

Setting up your Winogradsky Columns (~ 1 hour)

Materials (per team)

- Lots of Newspaper (to cover tables)
- Your site sediment and water samples
- Gloves for each team-member
- Sharpies for labeling
- Lab tape for labeling
- Bucket for making sediment suspension
- Mixing spoons (2x)
- 5 mL syringe and syringe filter for water filtration
- 15 mL falcon tube for filtered water sample
- 500g diatomaceous earth (DE)
- Large mixing container 2L for DE slurry
- Lab goggles (1x) for working with DE
- Face mask (1x) for working with DE
- Culture flasks (5x)
- Small beakers 400mL (5x)
- Wood sticks for stirring final DE slurries (5x)
- Weighing cups (**color-coded for each chemical**)
- 3 mL syringes and blunt needles (for N & P)

General Equipment

- Scales
- Gloves (S/M/L)
- Aluminum foil
- pH paper
- Spatulas (1 per chemical, color-coded)
- Paper towels (in case of spills)

Ingredients

- Sodium bicarbonate (NaHCO_3)
- Yeast Extract
- Cellulose powder
- Shredded paper
- Magnesium Sulfate (MgSO_4)
- Elemental Sulfur (S)
- Iron hydroxide (FeOOH)
- 1M NH_4 solution
- 100mM PO_4 solution
- 2L degassed MQ water (as needed)

Instructions

1. Lay out a few layers of newspaper on table to protect the surface from spills. Wear gloves for all steps!
2. Prepare the sediment suspension: in the bucket, mix your sediment (~500mL) with the site water (~2L), stir gently to suspend the sediment in the water, let stand for 5-10min so large sediment particles settle out.
3. In the meantime:
 - A. prepare the 2L mixing container by adding **five times** the amount of the ingredients that are **mixed (M)** into **ALL** of your 5 conditions (do NOT add any ingredient that is not mixed into all 5 conditions). Use your checklist in the experimental plan to make sure you keep track of everything that was added.
 - B. prepare the culture flasks for your 5 conditions by adding the appropriate ingredients that go on **the bottom (B)** of each. Use your checklist in the experimental plan to make sure you add everything. Most importantly, make sure to label each flask clearly on the narrow side with lab tape and sharpie.
4. Once your suspension has settled, withdraw some water with the 5mL syringe, attach the syringe filter and push it through the filter into the 15mL falcon tube (blue cap). Make sure to label the tube well with your team name and the sample site and the words "filtered suspension water" for later analysis.
5. Use a pH strip to measure the pH of your suspension water and record it on your worksheet.
6. Prepare your 5 conditions, the following steps can be done in parallel by different team members:
 - A. Make a silica slurry with diatomaceous earth (wear goggles and face mask to avoid inhalation! the fine powder is a strong irritant while dry): add **1.3 L** of your sediment suspension (with as little sediment as possible) to the 2L mixing container and then slowly add **500g diatomaceous earth (= whole container) while stirring**. This should produce a slurry with the consistency of a thin milk shake.
 - B. Label your 5 beakers 1-5 for your 5 conditions with tape and a sharpie. Weight out and add the remaining missing ingredients that go into the **mix (M)** for each into the corresponding beaker. Again, use your checklist in the Plan to make sure you add everything.
7. Divide the slurry between your 5 beakers, a little over 250mL each (keep stirring the big slurry in between pouring so it stays well mixed!). Stir each beaker well with one of the wood sticks.
8. Pour the right slurry into each culture flask. Pause frequently to stir the slurry in the beaker so it remains suspended. Stop when the slurry reaches the top of the culture flask just below the neck of the lid.
9. Securely cap the flasks and **tap the sides release trapped air bubbles from the diatomaceous earth**. The slurry will compact by gravity overnight.
10. Those intended for incubation in the dark should be wrapped in aluminum foil. Those for incubation in the light should be placed in the light incubator.
11. **Cleanup**: please add all residual site water back into one of your original site water sampling containers, and all remaining sediment sample and diatomaceous earth mixtures into the other site water sampling container. Put all materials back into your team tub (plastics and paper waster can go into the trash).