Lettuce Seed Bioassay

Lab Procedure

(experimenting with different solutions that may impact growth and development)

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What is the purpose of this bioassay?

To evaluate the effect of different solutions on the germination of lettuce seeds, and raise awareness about the effects of contaminants on living things.

Solution Information:

- You can choose a variety of solutions to use in this experiment. However we chose:

    i. Copper Sulfate 1000 mg/L

    ii. Iron Sulfate 1000 mg/L

- Iron and copper can be found in the natural environment, including in groundwater. This makes them good candidates for this experiment as they produce results relevant to our well water sampling project. Iron and copper are two metal contaminants of well water that students will find in their own well water datasets.

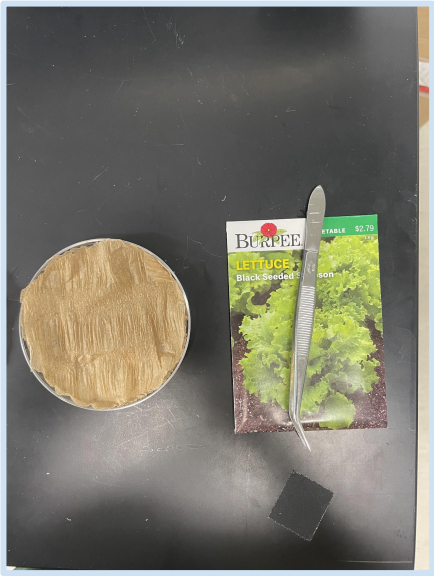
Materials: Here is what everything looks like!

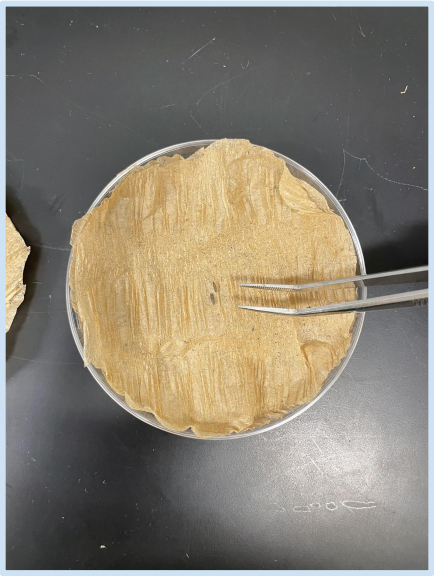
- 9 Petri Dishes with color-coded labels
- Bleach-Free paper towels or coffee filters, cut into petri dish-sized disks
- 90 Lettuce Seeds
- Reverse-Osmosis or distilled H_2O (about 40 mL)
- $CuSO_4$ 1 M solution– (about 40 mL)
- $FeSO_4$ 1 M solution (about 40 mL)
- pipettors, one for each solution
- Beaker for H_2O, rack for tubes of other solutions
- Wet paper towels to put in large plastic tupperware with lid to serve as moist chamber so dishes do not dry out

Step 1: Prepare Bleach-Free Paper Towels or Coffee Filters

Using your petri dish as a guide, trace four circles on coffee filters or brown paper towel, and cut to size

