



“Analysis of Urine Dilutions on the Growth of Swiss Chard in 50/50 Earth-Lunar Simulant”

The MAJOR MOONERS
8th Grade Participants
Grace Lutheran School Pocatello, Idaho
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BACKGROUND/REASONING: Why did you choose your experiment category (Moon simulant) and your particular independent and dependent variables?

Two years ago our school celebrated the launch of Artemis I and was excited to follow the journey back to our moon. We chose the lunar simulant because we are following the Artemis mission and excited to contribute in whatever way possible. We considered what it would take to grow food for astronauts in space and didn't want to add to the payload, so we researched low-cost, sustainable and renewable gardening methods. Using urine as a fertilizer would allow the astronauts to recycle their waste and conserve precious cargo room. Therefore, we agreed to use urine dilutions as the independent variables in our experiment.

Urine as a fertilizer has been utilized for many years worldwide. Rather than flushing urine away, it could be used to supply precious minerals and nutrients to plants like nitrogen, phosphorus, potassium, magnesium, calcium, and sulfur. According to the Rich Earth Institute, one gallon of urine contains 0.05 pounds of nitrogen, 0.008 pounds of phosphorus, and 0.017 pounds of potassium. In another study reported by Peter Morgan, urine was applied to several crops including spinach and other nitrogen loving plants and plant growth was up to seven times improved. They used a 1:3 urine dilution and applied it more frequently than our plans could accommodate. We were also wary about using too much urine as that could cause nutrient burn and harm the plants.

The dependent variables were chosen based on the idea that the following plant growth parameters, plant height, leaf and stem length, number and color of leaves and overall plant mass, would show improvement with the addition of urine because of its high nitrogen and mineral value. Nikhil Kishor et al. reported diluted urine at 30% improved plant leaf growth and amount of chlorophyll, which we understood to be leaf growth. Looking at photographs in other studies like Peter Morgan's, we noticed that the increase in plant mass was visually evident.

Initially, we were interested in studying spinach because it has been used in a variety of space gardening experiments because of its reliability and nutritional value. We then discovered Swiss Chard as an alternative because it had a very similar germination rate but was not day-length responsive, meaning it would not bolt if subject to changing environmental conditions like light and temperature. Swiss Chard is hearty, easy to grow, and offers more vitamin C, choline and B5 than spinach. Finally, it offers the astronauts a variety!

VARIABLES:

INDEPENDENT VARIABLE(s): The independent variables are the additions of urine as a fertilizer due to its high nitrogen content. The following dilutions of urine with distilled water will be applied to the seedlings twice during the growing period: Distilled water (control), Undiluted urine, 1:1, 1:4 and 1:8.

DEPENDENT VARIABLE(s): The dependent variable is the observed growth of the Swiss Chard seeds. This will be measured as the height of the plant (cm), length of longest leaves (cm), length of stem (cm), number and color of leaves, final plant mass and overall health.

CONTROLS/CONSTANTS:

- * soil mixture (50/50 lunar regolith simulant & Miracle Grow Garden Soil: Vegetable & Herbs)
- * 300 grams of soil mixture per pot
- * filtered water from fountain (all pots received the same amount of water)
- * two seeds per pot planted at a depth of 1/2 inch
- * same light, temperature and humidity exposure
- * Swiss Chard Ruby Red (*Beta vulgaris subsp. vulgaris*) from Organo Republic www.organorepublic.com

HYPOTHESIS:

We think that by adding diluted urine as a source of nitrogen will enhance the growth of swiss chard because swiss chard is a nitrogen-needy plant. Urine is an excellent fertilizer when used correctly. According to the Rich Earth Institute, no standard urine:water dilution exists but a range of 1:3 to 1:5 is the most common. Therefore, we hypothesized that the 1:4 dilution will work best without the fear of overfertilizing the plants.

EXPERIMENTAL RATIONAL: What is the overall value your experiment brings to the scientific community?

By experimenting with alternative fertilizers, lunar soils, and various vegetable species, we aim to provide the scientific community with innovative options for establishing living colonies on the moon. A 50/50 mix of lunar simulant and Earth soil can minimally support plant growth. However, when supplemented with a 1:1 urine-to-water additive, the plants exhibited robust growth. Even weaker dilutions demonstrated enhanced growth compared to no fertilizer at all.

