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Effect of substrate composition on heavy metal uptake in the alga *Chara australis*

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Introduction

Chara australis, a macroalga, has been shown to have the potential to take up ("phytoremediate") Cadmium, a toxic heavy metal contaminant, from the environment at concentrations greater than one part per million[1,2], a figure well above the naturally occurring level. In the experiments characterizing Cd uptake, *Chara* had been grown in either store-bought "Garden Magic" soil or river sediment collected from the Buffalo River.[1,2,3,4] Substrates are a mixture of particles of varying sizes, as well as organic components, and these components have varying ability to hold heavy metal cations.[6] To follow up on the findings of these studies, it would be prudent to determine any difference in the composition of the substrates, and see if the uptake of metals correlates to the composition.

Objectives

1. Determine the composition of each sediment in terms of sand, silt, and clay particles by percent.
2. Compare the Cd uptake of each substrate and see if it correlates with the composition of the substrate.
3. Determine the depth the rhizoids of *Chara* can reach to see how deep it may be able to reach for contaminants in a practical setting.

Methods

Experiment 1: Cd Uptake in Varying Substrates

- 6 tanks of 6 explants each grown in 750g of substrate; 2 with "Garden Magic" soil, 2 with Buffalo River sediment, 2 with Ellicott Creek sediment.
- 1 of each replicate had Cd added to a concentration of 10 parts per million (ppm).
- Samples were harvested from each tank at the middle and end of semester.
- Samples were acid digested based on EPA Methods 3050B.[5]
- Inductively coupled plasma mass spectroscopy (ICPMS) was used to determine Cd concentrations the samples.

Experiment 2: Substrate particle composition

- Experimental procedure obtained from Colorado State University.[7] Each substrate was pulverized, then mixed with water and detergent to facilitate particle separation. The substrates were mixed for 15 minutes and left to settle. This was repeated with a total of 5 replicates.
- The amount of settled substrate was measured at 2 minutes, 2 hours, and 3 days to determine sand, silt, and clay content respectively.

Experiment 3: *Chara australis* rhizoid penetration

- 1 *Chara* explant was placed in a sealed PVC pipe filled with 20cm of river sediment (Figure 1).
- At the end of the semester, pipe was drained and the seal was removed from the bottom.
- Sediment was removed and separated into equal sized slices.
- Each slice was searched for rhizoids.

Results

Experiment 1: Cd Uptake in Varying Substrates

- Average concentration of Cd in controls was less than 3 ppm except in the *Chara* grown in topsoil (figure 2).
- Average concentration of Cd in *Chara* grown in B. River and E. Creek sediments was roughly the same (just below 6 ppm).
- *Chara* grown in Cd-doped soil had 83.8 ppm of Cd (not shown) - more than 10 times the Cd present in E. Creek and B. River doped tanks.
- *Chara* grown in Cd-doped soil did not grow after first harvest.

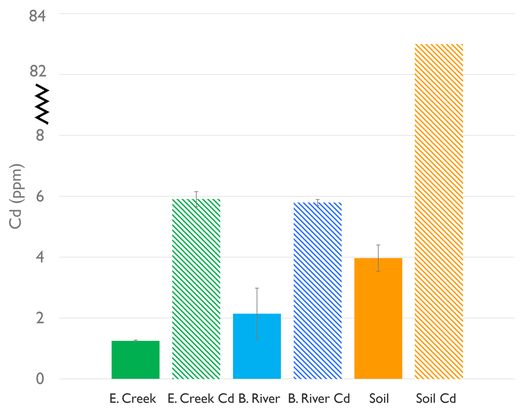

Figure 2. Average of Cd concentration from both harvests.

Experiment 2: Substrate Particle Composition

- All three substrates are mostly (>90%) sand (figure 3, table 1).
- B. River and Soil contain similar amounts of clay, while E. Creek has less.

Table 1. Composition of each substrate with standard deviations. Percentages are an average of five replicates.

%	E. Creek	B. River	Topsoil
Sand	95.6 ± 2.8	91.5 ± 4.6	91.9 ± 4.0
Silt	1.6 ± 2.5	3.4 ± 0.9	1.7 ± 1.4
Clay	2.7 ± 2.5	5.0 ± 3.8	6.1 ± 4.3

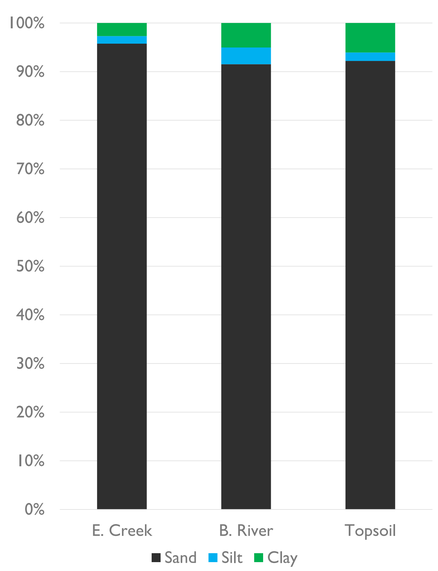

Figure 3. Visual Representation of substrate composition.

Experiment 3: *Chara australis* rhizoid penetration

- Rhizoids found as low as slice 4 (Table 2).
- Slice 4 contained the sediment from 11.43 cm to 8.56 cm deep.
- Rhizoids of *Chara* may reach up to 11.43 cm.

Figure 1. Tube used to determine rhizoid length. Tube was placed directly under lamp to ensure normal exposure. The bottom is removable.

Table 2. Starting depth for each slice. Rhizoid was first found in slice 4

Slice #	Depth (cm)
1	20.0
2	17.1
3	14.3
4	11.4
5	8.6
6	5.7
7	2.9

Discussion

Experiment 1: Cd Uptake in Varying Substrates

- Previous experiments reported that Cd concentration in *Chara* from Cd-doped tanks ranged from 5-18 ppm and the concentration of Cd in *Chara* grown in control tanks (no Cd) was below 1 ppm.[4]
- *Chara* Cd concentration in the soil control tank and the very high *Chara* Cd concentration in the Cd-doped soil tank could be the result of contamination, or error in setting the tank up, digestion, or the ICPMS procedure.
- Failure of *Chara* to grow after first harvest from Cd-doped soil tank is consistent with the high Cd concentration reported due to toxicity.
- *Chara* grown in Ellicott Creek and Buffalo River sediment took up similar amounts of Cd.

Experiment 2: Substrate Particle Composition

- All substrates are highly sandy.
- Method should be tested with substrates with known composition to verify results.

Experiment 3: *Chara* Rhizoid Penetration

- Rhizoids can reach 11 cm in this experimental setting.

Future Work:

- Examine cause of very high Cd in soil grown *Chara*
- Quantify organic component of substrates to better describe their cation-carrying ability
- Determine penetration of Cd into contaminated areas and compare the *Chara's* rhizoid length

References

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