PTM Final Paper - How well will Water Lilies (*Nymphaea tuberosa)* grow in lunar regolith?

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Background:

Lunar regolith is a mixture that consists of rock chips, mineral fragments, glasses, and agglutinates, and thus "[contains] no organic matter" (Noble 2009). Because of the lunar regolith's lack of nutrients, the team had to decide which plants were best suited for this project and whether or not these plants could rely on lunar regolith. This project was completed because; so far as seen in the history of *Plant The Moon*, no one involved in the program has yet to do any kind of aquatic plant. The idea was created due to the various advantages and positive benefits of growing underwater plants, as well as the possibilities of the idea. First off, astronauts would be able to have aquatic life on the moon or other extraterrestrial places in the future. Secondly, plants had continued to show growth when placed within 100% lunar regolith, which was completely unexpected, as the contents of lunar regolith are not sufficient for the survival of most plants. One major disadvantage to lunar regolith is that the pH is somewhat high. As an article on the discussion of pH of Bio-Stratum and lunar regolith claims, "Studies report that the Martian and Lunar simulants have a pH above 6, and have alkaline properties in some cases…" (Gilrain et al., 1999; Zaets et al., 2011; Wamelink et al., 2014; Caporale et al., 2020; Eichler et al., 2021). Despite this, however, the water lily continues to grow. The theory for why this growth had appeared, despite the pH, was that the water the American Water lilies were submerged in were what dictated the required pH for the lily to thrive. This means that all the water lilies should have grown, and any that didn't were possibly outliers. Next, aquatic plants have different structures than plants that thrive on land, enabling them to grow in unique ways and in unique environments. Thirdly, aquatic plants will be able to provide nutrients for astronauts that would have not been possible if an earth-bound plant was grown instead. The plant *Glossostigma elatinoides* was originally chosen first; they either could not have grown from a seed or would have taken a long amount of time to grow. The supervisor of this experiment informed the team about American Waterlilies (*Nymphaea tuberosa*) then, and the team decided to plant *Nymphaea tuberosa* in place of the plant originally chosen. The hope of this experiment was to see how well the plants would grow in lunar regolith.

Experiment Design:

To start off the experiment, 6 pots were set to be prepared in the designated classroom aquarium in order for the research to commence. All of these pots were filled with pebbles up to the amount of roughly 0.5 inches above the bottom of the pot; this was an attempt to weigh things down in order for the procedures to follow thereon out to be in control. Then, the pots were filled with the experimental levels of Bio-Stratum to lunar regolith each required. One pot was filled with 100% Bio-Stratum, and four other pots were filled with 50% Bio-Stratum and 50% lunar regolith, while the final pot was different compared to the other pots, as it was instead filled with 100% lunar regolith. To make the 50% Bio-Stratum and

50% lunar regolith mixture for the four pots, the two substances were placed into a bag in a 1:1 ratio, where it was then shaken around until the two selected substances had combined into a single uniform mixture. Once this was completed, the specific mixture was split into the four pots. After all six pots were filled with the respective soil/regolith ratio required for each, the American Waterlilies were placed into the mixture. Afterwards, a final layer of rocks were placed on top of the soil, thus completing the pot and allowing the plants to be grown. These pots were then submerged within water, where the plants were observed and recorded quantitatively (measured by numbers instead of by quality). Over the course of the following weeks, the plants were regularly measured for the length of the stalk, as well as the largest leaf of each plant. Additionally, the lid of the aquarium the plants are being grown in contains a light attached to the bottom of it, which projects a certain kind of light onto the Waterlilies, so the plants can get the appropriate amount of light needed in order to thrive.

Research Question and Hypothesis

Research Question: How well will Water Lilies (*Nymphaea tuberosa)* grow in lunar regolith?