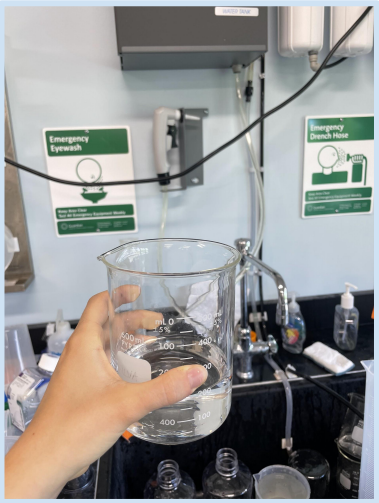
Step 2: Sort Seeds into Dishes

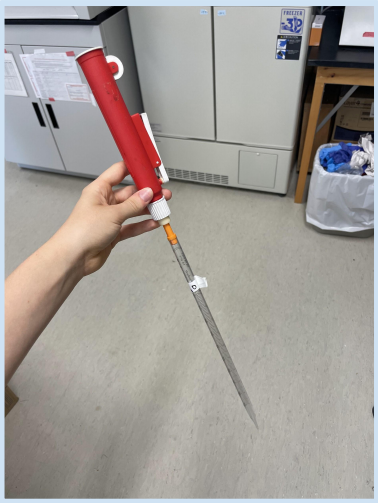


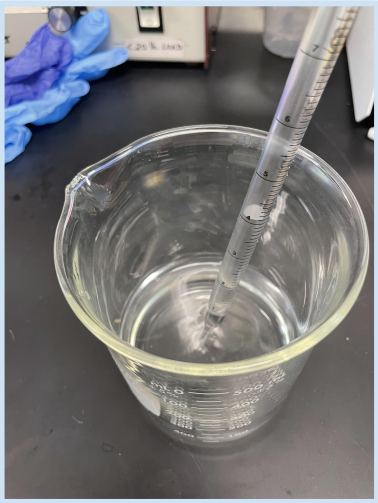


Stack one or two paper disks (depending in thickness and absorbance) on the inside of the Petri dish. Set one or two more paper disks to the side. Gently distribute ten lettuce seeds evenly across the center portion of the paper disk.

Step 3: Gather materials and Prepare Solution







Fill a beaker with >50 mL of Reverse Osmosis (RO), deionized, or other lab-grade water. This is your control. Prepare Copper or Iron Sulfate solutions by measuring out 1000 milligrams of Copper or Iron Sulfate crystals and mixing with 1000 milliliters of control water. Practice pipetting with control water if needed!

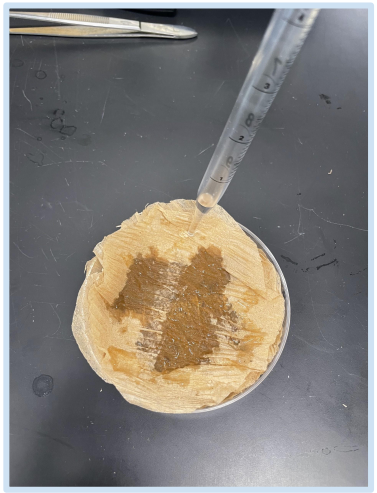
Step 4: Add Solution to Seeds

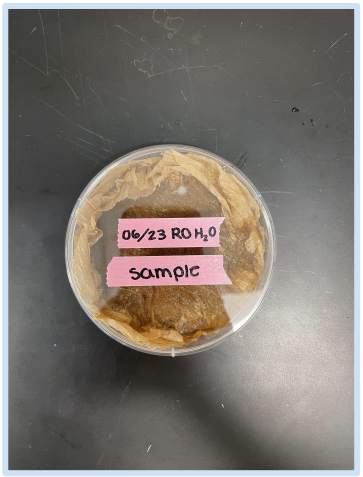
Evenly and gently pipette **3 mL** of liquid over the seeds. If you want you can add the solution to the paper disks first and then add the seeds. Either way works!

Step 5: Put an additional filter disk on top each set of seeds

Gently press the disk of paper on top of the seeds, allowing the solution to saturate the paper layers.

Step 6: Add More Solution to Ensure Filters are soaked



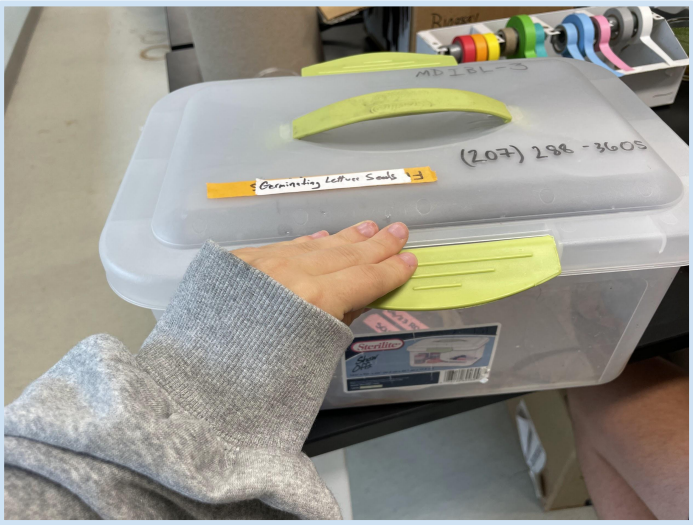


Pipette an additional **2** mL of liquid over the new paper disks (if needed, you don't want the seeds to dry out, but you don't want them to drown either!) and gently press down on the papers, enabling the solution to spread throughout the dish. At this point, the paper should be saturated to some degree. If the paper is not fully saturated, add more solution at your discretion, avoiding pooling. Replace labeled lid.

