Investigating the Growth of Green Bean Plants in Lunar Regolith Supplemented with Urea

Academy of Science

S. Rao, M. Dasari, E. Garcia, M. Li, T. Manu, M. Payne, E. Ymeri, L. Acker, A. Chatterjee, M. Wang, N. Molina, M. Tyagi, A. Pamulapati, E. Bogusz, M. Vemulapalli, N. Freire, S. Byreddy, V. Rathakrishnan, P. Mondreti, R. Govardhanam, S. Laks, T. Chitturi, A. Muju, D. Joshi, B. Alluri, S. Bhaskaruni, S. Nadimpalli, S. Sadhu, W. Stehn, S. Khan, M. Sosale, D. Admala, R. Kalluri, R. Singh, V. Togaru, R. Kulkarni, M. Lu

Team Name: Lean Green Demoons

Team Number: 9287

Challenge Category: High School

Challenge Identification: Plant the Moon

1 | Background:

One of the primary challenges impeding the ability for astronauts to return to the Moon and continue exploration of the lunar surface is the issue of resources, particularly food. Especially for long-duration missions, it is unattainable for astronauts to bring all the necessary food or water, which greatly hinders the potential crew size and the length of the stay. Therefore, effective use of lunar resources is essential for efficient missions to the Moon. This study aims to investigate the growth of green beans in lunar regolith supplemented with urea and potting soil.

Green beans are nutrient dense and can easily be grown in less desirable conditions. They are a rich source of folic acid, which helps oxygenate the blood, and contain high amounts of protein, which is beneficial for energy sustenance, and fiber, which aids digestion and lowers cholesterol. Green beans also contain vitamins A, C, and K, which are powerful antioxidants. These factors make green beans advantageous to sustain human inhabitation on lunar soil.

The key ingredients in potting soil—such as 60-70% processed forest products, sphagnum peat moss, perlite, fertilizer, and a wetting agent—help the plant grow healthy root systems, which is vital to the longevity and durability of plants (Wamelink, 2019).

The inclusion of urea in this potting soil is to test whether green beans can be grown in human urine. Urea is a nitrogen based fertilizer that can help plants grow in nutrient-lacking soils. A study conducted by Wageningen University and Research verified the idea that green beans can grow efficiently in lunar regolith, after the addition of human urine (Wamelink, 2020). Additionally, a study done in Cairo by the National Research Center supports this theory as they were able to grow sweet peppers in clay loam fertilized with urea and increased the plants' length, number of leaves and shoots, and weight (El-Al, 2009). Furthermore, urea is easily accessible to astronauts, making it an ideal additive to lunar regolith (Stephens, 2018).

2 | Experimental Design:

This study was conducted in the Academies of Loudoun greenhouse. First, six pots with varying concentrations of moon soil, potting soil, and urea were placed aside. The first pot, a control, was composed of 50% moon soil and 50% potting soil (BM1), while the second pot, also a control, was composed of 75% moon soil and 25% potting soil. The third pot had 50% moon soil, 49.5% potting soil, and 0.5% urea. The fourth pot had 0.5% urea (Easy Peasy Urea Fertilizer, Easy Peasy Plants), but 75% moon soil and 24.5% potting soil. Group 5 was composed of 50% moon soil, 48% potting soil and 2% urea. Finally, Group 6 also had 2% urea, but 75% moon soil and 23% potting soil.

In order to plant these pots, 0.5 kg of lunar regolith was added to each of the pots. The amounts of each component were calculated by multiplying the aforementioned proportions by a total of 800 mL. For example, since the total volume was 800 mL, to calculate how much moon soil to add for the first pot, 0.50 was multiplied by 800 mL, so the total moon soil added was 400 mL. The moon soil was then measured using a 1000 mL beaker to ensure accurate volume. Then, three green beans were placed in each pot. After potting, the soil was first drenched with filtered greenhouse water, and then subsequently watered with half cup of spring water every 2-4 days. Finally, urea was added on top to the pots 3, 4, 5, and 6 based on the concentrations determined before. Data collection occurred weekly to measure plant mass, the length and height of the leaves, and the pH.

