An Analysis of *Azotobacter vinelandii* and Its Effect on the Fertility of the Lunar Simulant Soil

Shruti Gnanasekar

Freedom High School

Team 2 - #20654

Research Question: How does the addition of Azotobacter vinelandii affect the fertility of lunar soil?

Hypothesis: If *Azotobacter vinelandii* is inoculated to the lunar soil, then the fertility, specifically the nitrogen content, of lunar soil will increase due to the nitrogen-fixing properties of *Azotobacter vinelandii.*

BACKGROUND

The purpose of this experiment is to increase the fertility of lunar soil through the addition of *Azotobacter vinelandii*. This can be useful for scientists as improving the fertility of the simulant can help develop methods to turn lunar regolith into a fertile soil using different additions such as bacteria. Additionally, improving the fertility of the lunar simulant can aid in improving the fertility of other soils such as Martian soil. Overall, this experiment can add knowledge and findings to the field of lunar soil and plant growth, which furthers the understanding of the Earth and the Moon.

EXPERIMENTAL DESIGN

The aim of this project is to increase the fertility of lunar simulant through the addition of *Azotobacter vinelandii* and the addition of this bacteria will be the independent variable. Lunar soil lacks nutrients, especially nitrogen, which is crucial for plant growth. *Azotobacter vinelandii* is a nitrogen-fixing bacteria, so by adding this bacteria, I hope to increase the fertility of lunar soil. I will begin the experiment by testing the initial amount of nitrogen in the simulant through the use of a colorimeter. Almost everything that is done with the lunar simulant will also be done

to Earth soil, also known as terrestrial soil. This will be the control variable and is for the purpose of making comparisons. The bacteria, lunar simulant, and terrestrial soil will be observed under a microscope. Then, the bacteria will be added to the lunar simulant, however will not be added to the terrestrial soil because this bacteria is already present in terrestrial soil. Spinach seeds will be added to modified lunar soil, terrestrial soil, and only lunar soil. After plants have grown, the nitrogen content will be measured again using the colorimeter and the change in nitrogen content will be the dependent variable. Finally, the spinach plants grown in each type of soil will be observed under a microscope to see if any changes have occurred on a microscopic level.

I am hoping to collect quantitative and qualitative data from this experiment. The height of the plants will be measured every week and factors such as color and shape will be observed and documented to measure any changes that might occur. Additionally, pictures will be taken from the microscope before and after the addition of the bacteria in the lunar soil. Furthermore, the nitrogen content observed from the colorimeter will also be documented. I will be making bar graphs to illustrate the progress of growth and the contrast between the different types of soil regarding the nitrogen content.

BACKGROUND INFORMATION

There are six factors that affect plant growth: “light, mechanical support, heat, air, water, and nutrients” (Ming, Galindo & Henninger, 1990, par. 6). A deficiency of nutrients such as “nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur” in soil limits plant growth (Ming et al., 1990, par. 7).

Lunar soil is the combination of lunar regolith and lunar dust. This was collected by astronauts throughout several Apollo missions conducted by NASA and is heavily guarded. Therefore, through different combinations of rocks and glass, NASA created a simulant to mimic the "physical and chemical properties" of lunar soil (Ming, 1989, par. 3). Factors that are considered when manufacturing the simulated lunar soil include "nutrient retention, aeration, moisture retention, and mechanical support" (Ming, 1989, par. 8). Nutrient retention is a system of recycling nutrients in order for the soil to hold onto nutrients. Aeration is the process of breaking up soil compaction so oxygen and water can reach the roots of a plant. Moisture retention is the soil's ability to retain water. Finally, mechanical support ensures that the plant's stem supports the plant and holds it upright.

However, the lunar simulant lacks nutrients, especially nitrogen, which is crucial for plant growth. Thus, several researchers have attempted to combat this problem by adding nutrients in different forms. For example, an experiment was conducted to improve the fertility of the lunar simulant by adding a nutrient solution. The nutrient solution consisted of "peat moss, vermiculite, compost, red clay, GardenTone Vegetable fertilizer, and a fertile soil control" (Thorsen, Petollari, Debrezion, Thabit-Yousef & Eeds, 2023, par. 2). Crops such as "common buckwheat, hard red spring wheat, iona pea, and glacier tomato" were used and were grown in the modified lunar simulant and regular terrestrial soil (Thorsen et al., 2023, par. 5). As expected, the fertility of the lunar simulant performed similar to, if not better than the plants grown in the terrestrial soil.

