

# Utilizing EGCG to Mitigate Heavy Metal Stress Induced *Chlorella vulgaris*

## Research Plan/Project Summary Instructions

The Research Plan/Project Summary should include the following:

a. **RATIONALE:**

Heavy metal stress, especially aluminum toxicity, is known for its detrimental effects on the human body and ecosystems around the world. For instance, the toxicity resulting from aluminum exposure can lead to neurodegenerative diseases in humans or mutations in plants. Mutations from aluminum exposure are a result of oxidative stress exposure to the cell which causes damage to DNA and RNA, therefore distracting the cell from performing its physical functions. In plants, these physical functions include photosynthesis, root growth/formation, and leaf coloration. These oxidative stresses, however, can be mitigated with the utilization of EGCG, an antioxidant. EGCG is a naturally recurring antioxidant found in green tea and has been found to mitigate oxidative stress and repair cellular anomalies such as misfolded proteins. However, the resulting efficiency in photosynthesis is unknown after exposing EGCG to heavy metal stress induced algae cells. The algae cells to be utilized is *Chlorella vulgaris* due to its classification as phytoplankton which is responsible for around half of the worlds oxygen production through photosynthesis.

b. **RESEARCH QUESTION(S), HYPOTHESIS(ES), ENGINEERING GOAL(S), EXPECTED OUTCOMES:**

If *Chlorella vulgaris* is exposed to heavy metal stress and population growth decreases, then a treatment of EGCG will restore population growth.

c. Describe the following in detail:

● **Procedures:**

1. Pour 100mL of Alga-Gro Solution into two 125 mL flasks.
2. Flame an inoculating loop and test tube containing the *Chlorella vulgaris*
3. Scrape algae from the Protease agar in the test tube with the inoculating loop and swirl the loop inside the Alga-Gro flasks. Repeat 3 times for each flask.
4. Place a parchment lid with holes to allow for CO<sub>2</sub> transfer for photosynthesis.
5. Expose *C. vulgaris* to light with a lamp for an 18hr light/6 hour dark cycle.
6. Allow for flasks to incubate for 3 days.
7. Add 1.0 mL of 0.1% Sulfuric Acid solution to 1 liter of distilled water along with 0.705 g of Bold's Basal Medium.
8. Autoclave mixture for 15 minutes at 15 pounds of pressure.
9. Prepare 24 sterilized 100mL test tubes.
10. Transfer 25 mL of the new solution into each test tube.
11. Transfer 5 mL of *C. vulgaris* into each test using a pipette.
12. Divide the 24 test tubes into 4 groups of 6 test tubes and label group number.
13. Incubate the test tubes under the lamp for 3 days on shaking incubator.
14. Insert pH probe into each test tube and make sure the pH probe is cleaned between each test tube testing.
15. Quantify a baseline pH for each group by averaging the pH's using the LabQuest device.
16. Place each test tube inside the UV spectrometer to determine a baseline cell density for each group and record the values.
17. Place 8.0 mg of Aluminum into each test tube of groups 3 and 4.

18. Place 4.0 mg of EGCG into each test tube of group 2 and 4.
19. Incubate the test tubes under the lamp for 3 days on shaking incubator.
20. Insert the pH probe into the test tubes after the incubation period is over and quantify the pH.
21. Place the test tubes under a UV Spectrometer, determine the light intensity based on the calculated measurement by the machine, and record the data.
22. Transfer all of the data from the LabQuest device onto Microsoft Excel and perform an ANOVA and Tukey's Post Hoc.
23. Transfer recorded data about population density from the UV Spectrometer and calculate an ANOVA and Tukey's Post Hoc on Microsoft Excel.

- **Risk and Safety:**

Aluminum Nitrate:

- Skin contact
  - Rinse immediately
  - Discard clothing
- Eye contact
  - Rinse with plenty of water
  - Restrict contact lens use
- Ingestion
  - Drink glasses of water
  - If necessary, contact physician
  - Administer activated charcoal (20-40g in 10% slurry)
- Cover drains
- Keep away from open flames

EGCG:

- Skin contact
  - Rinse with soap and water
  - Consult physician
- Eye contact
  - Flush eyes with water
- Ingestion
  - Do not give to an unconscious person
  - Rinse mouth with water
  - Consult physician

Chlorella vulgaris:

- No safety information needed according to seller
- Instructions for disposal:
  - Treat with 10% bleach for 24 hours and rinse solution down a drain until bleach odor is not detected.

- **Data Analysis:**

The average transmittances and pHs can be calculated to find the standard deviation of the data accordingly. By analyzing the cell survival rates and rate of photosynthesis, an ANOVA can be performed. An ANOVA would allow for comparisons within groups and the Tukey's Post Hoc would allow for comparisons between the groups. By acquiring such numerical values, the hypothesis can be supported or not.

#### d. BIBLIOGRAPHY:

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1. Human Participant Research: N/A
2. Vertebrate animal research: N/A
3. Potentially hazardous biological agents research: N/A
4. Hazardous chemicals, activities & devices:

Aluminum nitrate:

- Risks:
  - Skin contact
    - Rinse immediately
    - Discard clothing
  - Eye contact
    - Rinse with plenty of water
    - Restrict contact lens use
  - Ingestion
    - Drink glasses of water
    - If necessary, contact physician
    - Administer activated charcoal (20-40g in 10% slurry)
  - Cover drains
  - Keep away from flames
- Safety precautions:
  - Wear gloves
  - Use eye protection
  - Protective clothing
- Methods of disposal:
  - Dispose through a biohazard bin

EGCG:

- Risks:
  - Skin contact
    - Rinse with soap and water
    - Consult physician
  - Eye contact
    - Flush eyes with water
  - Ingestion
    - Do not give to an unconscious person
    - Rinse mouth with water
    - Consult physician
- Safety precautions:
  - Wear gloves
  - Use eye protection
- Methods of disposal:
  - Dispose through a biohazard bin