Step 7: Repeat!

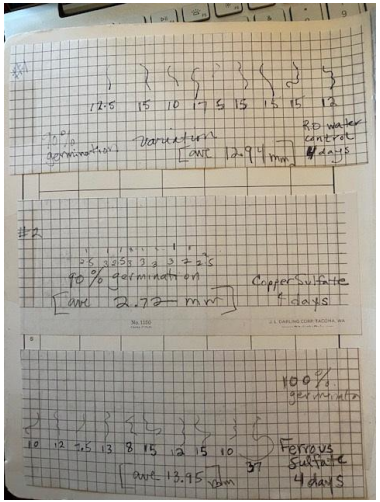
Repeat! Prepare three Petri dishes for each solution, assuring that lids are not mixed up. For extra quality control, use a sharpie marker to put the same label on the side or bottom of the bottom dish!

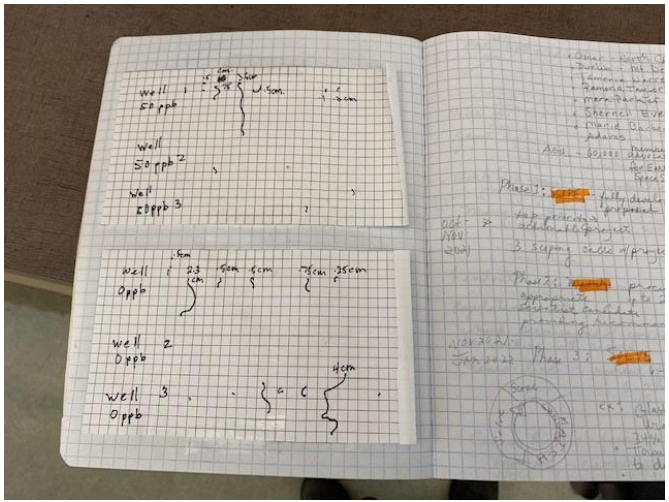
Step 8: Construct Moist Chamber



Place two layers of paper towels in the bottom of the plastic tupperware, then using tap water, saturate the towels. Next, stack the Petri dishes in the tupperware and add the lid, sealing it shut. A Ziploc bag works too!

Step 9: Wait 4 or More Days (then measure roots using a piece of graph paper)





Each box on this graph paper is .5 cm, so after tracing each root it is easy to estimate the measurements by adding up the number of .5 cm boxes. Note that by multiplying by 10 you can convert cm to mm. You will see from the notes on the photo at the right that this experiment involved using well water instead of a copper or iron solution!

Step 10 Analyze Your Data

This dataset was analyzed in Tuva…there is data from four different groups of students; the black group the blue group, the green group, and the red group! You can find this dataset at https://arsenicdata.tuvalabs.com/

Discussion Questions

- Why do you think constructing a moist chamber is important?
- Why do you thinking adding the same amount of a given solution each petri dish is necessary?
- How do you think the different solutions will influence the growth of the lettuce seeds?
- What did you like about this lab? What did you dislike?
- What was easy about conducting this lab? What was difficult?
- Where might errors occur? How would this skew results?
- What can you conclude from the graph on the previous slide?
- How did your graph compare with the graph in this presentation?

Sources

The idea to use copper sulfate came from this source, which also suggests making filters to take the copper sulfate out of solution and test the effectiveness of filters:

https://www.wwoa.org/education/public-library

There are lots of bioassay ideas at the Cornell site:

http://ei.cornell.edu/toxicology/bioassays/lettuce/