Azotobacter vinelandii is a type of bacteria present in terrestrial soil. This bacteria helps with "nitrogen fixation" in terrestrial soil, which is when nitrogen in the atmosphere is converted into nitrogen in the form of ammonia (Knutson, 2022, par. 7). *Azotobacter vinelandii* has been

used for several lab studies. For example, it has been used in “transgenic expression studies” which is when the DNA that surrounds the outside of an organism is translated into a protein (Knutson, 2022, par. 7). The sequences of *Azotobacter vinelandii* have also been used in “comparative genomics”, where the genome sequences of different species are compared using different data tools (Knutson, 2022, par. 7).

VARIABLES AND CONTROLS

The addition of *Azotobacter vinelandii* will be the independent variable and the nitrogen content of the modified lunar soil will be the dependent variable. The control group will be the plants grown in the terrestrial soil and this will be used to make comparisons.

METHODS AND MATERIALS

- 1.875 kg of Lunar Soil
- 1.250 kg of Terrestrial Soil
- Tube of *Azotobacter vinelandii*
- 20 Spinach Seeds
- 10 Burk’s Medium Agar Plates
- 20 Small Planting Pots
- Black Marker
- 2.0 M Potassium Chloride (KCl)
- 22 Count of 50mL Centrifuge Tubes
- Shaker
- 22 Pieces of Filter Paper

- Colorimeter
- Cuvettes
- Distilled Water
- Kimwipes
- Coffee Grounds
- 2 Trays
- Grow Tower

Procedures

In a centrifuge tube, mix 5 mL of 2.0 M potassium chloride (KCl) with 33 mL of distilled water and 5g of lunar soil and coffee grounds. In another centrifuge tube, mix 5 mL of the KCl with 33mL of distilled water and 5g of terrestrial soil. Place the two tubes on a shaker and shake for 15 minutes. Turn on the colorimeter and let it sit for 10 minutes so the machine warms up. Filter the solution from the tube with lunar soil into a cuvette which will be placed into the colorimeter. Add KCl to the blank which is the control solution. Wipe the outside of the cuvette with a Kimwipe to make sure the dust does not interfere with the reading. Set the colorimeter at 635 nm. Place the blank into the colorimeter and press the calibrate button to set the blank to 0. Record the value of the blank and remove to see if the machine is properly calibrated. The value should remain 0 even after the blank is removed. Place the cuvette with the lunar soil into the colorimeter. Wait 10 seconds before recording the value. Remove, place, and record the value three more times to get a more accurate reading. Average the four numbers for the value of nitrogen in the lunar soil. Once a value of the nitrogen content in the lunar soil has been determined, dispose of the solution and clean the cuvette. Filter the solution from the tube with

terrestrial soil into the cuvette. Repeat the steps from before to get a value of the nitrogen content in the terrestrial soil. Turn off the colorimeter. Dispose of the solution and clean the cuvette.

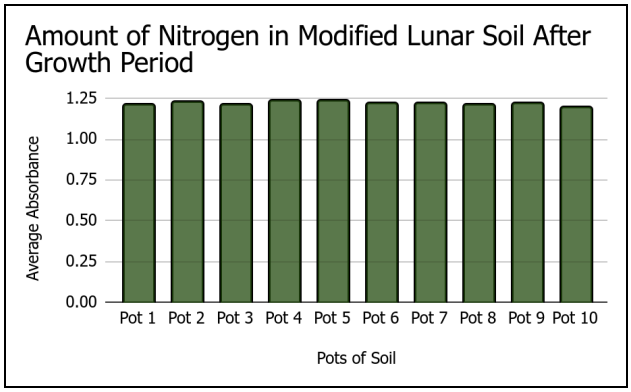
On an agar plate, spread two grams of *Azotobacter vinelandii* from the tube. Repeat until ten agar plates are used in total. Incubate the agar plates for 72 hours at 25°C. Add an agar plate to the bottom of a pot. Mix 18.75 g of coffee grounds with 125g of lunar soil and add the mixture into the pot. Plant one seed of spinach into the pot. Repeat the previous steps multiple times until there are 10 total pots with the modified lunar soil. Label each pot with the name "Modified Lunar Soil" and assign a number to each pot. In another pot, mix 18.75g of coffee grounds and 125g of only lunar soil and plant one seed of spinach into the pot. Repeat so there are 5 total pots of only lunar soil. Label each pot with the name "Lunar Soil" and assign a number to each pot. In another pot, add 125g of terrestrial soil and plant one seed of spinach into the pot. This will be the control group for the experiment. Repeat the steps so there are 5 total pots of terrestrial soil. Label each pot with the name "Terrestrial Soil" and assign a number to each pot. Transfer the pots to a tray and transfer the trays to a grow tower. Add 175g of distilled water in each pot. Turn on the grow tower's light and turn it off at the end of the day.