Urine is an economical and accessible fertilizer that should not be overlooked. The students involved in this study dedicated their time and effort in the hope that their findings will contribute to future experiments and support advancements in space exploration and sustainable farming.

GROW POT SETUP:

Pot label:	U1	U2	U3	U4	U5	U6	U7	U8	CA	CB
Substrate	300 g 50/50 soil/lunar mix	300 g 50/50 soil/lunar mix	300 g 50/50 soil/lunar mix	300 g 50/50 soil/lunar mix	300 g 50/50 soil/lunar mix	300 g 50/50 soil/lunar mix	300 g 50/50 soil/lunar mix	300 g 50/50 soil/lunar mix	300 g 50/50 soil/lunar mix	300 g 50/50 soil/lunar mix
Amendment (2x)	Pure Urine	Pure Urine	1:8	1:8	1:4	1:4	1:1	1:1	none	none

PROCEDURES: Describe the grow setup, schedule and method your team used to test the hypothesis and record data.

SET UP

- Put on our masks and gloves and prep a draft free area to mix soils.
- Using a digital scale, measure and thoroughly mix 1500 grams each of lunar regolith and Miracle Grow soil creating 3000 grams of soil mixture.
- Measure 300 grams of soil per pot and label each pair of pots as follows: control, 1:1 urine, 1:4 urine, 1:8 urine, pure urine.
- Plant 2 SWISS CHARD seeds 1/2 inch into each pot using a marked glass rod.
- Water each pot with 20 ml of water. Spray each surface 10 times.
- Put pots inside the classroom greenhouse. Monitor each pot for signs of germination and moisture levels.

MAINTENANCE/ WEEKLY PROTOCOL

- Water plants Mon-Wed-Fri using graduated cylinders and filtered water from the hall fountain. Amount will change as needed with all pots receiving the same amount each time. Average increase was about 10 ml per week.
- Record the following when watering: volume of water in mL, temperature degrees F using digital thermometer, % humidity using the digital thermometer, pH using the probe, germination and interesting observations.
- Record the following data on Wednesdays into data chart: plant height (cm), stem height (cm), longest leaf length (cm), # of leaves, leaf color, and overall health.
- Clean up area.

FINAL DATA GATHERING

- Each plant was trimmed at the very base and the mass was measured immediately in gram to lessen the effects of water loss. Our balance only measured the whole number. Averages were calculated.

EXPERIMENT

- Obtain 100 ml clean, sterile urine sample (from a drug/medicine free adult) for use that day. Freeze the rest for the second application.
- The amendments were created using the following ratios:

Pure Urine (U1-2)	1:8 (U3-4)	1:4 (U5-6)	1:1 (U7-8)	Control (CA, CB)
—	40 ml distilled water	20 ml distilled water	15 ml distilled water	20 ml distilled water
20 ml urine	5 ml urine	5 ml urine	15 ml urine	—

- Use a plastic pipette to apply 20 ml of the amendment, 1 inch deep into the soil so that the urine does not evaporate and/or smell.
- Repeat one week after initial application using thawed urine sample. Our application weeks were 5 & 6 as we wanted to fertilize once they were well established.