However, after a few weeks of data collection, it was observed that the urea stunted the growth of the seeds in the pots 3, 4, 5, and 6, which led to the decision to plant three new pots, one control pot of 50% lunar regolith and 50% potting soil, and two experimental pots each with ½ teaspoon of urea. In the new pots, urea was added after the first true leaves formed onto the top of the soil to reduce the risk of fertilizer scalding. To ensure the seeds grew properly, the urea was left around the drip line of the plants, compared to what was done the first time, which was mixing the urea with the soil inside the pot. The proportion of soil in all three new pots was kept constant at 50% lunar regolith and 50% potting soil because those green beans grew the best from the original pots.

3 | Hypothesis:

The original hypothesis was that if green beans are grown in lunar regolith enriched with 48% potting soil and 2% urea, the plant will grow more efficiently, in comparison to the growth of the green beans grown in the control soils or with less of the additives. This is because in addition to the presence of moisture retention and numerous nutrients in potting soil, the addition of urea acts as a fertilizer by providing nutrients to further enrich the regolith.

After replanting, it was hypothesized that the pots with ½ a teaspoon of urea and 50% lunar regolith and 50% potting soil would grow the most efficiently compared to the control pot, because the new method of mixing the urea in would be more effective.

4 | Independent Variable or Parameters:

The independent variables are soil amendments, namely urea fertilizer and potting soil, both chosen due to their nutritional benefits and effectiveness in previous studies.

5 | Dependent Variable:

The dependent variable in this study is the growth of the green beans. The mass of the beans and the height and length of the leaves were measured to analyze overall bean growth.

6 | Measurements:

The plant mass (measured in grams with an electronic scale), length and height of the longest leaf (measured in centimeters using a ruler), and pH (measured by inserting a pH meter into the soil) were determined at the end of each week before watering to minimize error from water weight. At the end of the ten week experimental period, the dry mass, length and height of the longest leaf, and pH of the green bean crops were measured to make final conclusions.

7 | Controls:

The control groups for this experiment are the pots with only moon (or lunar) soil and potting soil. They contain no urea, so can be used as comparison in order to assess our results.

8 | Results:

8.1 | Writer Observations:

During the latter half of the experimental period, several members of the writing group visited the greenhouse to oversee the data collection process and recorded notable observations. Overall, factors that could contribute to error in the data and results were thoroughly controlled and thought of prior to the experiment. This included the care taken by the waterers to uniformly distribute the water over the pots, as to not disturb the soil; the measurement process of leaf length and water application; and the use of a pH probe to measure said variable.

8.2 | Data:

The collected data was compiled into the following tables:

Table 1: Change in Mass of Pots (g) over time

Time:	**Change in Mass (g)**		
	Pot 1	Pot 2	Pot 3 (control)
Week 6	50.37	47.35	19.95
Week 7	84.85	87.31	88.65
Week 8	39.34	42.7	1.63
Total	**174.56**	**177.36**	**110.23**

Table 2: Plant Height (cm) over time

# of days	Height (cm)		
	Pot 1	Pot 2	Pot 3 (control)
8	0	0	0
12	0.9	4.5	7.6
14	1.2	6	10
16	7	7.6	12.4
22	10.3	9	15.8

Table 3: Leaf Length (cm) over time

# of days	Leaf Length (cm)		
	Pot 1	Pot 2	Pot 3 (control)
12	0	0	0
14	0	0	4.5
16	3.2	2.8	7.9
22	6.7	4.7	9

Note that in the following table, the data in red shows a break in the trend.