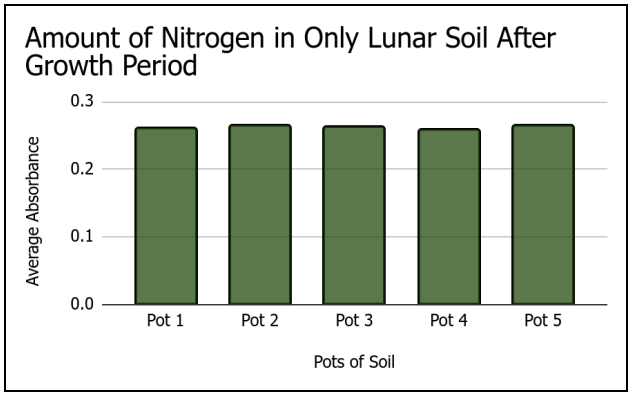
Monitor the growth of the plants by taking pictures. Add 57g of distilled water every two days. Turn on the light of the grow tower at the start of the day and turn the light off at the end of each day. In 4 weeks, the spinach should be fully grown.

After the four week period, repeat the KCl extraction method again. In a centrifuge tube, mix 5 mL of 2.0 M potassium chloride (KCl) with 33 mL of distilled water and 5g of the modified lunar soil. Repeat for the other pots of modified lunar soil. In another centrifuge tube, mix 5 mL of the KCl with 33mL of distilled water and 5g of only lunar soil. Repeat for the other pots of lunar soil. In another centrifuge tube, mix 5 mL of the KCl with 33mL of distilled water

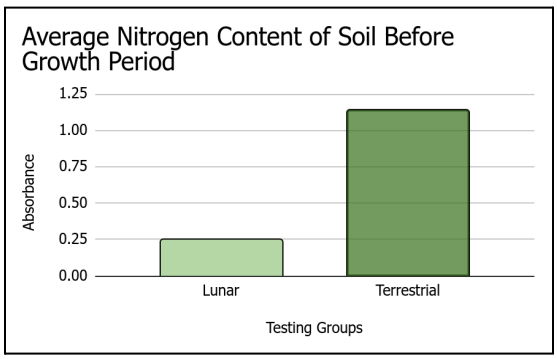
and 5g of terrestrial soil. Repeat for the other pots of terrestrial soil. Place four tubes on the shaker at a time and shake for 15 minutes. After 15 minutes, add another four tubes. While those tubes are on the shaker, test the nitrogen content of the four tubes that have already been on the shaker. Turn on the colorimeter and let it sit for 10 minutes so the machine warms up. Filter the first modified lunar soil solution from the tube into a cuvette which will be placed into the colorimeter. Add KCl to the blank which is the control solution. Wipe the outside of the cuvette with a Kimwipe to make sure the dust does not interfere with the reading. Set the colorimeter at 635 nm. Place the blank into the colorimeter and press the calibrate button to set the blank to 0. Record the value of the blank and remove to see if the machine is properly calibrated. The value should remain 0 even after the blank is removed. Place the cuvette with the modified lunar soil into the colorimeter. Wait 10 seconds before recording the value. Remove, place, and record the value three more times to get a more accurate reading. Average the four numbers for the value of nitrogen of the modified lunar soil. Once a value of the nitrogen content in the modified lunar soil has been determined, dispose of the solution and clean the cuvette. Repeat the above steps for the other solutions of modified lunar soil once they have been on the shaker. Filter the solution from the flask with terrestrial soil into the cuvette. Repeat the steps from before to get a value of the nitrogen content in all of the different solutions with terrestrial soil once they have been on the shaker. Filter the solution from the flask with only lunar soil into the cuvette. Repeat the steps from before to get a value of the nitrogen content in all of the lunar soil solutions once they have been on the shaker. Turn off the colorimeter and dispose of all of the solutions.

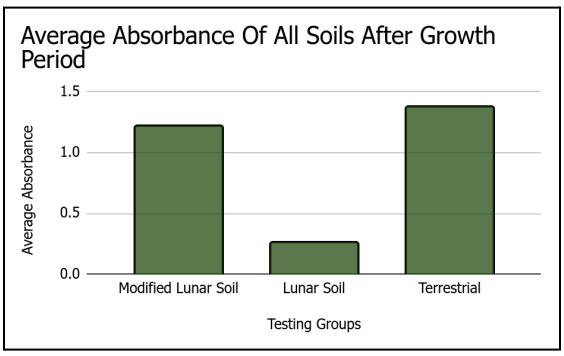
RESULTS





The above graphs illustrate the amount of nitrogen in each type of soil: modified lunar soil, only lunar soil, and terrestrial soil. This includes the average absorbance in each pot of soil which is directly proportional to the amount of nitrogen in the soil. The pots that share the same soil have around the same amount of nitrogen.