week	Day	Water	Temp F	Humid %	pH	Initials	Fert- ilizer	Observations/Notes
Wk1	9/30	10 ml & 10 seeds	74		7.5	cc,sv		
	10/2	10 ml & 10 seeds	72	39	7	nh,jc,ow		
	10/4	10 ml & 10 seeds	68.9	41	7.5	jc,sv		CB, U7, U1 germinated!
Wk 2	10/7	10 ml	74	42	7.5	nh,sv		"decide not to spritz- no gravity in space."
	10/9	10 ml				ow,nh		
	10/11	20 ml	73.3	43	8	Ap, ow, ls, nh		
Wk 3	10/14	15 ml	72.2	38	8	cc		
	10/16	15 ml	72.9	40	8	nw,pe		
	10/18	20 ml	70	40	7	nh,sv		
Wk 4	10/21	20 ml				jc,ap		
	10/23	20 ml	72.2	39	8	sv,nh		
	10/25	20 ml	76	39	7.9	cc		
Wk 5	10/28	30 ml	69.8	39	7	ow,jc		
	10/30	30 ml				nw,pe	XXX	
	11/1	50 ml	74	41	7.8	ls,ap		
Wk 6	11/4	40 ml	67.9	38	7.6	sv		
	11/6	50 ml	74.7	35	7.5	sv,pe,sw	XXX	"U8 got more fertilizer, U7 got less"
	11/8	50 ml	69.8	39	8	ls,ap,nh		
Wk 7	11/11	50 ml	73.3	36	8	ls,ap,nh		
	11/13	70 ml	75.6	35	8	ls,nh,jc		
	11/15	70 ml	74	36	7.6	cc,jc,sw		Science fair
Wk 8	11/18	70 ml	73.8	35	7.7	nh,sv		
	11/20	70 ml	74.6	37		ls,nh,jc		
	11/22	80 ml				cc		Thanksgiving break-

DATA COLLECTION: List and organize your results.

#	Week 1 10/2	Observers	Plant Height (cm)	Length of Stem (cm)	# Leaves	Leaf Color	Longest Leaf Length (cm)	General Observations
2	U1 Pure Urine	PE & RW	0	0	0	none	0	none
3	U2 Pure Urine	PE & RW	0	0	0	none	0	none
4	U3 1:8	PE & RW	0	0	0	none	0	none
5	U4 1:8	PE & RW	0	0	0	none	0	none
6	U5 1:4	PE & RW	0	0	0	none	0	none
7	U6 1:4	PE & RW	0	0	0	none	0	none
8	U7 1:1	PE & RW	0	0	0	none	0	none
9	U8 1:1	PE & RW	0	0	0	none	0	none
10	CA none	PE & RW	0	0	0	none	0	none
11	CB none	PE & RW	0	0	0	none	0	looks pretty good

#	Week 2 10/9	Observers	Plant Height (cm)	Length of Stem (cm)	# Leaves	Leaf Color	Longest Leaf Length (cm)	General Observations
2	U1 Pure Urine	O W	4.5	3	2	green	3	healthy
3	U2 Pure Urine	O W	4.5	3	2	green	1	healthy and short leaves
4	U3 1:8	O W	5	2.5	2	green	2	healthy
5	U4 1:8	O W	5.5	4	2	green	2.5	healthy and floppy
6	U5 1:4	O W	6.5	4.5	2	green	2	healthy
7	U6 1:4	O W	4.5	4	2	green	1.5	healthy
8	U7 1:1	O W	5	4	2	green	2.5	healthy and a lot of sprouts
9	U8 1:1	O W	4.5	3.5	2	green	2.5	healthy
10	CA none	O W	3	2.5	2	green	2.5	healthy and less sprouts and no germination
11	CB none	O W	5	4	2	green	3	healthy small leaf

#	Week 3 10/16	Observers	Plant Height (cm)	Length of Stem (cm)	# Leaves	Leaf Color	Longest Leaf Length (cm)	General Observations
2	U1 Pure Urine	PE & RW	7.6	4.5	18	green	5	Growing well
3	U2 Pure Urine	PE & RW	7.5	4.3	21	green	3.4	Growing well
4	U3 1:8	PE & RW	10.3	4.5	16	green	4.4	Growing well
5	U4 1:8	PE & RW	8.2	4.4	20	green	3.5	Growing well
6	U5 1:4	PE & RW	9.3	5.3	15	green	3.6	Plants are a little flattened
7	U6 1:4	PE & RW	7.9	5.1	20	green	3	growing very well
8	U7 1:1	PE & RW	9.2	5.2	28	green	3.7	growing very well
9	U8 1:1	PE & RW	8.1	4	17	green	4	growing pretty well not many new
10	CA none	PE & RW	7.7	4.1	8	green	2.3	plants
11	CB none	PE & RW	9.3	4.4	15	green	3.6	growing pretty well