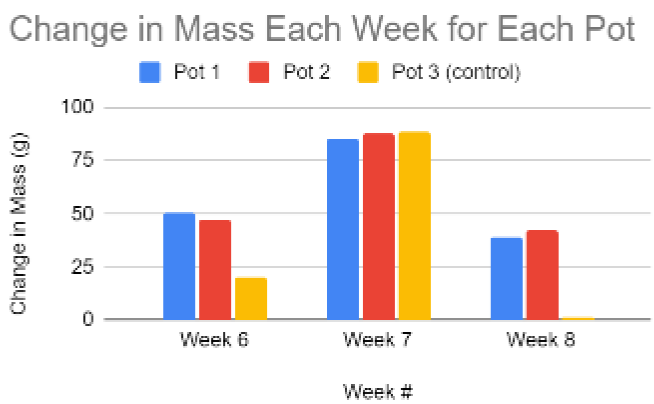
Table 4: pH of the Soil over time

Time:	pH		
	Pot 1	Pot 2	Pot 3 (control)
Week 6	6	6	6
Week 7	7.9	7.9	7.5
Week 8	7	7	6.9
Week 9	7.8	7.9	8

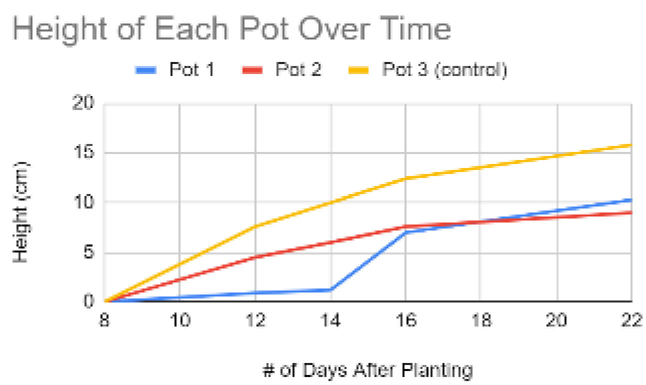
8.3 | Results:

The data above was then graphed:

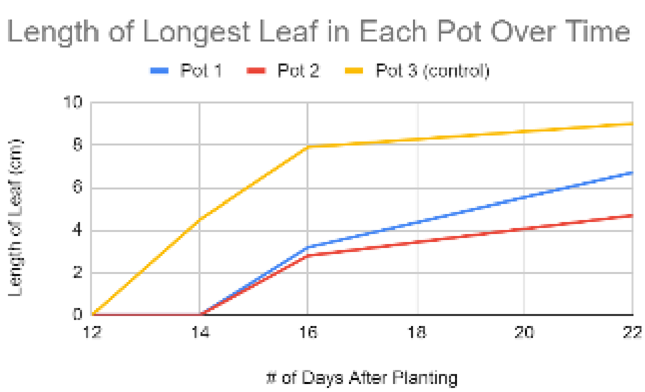
Graph 1: Change in Mass per Pot (g)



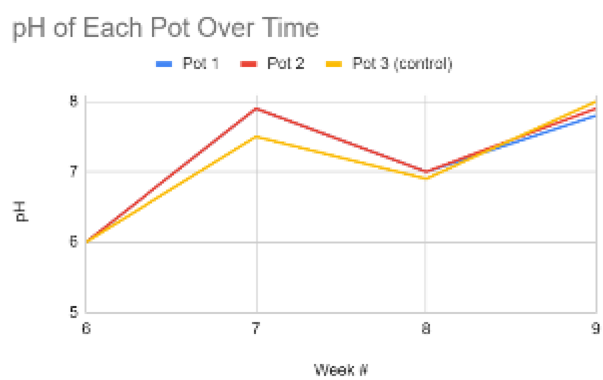
Graph 2: Plant Height (cm)



Graph 3: Leaf Length (cm)



Graph 4: pH of the Soil



8.4 | Statistics:

Table 5: Kruskal-Wallis Test for Pot Weight

Kruskal-Wallis Test (Nonparametric ANOVA)

The P value is 0.4360, considered not significant.
Variation among column medians is not significantly greater than expected by chance.
The P value is exactly correct (no approximations).