Hypothesis: The American Waterlilies will grow somewhat poorly in the lunar regolith, grow decently in the 50% lunar regolith and 50% Bio-Stratum, and grow well in 100% Bio-Stratum. This is because the plant lives on earth, and plants grown in lunar regolith have shown some strain when growing, mostly because lunar regolith is just ground-up rocks, and doesn't truly have all of the proper nutrients for plants to thrive. The hope was that the pot containing 100% lunar regolith would be able to grow well enough to survive, and potentially even outstrip the growth of the 0% lunar regolith (100% Bio-Stratum) pot due to the fact that the former contained an aquatic plant, which has never been recorded with a soil base of lunar regolith in the history of Plant The Moon before.

Independent Variable: The Independent Variable in this experiment is the percentage of lunar regolith, which are 0% Lunar regolith (Labeled 100% ES for Bio-Stratum), 50% lunar regolith, and 100% lunar regolith. These are the variables that will change how much the plant grows, which is the Dependent Variable in this experiment (See Dependent Variable).

Dependent Variable: The dependent variable is the height of the Water Lily from the pebbles placed at the bottom to the top of the plant being measured. The width of the largest leaf is also measured in centimeters. The data that is being taken is only quantitative data, which is based on how tall the plant grows.

1. Acquire the seeds of the *Nymphaea tuberosa* and the materials needed. The project will require:
    a. An aquarium tank
    b. *Nymphaea tuberosa* seeds
    c. Pots in order to maintain the plants
    d. Lunar regolith
    e. Bio-Stratum soil
    f. Bowl in order to mix Bio-Stratum and regolith in

2. Pour 50% lunar regolith and 50% Bio-Stratum soil into a bowl and mix carefully.
3. Put a layer of rocks in the bottom of the pots to prevent soil leakage.
4. Put the *Nymphaea tuberosa* seed into the pots.
5. Fill one pot with 100% Bio-Stratum, two pots with a mixture of 50% lunar regolith and 50% Bio-Stratum each, and the last pot with 100% lunar regolith. Label the pots accordingly with tape.
6. Add more rocks on top of the soil so the mixture does not escape.
7. Fill up the aquarium with water.
8. Put in an additive to remove chlorine.
9. Put the pots into the water and record the growth of the plants every A-Day (every other day) in class.
10. With a ruler, measure the plants by placing the ruler in the water at the base of the plants all the way to the leaves. With the same ruler, measure the biggest leaf of each plant from end to end (diameter). All measurements are done in centimeters.
11. Data is measured and recorded on A days (every other day)
12. After the 8-week growth period has ended, transfer the plants into a bigger tank for more room.
13. Empty the new container and prepare it by cleaning it with water and a sponge.
14. Put the new container onto the counter then fill it with water
15. Transfer plants to the new tank, one by one
16. No more measuring needs to be done now that the project is over

Controls:

The controls in this experiment are: the water that the plants are submerged in, the temperature of the water in the tank, the seed of the plant that is being grown, the pot that the plants are being grown in, the rocks that the plants' Bio-Stratum soil are layered with, the ruler that is used to measure the plant, and the environment around the plant in question. To prevent the soil from mixing with the water while simultaneously allowing the soil to absorb the water, rocks are layered on the bottom and on the top of the soil.

Results:

The hope of this experiment was to see how well the plants would grow in lunar regolith. Final measurements were taken on March 28th in which the data collectors were tasked to compile the collected data into graphs, tables, and charts. Of all the plants, only one of the plants (the 50/50 Front Right) did not show any growth. The *Nymphaea tuberosa* planted in 100% Bio-Stratum soil showed a noteworthy amount of growth, but lower than the 50% lunar regolith and 50% Bio-Stratum soil plants. On the other hand, the *Nymphaea tuberosa* grown in 100% lunar regolith grew the shortest (around 6.3 cm) and was shorter than the 50% lunar regolith and 50% Bio-Stratum soil plants. The 50/50 plant that grew the most is the 50/50 Front Left at 26.5 cm; on the other hand, the 50/50 Back Left grew the least at 23.3 cm.