The graph on the left indicates the nitrogen content before the growth period and the graph on the right illustrates the nitrogen content of the testing groups after the growth period. There has been an increase in nitrogen for all testing groups, however, while the terrestrial soil has the most amount of nitrogen in it, the data clearly indicates that there has been a great increase in the amount of nitrogen in the modified lunar soil compared to the regular lunar soil.

<u>Visual Observations</u>

	Modified Lunar Soil	Terrestrial Soil	Lunar Soil
Week 1	No growth	No growth	No growth
Week 2	No growth	Visible growth from 2 pots: Pots 2 and 4	No growth
Week 3	No growth	Visible growth from 2 pots: Pots 2 and 4	No growth
Week 4	No growth	Visible growth from 2 pots: Pots 2 and 4	No growth

This table indicates the visual observations of the growth process. As the table mentions, there was no growth from the pots of modified lunar soil and regular lunar soil. In the terrestrial soil, only two pots exhibited growth.

DISCUSSION AND CONCLUSIONS

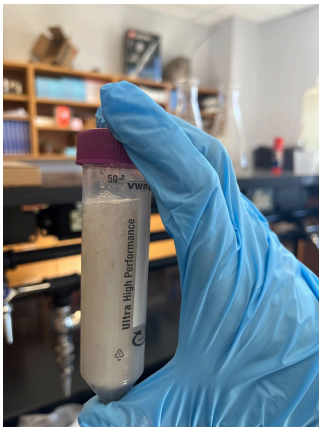
There has been an increase in the nitrogen content in the modified lunar soil compared to the regular lunar soi. This indicates that the bacteria greatly aided in nitrogen-fixation, therefore, it can be concluded that the addition of *Azotobacter vinelandii* does improve the fertility of lunar soil.

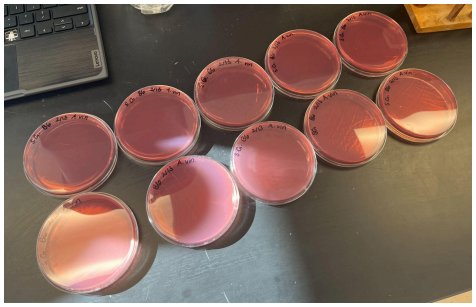
However, throughout the growth period, there was no growth observed in the pots with the modified lunar soil or the regular lunar soil. Similarly, in only two pots of the terrestrial soil were there significant growth. This can be for several reasons. There was no germination done before planting the seeds into the soil, so germination could have helped with the growth of the plants. Additionally, the coffee grounds added to the lunar soil could have prevented the growth. Another possible explanation is that the spinach seeds could have been damaged which is why it

did not germinate and grow. However, it is difficult to determine the exact reason for why the growth was stunted.

For further experimentation, it would be beneficial to have pots of lunar soil without coffee grounds to observe if the coffee grounds had a negative impact on the growth. Additionally, using different types of seeds would benefit the purpose of this experiment as it would demonstrate if there were plants that were more viable in growing in lunar soil compared to spinach seeds.

PHOTOS





LITERATURE CITED

Knutson, C. M. (2022, June 1). *The Genetic Basis of Nitrogen Fixation and Carbon Metabolism in Azotobacter vinelandii*. ProQuest. https://www.proquest.com/openview/246708ae6a884a3af6c007605db67c5e/1?pq-origsite=gscholar&cbl=18750&diss=y

Ming, D. W. (1989). Manufactured soils for plant growth at a lunar base. *Lunar base agriculture: soils for plant growth*, 93-105.

Ming, D. W., Galindo, C., & Henninger, D. L. (1990, March). Solid-support substrates for plant growth at a lunar base. In *NASA. Ames Research Center, Controlled Ecological Life Support Systems: CELSS'89 Workshop*.

Pitts, R., Orourke, A., Li, W., Hummerick, M., Irwin, T., & Diaz, A. (2024). Lunar Soil Enrichment for Plant Production: WILD (Waste Improved Lunar Dirt).

Thorsen, A., Petollari, S., Debrezion, S., Thabit-Yousef, S., & Eeds, A. (2023). Growth of Four different Crop Species in Supplemented Loamy Lunar Regolith. *Journal of Student Research*, *12*(3)

Wamelink, G. W. W., Frissel, J. Y., Krijnen, W. H. J., & Verwoert, M. R. (2021). Crop growth and viability of seeds on Mars and Moon soil simulants. *Terraforming Mars*, 313-329