Calculation detail & Summary of Data:

Group	Number of Points	Sum of Ranks	Mean of Ranks	Median	Minimum	Maximum
New Pot 1	4	34.000	8.500	894.43	801.63	976.19
New Pot 2	4	21.000	5.250	813.51	722.50	722.50
New Pot 3 (C)	4	23.000	5.750	814.22	749.94	860.17

Kruskal-Wallis Statistic KW = 1.885
Post tests were not calculated because the P value was greater than 0.05.

Table 6: Kruskal-Wallis Test for Plant Height

Kruskal-Wallis Test (Nonparametric ANOVA)

The P value is 0.6162, considered not significant.
Variation among column medians is not significantly greater than expected by chance.
The P value is approximate (from chi-square distribution) because at least one column has two or more identical values.

Calculation detail & Summary of Data:

Group	Number of Points	Sum of Ranks	Mean of Ranks	Median	Minimum	Maximum
New Pot 1	7	69.000	9.857	0.90	0.00	10.30
New Pot 2	7	72.500	10.357	4.50	0.00	9.00
New Pot 3 (C)	7	89.500	12.786	7.60	0.00	15.80

Kruskal-Wallis Statistic KW = 0.9685 (corrected for ties)
Post tests were not calculated because the P value was greater than 0.05.

<u>Table 7: Kruskal-Wallis Test for Leaf Length</u>

Kruskal-Wallis Test (Nonparametric ANOVA)

The P value is 0.6168, considered not significant.
Variation among column medians is not significantly greater than expected by chance.

The P value is approximate (from chi-square distribution) because at least one column has two or more identical values.

Calculation detail & Summary of Data:

Group	Number of Points	Sum of Ranks	Mean of Ranks	Median	Minimum	Maximum
New Pot 1	7	72.500	10.357	0.00	0.00	6.70
New Pot 2	7	70.500	10.071	0.00	0.00	4.70
New Pot 3 (C)	7	88.000	12.571	0.00	0.00	9.00

Kruskal-Wallis Statistic KW = 0.9664 (corrected for ties)
Post tests were not calculated because the P value was greater than 0.05.

9 | Discussion & Conclusions:

Once results were compiled, a Kruskal-Wallis test was performed on the biomass, height, and leaf length data. A Kruskal-Wallis test is used for two or more different samples (in this case the varying concentrations of urea). The Kruskal-Wallis is appropriate for when the data is not distributed normally. For the biomass data, the resulting P value was 0.4360, which is greater than the alpha value of 0.05. The null hypothesis stated that the median biomass of each of the three pots was equal, and the P value states that there is a 43.60% chance of obtaining the results found. This is greater than the alpha value of 0.05, so the difference in biomass was not significant enough to reject the null hypothesis. Similarly, when the Kruskal Wallis test was performed on the height of the plants in each pot, there was a resulting P value of 0.6162. Once again, the P value was greater than 0.05, indicating that the null hypothesis failed to be rejected and that the median height of each of the three pots were equal. Lastly, the P value calculated for the leaf length data was 0.6168. Since this value was also greater than 0.05, there was not enough evidence to reject the null hypothesis and the median leaf length for each pot was equal. The results of all three statistical tests implied that the presence of urea mixed with lunar regolith did not contribute to any significant differences in biomass, height of the plant, or leaf length between the control and experimental groups.

While completing the Kruskal Wallis test, it was assumed that all samples were random and that allocation to the experimental group was random. All samples were independent of one another and the measurement scale was ordinal or could be ranked. Additionally, it was assumed that the data was continuous. During the experiment itself, it was assumed that all plants received the same amount of light and that no other external factors impacted any changes in the dependent variables (biomass, plant height, or leaf length).