While the data collectors were making the charts, the plant that didn't grow at all, the Front left pot with 50/50 lunar regolith and Bio-Stratum soil mixture, was eliminated from the charts as it didn't show any progress and would only interfere with the final result of the data.

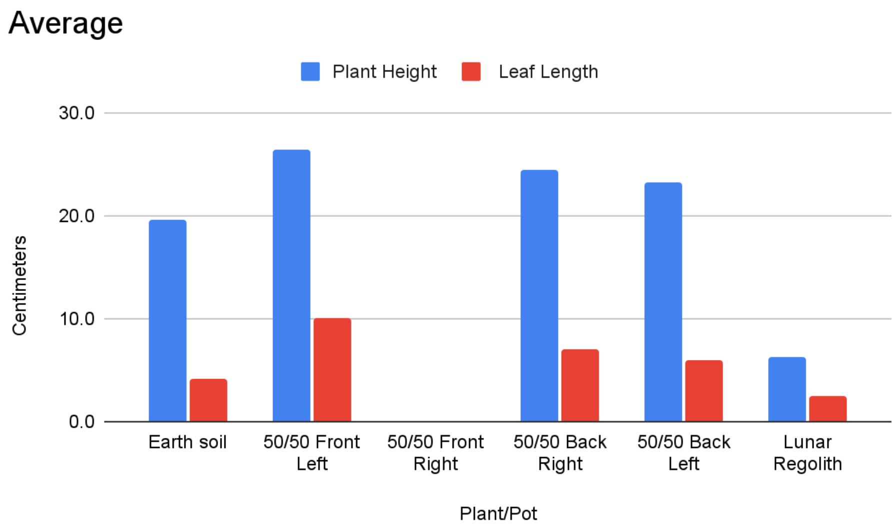


Figure 2 (above) presents a bar graph showing the height of each plant on average, as well as the length of each leaf.

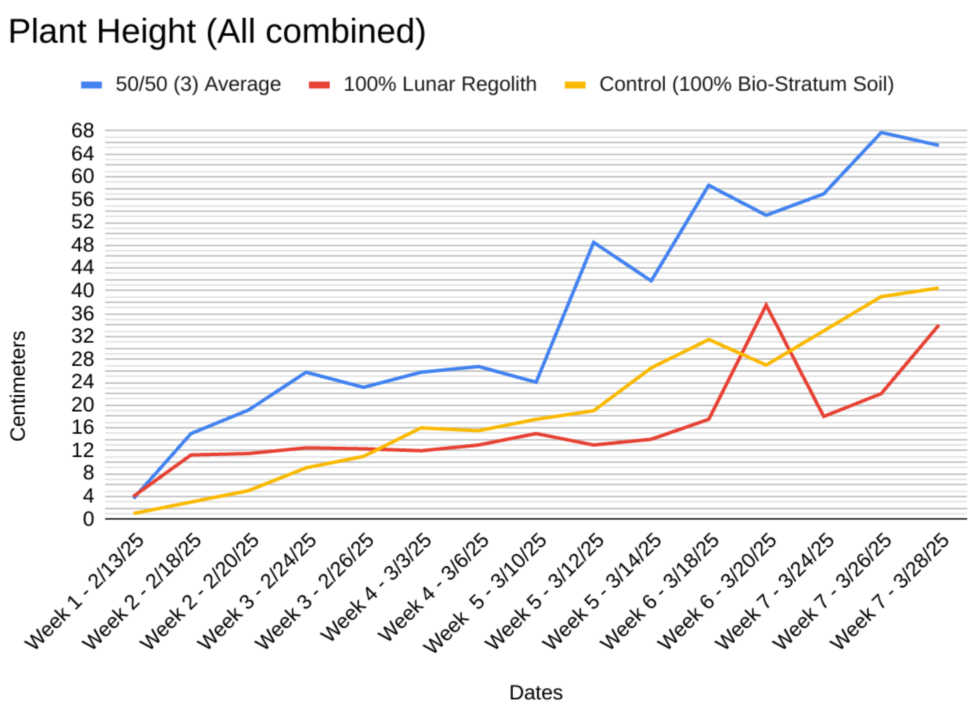


Figure 3 (above) presents a line graph of the growth of the lunar regolith plant, Bio Stratum control plant, and the average of the three 50/50 regolith and Bio-Stratum soil plants.