#	Week 4 10/23	Observers	Plant Height (cm)	Length of Stem (cm)	# Leaves	Leaf Color	Longest Leaf Length (cm)	General Observations
2	U1 Pure Urine	S V & Nsh	10.1	4.5	4	green	4	slightly less healthy
3	U2 Pure Urine	S V & Nsh	7.5	6.6	7	green	4.5	green + healthy
4	U3 1:8	S V & Nsh	10.6	5.5	4	green	5	green + healthy
5	U4 1:8	S V & Nsh	8	4.3	4	green	4	green + healthy
6	U5 1:4	S V & Nsh	9	3.5	4	green	4	green + healthy
7	U6 1:4	S V & Nsh	9.1	5.4	4	green	4	green + healthy
8	U7 1:1	S V & Nsh	7.6	6	4	green	4.5	healthiest
9	U8 1:1	S V & Nsh	8.8	4	4	green	4	green + healthy
10	CA none	S V & Nsh	8.3	3.4	5	green	4.5	least healthy
11	CB none	S V & Nsh	6	4.6	4	green	4.5	less healthy

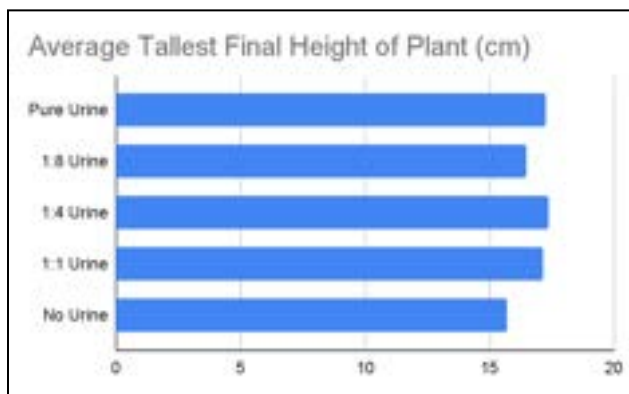
#	Week 5 10/30	Observers	Plant Height (cm)	Length of Stem (cm)	# Leaves	Leaf Color	Longest Leaf Length (cm)	General Observations
2	U1 Pure Urine	PE & RW	8.8	6.1	21	green	4.7	Some growing over edge, green
3	U2 Pure Urine	PE & RW	9.6	4.5	24	green	3.5	healthy and green, growing well
4	U3 1:8	PE & RW	12.2	5.7	23	green	3.8	Growing well, some are flattened
5	U4 1:8	PE & RW	9.1	4.2	26	green	3.4	green and healthy, growing well
6	U5 1:4	PE & RW	10	5.2	17	green	3.7	green and healthy, growing well
7	U6 1:4	PE & RW	7.9	5.6	21	green	3.6	green and healthy, growing well
8	U7 1:1	PE & RW	8.1	5	30	green	3.8	green and healthy, growing well
9	U8 1:1	PE & RW	8	4	18	green	3.5	looks like it's the healthiest - reaching
10	CA none	PE & RW	9	4.6	8	green	2.6	doesn't look very well
11	CB none	PE & RW	9.9	4.7	18	green	3.4	looks pretty good

#	Week 6 11/6	Observers	Plant Height (cm)	Length of Stem (cm)	# Leaves	Leaf Color	Longest Leaf Length (cm)	General Observations
2	U1 Pure Urine	AP & RW	11	4	4	brownish	4.4	Looks unhealthy one is dying and one is healthy
3	U2 Pure Urine	AP & RW	10.2	7	9	green	4	one is discolored and one is very healthy
4	U3 1:8	AP & RW	11.4	5	6	green	5	all plants are healthy
5	U4 1:8	AP & RW	12.1	7.5	5	green	4.5	all plants are healthy
6	U5 1:4	AP & RW	11.5	8	5	green	5	one is droopy the rest are healthy
7	U6 1:4	AP & RW	11	7	6	green	3	all are healthy
8	U7 1:1	AP & RW	11	4	6	green	4.5	one plant is discolored and the rest are healthy
9	U8 1:1	AP & RW	13	7.5	6	green/brown	5.5	all are healthy
10	CA none	AP & RW	11.5	7.5	6	green	4	unhealthy
11	CB none	AP & RW	11	7.5	6	green	8	looks healthy but has lost leaves

