microcosms. Use full aseptic precautions each time you open a microcosm. Wear gloves, goggles, and (if you really want to be fully protected) an N100 particle mask. Wash the gloves in soap and water before removing and discarding them, and then wash your hands thoroughly in soap and water. Always replace the lid of the microcosm immediately when you're not actually obtaining samples from it.

Ideally, you should begin this procedure the day after you build the 50 mL microcosms.

1. Put on your protective gear.

2. Open tube A, measure the temperature of the water, and record it in your lab notebook. (Do not stir the microcosm; simply dip the thermometer into the water and withdraw it once it registers the temperature.)

3. Use the tip of the thermometer to transfer one drop of the microcosm water to a piece of pH test paper. Record the pH value in your lab notebook.

4. Use a clean pipette to withdraw a drop of water from the surface of the microcosm. Transfer it to a flat slide, add a drop of methylcellulose to slow down the fast movers, and put a coverslip in place.

5. Observe the slide at low magnification (40X), and note as many different organisms as possible. Scan the full area under the coverslip to make sure you don't miss any species. If necessary, use medium magnification (100X) to verify the identity of small organisms. Using your notes and sketches from the preceding lab session, identify each species present and record it in your lab notebook.

6. Scan the entire populated area of the slide and record your impressions of the relative numbers of each species present as "very abundant," "abundant," "moderate," "rare," or "very rare."

If you have time, you can also use high-dry magnification (400X) to identify and do population estimates on bacteria and other tiny organisms.

7. Using clean pipettes, repeat the preceding steps using specimens from near the vegetation and from near the bottom sediment.

8. Repeat steps 2 through 7 for each of the other microcosms.

9. Repeat steps 1 through 8 each day at about the same time for several days or until the microcosms reach equilibrium.

Do not be surprised if one or more of your microcosms dies off within a day or two. We introduced significant levels of pollution into tubes B through E, and it's very likely that those pollutants will suffice to eradicate some or even most of the species present either directly or indirectly (for example, by killing off the food source for a species that was otherwise unaffected or only minimally affected by the pollutant in question). Conversely, one or more of the pollutants may provide a selective advantage for one or more of the species present by killing or inhibiting competitors, allowing some species to flourish at the expense of other species.

REVIEW QUESTIONS

Q1: In detail, what effects did you observe from the differing pollutants in the various microcosms?