During this course of this study, the research group determined that urea was not suitable for bean plant growth. Urea has shown to be a nitrogen-rich fertilizer and can therefore stunt the growth of green beans and other legumes. This is because beans have the ability to take in nitrogen themselves and release it into the soils, causing an excess buildup of nitrogen (Reinprecht et al., 2020). An excess amount of nitrogen can lead to less beans being produced and too much leaf growth in comparison. This was supported by the results in Graph 1, as there was a more significant change in biomass between weeks 6 and 7 in Pots 1 and 2, than in Pot 3. Pot 3 was the control variable and did not contain an urea. However, Pots 1 and 2, as part of the experimental group did contain larger amounts of urea in comparison. Pots 1 and 2 likely had less growth overall than did Pot 3, which contained a smaller amount of nitrogen. In future experiments when growing green beans in lunar regolith, a different additive should be considered due to the nature of urea and its compatibility with certain crops.

Additionally, there may have been issues with the water retention of the lunar regolith and soil mixture. Overwatering would limit the amount of oxygen in the soil needed for proper green bean growth. In the event that there was too much water present, the soil mixture would constantly be wet and there would not be enough air pockets for adequate plant growth. Pots 1-3 were all watered with spring water every 2 days and so there were no discrepancies between the control and experimental group. However, an excess amount of water may have contributed to a lesser number of beans present on the plants themselves. When conducting future experiments, the amount of water to be added should be adjusted according to the porosity of the soil mixture. This can be done by further researching the individual particle size of each component of the growth medium and observing the amount of water it is able to absorb.

Another change in the experiment was that all groups contained 50% soil and 50% lunar regolith. In this case, the control group became the group that did not have any urea supplemented. This potentially weakened the final results as we were not able to compare the growth of the green beans in the soil and lunar regolith mixture versus in just lunar regolith or just soil. However, the green beans were still able to grow significantly in the lunar regolith and soil mixture, which was the desired outcome.

References

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10 | Appendix:

10.1 | Raw Data

			CONTROL (50-50)	CONTROL (75-25)	0.5% UREA (50-50)	0.5% UREA (75-25)	2% UREA (50-50)	2% UREA (75-25)
Shrika	**Week 1**		**Pot 1**	**Pot 2**	**Pot 3**	**Pot 4**	**Pot 5**	**Pot 6**
Bhargavi		**Plant Mass (g)**	**961.46**	**1142.41**	**998.01**	**1085.9**	**941**	**1195.43**
Week 2	**Week 2**		**Pot 1**	**Pot 2**	**Pot 3**	**Pot 4**	**Pot 5**	**Pot 6**
Thrisha		**pH**	**3**	**3**	**3**	**1.5**	**8.5**	**9**
Anya		**Plant Mass (g)**	**1000**	**1250**	**1095**	**1100**	**1050**	**1225**
Week 3	**Week 3**		**Pot 1**	**Pot 2**	**Pot 3**	**Pot 4**	**Pot 5**	**Pot 6**
Dwij		**pH**	**7**	**7**	**6.5**	**7.5**	**5**	**5**
Sahvir		**Plant Mass (g)**	**1033.29**	**1287.35**	**1146.48**	**1123.52**	**1101.73**	**1246.84**
Week 4	**Week 4**		**Pot 1**	**Pot 2**	**Pot 3**	**Pot 4**	**Pot 5**	**Pot 6**
Bhargavi		**pH**	**7**	**6.8**	**6.5**	**6.4**	**5.1**	**5.2**
Shrika		**Plant Mass (g)**	**971.21**	**1233.91**	**1060.71**	**1088.86**	**1055.65**	**1231.77**
Week 5	**Week 5**		**Pot 1**	**Pot 2**	**Pot 3**	**Pot 4**	**Pot 5**	**Pot 6**
Thrisha		**pH**	**7.6**	**7.1**		**7**	**5**	**6.5**
Anya		**Plant Mass (g)**	**889.72**	**1156.67**	**1009.91**	**1048.36**	**1008.77**	**1190.4**