Discussion & Conclusions

The experiment was designed to research if lunar regolith can sustain aquatic plants, such as the *Nymphaea tuberosa*, and how well the plant can grow in lunar regolith. All *Nymphaea tuberosa* plants sustained throughout the whole 8 weeks–excluding the Front Right lily shown in the Average Chart (figure 2)–with the 50% Bio-Stratum soil and 50% lunar regolith plants showing the most growth. The Front Left water lily grew an average height of 26.5 cm with an average leaf length of 10.1 cm, the Back Right lily grew an average height of 24.4 cm with an average leaf length of 7 cm, and the Back Left grew an average height of 23.3 cm with an average leaf length of 6 cm. Meanwhile, the 100% Bio-Stratum soil plant grew an average height of 19.6 centimeters and an average leaf length of 4.2 cm, and the 100% lunar regolith plant grew an average height of 6.3 centimeters and 2.5 cm for average leaf length. Overall, most plants showed promising results in terms of sustainability and growth, particularly the plants in the 50:50 mixture, which grew the most in terms of both height and leaf length.

This experiment helps solve one of the many problems of bringing life to extraterrestrial planets. Because these lilies continued to show growth despite the composition of regolith they were placed in, it is possible that they can be used as some of the first known life outside of earth that can live there for long periods of time. Another benefit by bringing such plants to the moon is it opens the opportunity to bring other lifeforms to the moon to farm. Simple aquatic animals such as fish and shrimp can possibly be brought outside of earth and raised using these water lilies as nutrients. This would open up a new world of sustenance for long term researchers on the moon and other planets. This means that astronauts and scientists could stay in space longer, as the issue of protein and meat is no longer a problem.

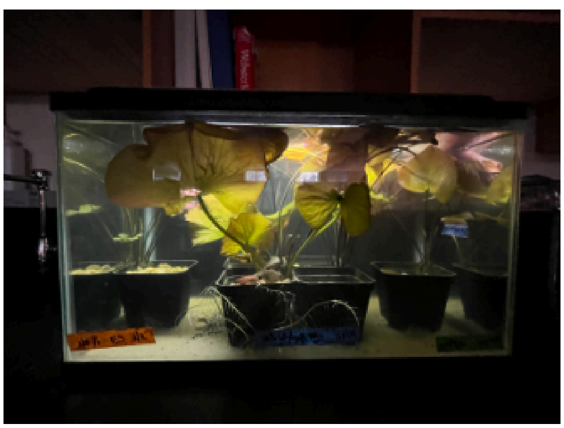
There are many limitations that arise when doing our experiment. The most important difference between the experiment in this report and one in a professional setting with better funding is that this lab was completed in a school. This means that many restrictions from working in a school applied. For example, outside temperature could not be controlled due to reasons outside of the experiment. For example, the temperature in a school is controlled to help students focus and not worry about temperature. It is possible that changing this temperature can make students lose focus, and this would become an issue for the school. Another issue that arises from working in a school is the budget. In a professional setting, a larger budget can be used, as well as better scientific tools such as a form of water heater or tested waters. Despite that, this experiment still provides good data on a basis for future research. These tests show that the water lily is in fact able to grow in percentages of lunar regolith, providing adequate background research for other experiments and reports to be created.

All in all, this experiment provided extremely interesting and useful information. It showed that water lilies are still able to grow within some percentage of lunar regolith, opening more opportunities to more research in water lilies' ability to grow outside of earthly conditions. It is also important to note that it appears the water lilies grew best in the 50/50 mix of lunar regolith and Bio-Stratum. This is important because it means that the water lilies grow better in this than pure Bio-Stratum, leading to some other areas of possible research.

Photos:





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