	Pots 1-3 replanted				**50/50 soil/regolith**		
			50-50 control	**75-25 control**	**New Pot 1**	**New Pot 2**	**New Pot 3 (control)**
Week 6	**Week 6**		**Pot 1**	**Pot 2**	**Pot 3**	**Pot 4**	**Pot 5**
Dwij		**pH**	**6**	**6**	**6**	**6**	**6**
Sahvir		**Plant Mass (g)**	**855.09**	**1160.48**	**801.63**	**722.5**	**749.94**
Week 7	**Week 7**		**Pot 1**	**Pot 2**	**Pot 3**	**Pot 4**	**Pot 5**
Bhargavi		**pH**	**7.9**	**7.6**	**7.9**	**7.9**	**7.5**
Shrika		**Plant Mass (g)**	**819.09**	**1109.66**	**852**	**769.85**	**769.89**
Week 8	**Week 8**		**Pot 1**	**Pot 2**	**Pot 3**	**Pot 4**	**Pot 5**
Thrisha		**pH**	**7.4**		**7**	**7**	**6.9**
Anya		**Plant Mass (g)**	**975.91**	**1225.52**	**936.85**	**857.16**	**858.54**
Week 9	**Week 9**		**Pot 1**	**Pot 2**	**Pot 3**	**Pot 4**	**Pot 5**
Dwij		**pH**	7.5	7.8	7.8	7.9	8
Sahvir		**Plant Mass (g)**	1058.02	1283.97	976.19	899.86	860.17

Note: The stats test for the mass only included data of the replanted plants.

		CONTROL (50-50)	CONTROL (75-25)	0.5% UREA (50-50)	0.5% UREA (75-25)	2% UREA (50-50)	2% UREA (75-25)
Date		**Pot 1**	**Pot 2**	**Pot 3**	**Pot 4**	**Pot 5**	**Pot 6**
2/23	**Height (cm)**	**3**	**1**	0	0	0	0
	Length of Leaf (cm)	**2.5**	**0**	0	0	0	0
3/1	**Height (cm)**	**13**	**10**	0	0	0	0
	Length of Leaf (cm)	**7.7**	**7**	0	0	0	0
3/3	**Height (cm)**	14	11.3	0	0	0	0
	Length of Leaf (cm)	8.7	7.1	0	0	0	0
3/10	**Height (cm)**	14.5	10	0	0	0	0
	Length of Leaf (cm)	9.4	9.3	0	0	0	0
3/18	**Height (cm)**	14	11	0	0	0	
	Length of Leaf (cm)	6.5	7	0	0	0	
3/22	**Height (cm)**	21.5	14.5	0	0	0	
	Length of Leaf (cm)	10	10.6	0	0	0	
3/24	**Height (cm)**	23.4	16.3	0	0	0	
	Length of Leaf (cm)	10.4	10.6	0	0	0	
3/28	**Height (cm)**	23.9	15.7	0.9	4.5	7.6	
	Length of Leaf (cm)	10.4	10	0	0	0	

3/30	**Height (cm)**	21.5	15.5	1.2	6	10	
	Length of Leaf (cm)	9.6	9.8	0	0	4.5	
4/1	**Height (cm)**	26.3	14.3	7	7.6	12.4	
	Length of Leaf (cm)	10.4	9.9	3.2	2.8	7.9	
4/7	**Height (cm)**	22.8	14.9	10.3	9	15.8	
	Length of Leaf (cm)	10.5		6.7	4.7	9	

10.2 | Images

Image 1: Academies of Loudoun Greenhouse

Image 2: Doing Calculations for Soil Proportions

Image 3: Creating Soil Mixture

Image 4: Initial Planting

Image 5: Data Collection

Image 6: Watering

Image 7: Replanting

Image 7: Replanting