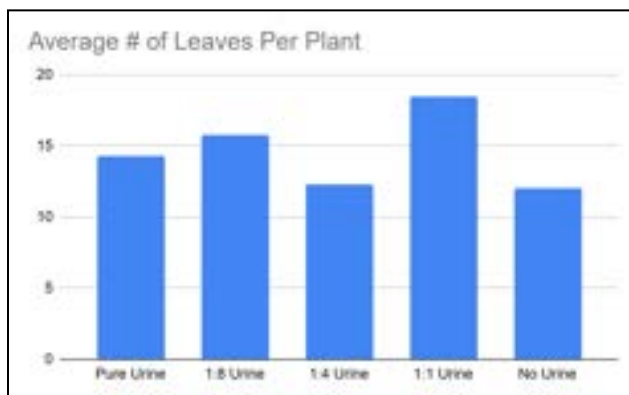
#	Week 7 11/13	Observers	Plant Height (cm)	Length of Stem (cm)	# Leaves	Leaf Color	Longest Leaf Length (cm)	General Observations
2	U1 Pure Urine	TS & SV	13.6	7.5	29	Green + Purple	4.5	Appearing healthy, but going downhill
3	U2 Pure Urine	TS & SV	14.2	7	31	Green + Purple	6.8	Good overall, some leaves look unhealthy
4	U3 1:8	TS & SV	14.3	8.5	34	Green	9.2	leaf left off, remaining buds look healthy
5	U4 1:8	TS & SV	12.3	8.6	35	Green	7.3	Healthy, some leaves are discolored
6	U5 1:4	TS & SV	13.8	8	25	Green	8.4	Healthy, big and flourishing leaves
7	U6 1:4	TS & SV	12	8	29	Green	7.1	Healthy, green leaves
8	U7 1:1	TS & SV	12.3	6	30	Green + Purple	6.4	Healthy overall, some purple leaves
9	U8 1:1	TS & SV	13.2	7.8	31	Green + Purple	8.2	One plant is purple, but rest are healthy
10	CA none	TS & SV	14.8	8.9	12	Green + Brown	8	Purple and small leaves, otherwise healthy
11	CB none	TS & SV	15.3	8.8	24	Green + Brown	7.8	Healthy, slightly purple leaves

#	Week 8 11/20	Observers	Plant Height (cm)	Length of Stem (cm)	# Leaves	Leaf Color	Longest Leaf Length (cm)	General Observations
2	U1 Pure Urine	S V & Nsh	16	7	31	green & purple	9	puny but healthy
3	U2 Pure Urine	S V & Nsh	18.5	8	26	green & olive	6.6	healthy
4	U3 1:8	S V & Nsh	18	7.9	32	green & purple	9.1	nice leaves
5	U4 1:8	S V & Nsh	15	6.7	31	green	6.2	good
6	U5 1:4	S V & Nsh	16.3	8	22	green & brown	9.4	good leaves
7	U6 1:4	S V & Nsh	18.5	6.1	27	green	9.7	beautiful
8	U7 1:1	S V & Nsh	15.3	7	43	greenish	8.3	healthy
9	U8 1:1	S V & Nsh	19	8.8	31	green & purple	10.7	healthy
10	CA none	S V & Nsh	16	8.5	14	purple	6.4	dying
11	CB none	S V & Nsh	15.4	6	22	purple	7.7	dying

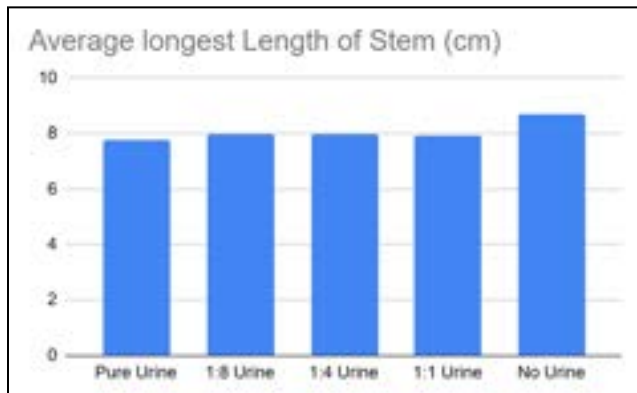
ANALYSIS: Summarize and draw meaning from your findings and results.



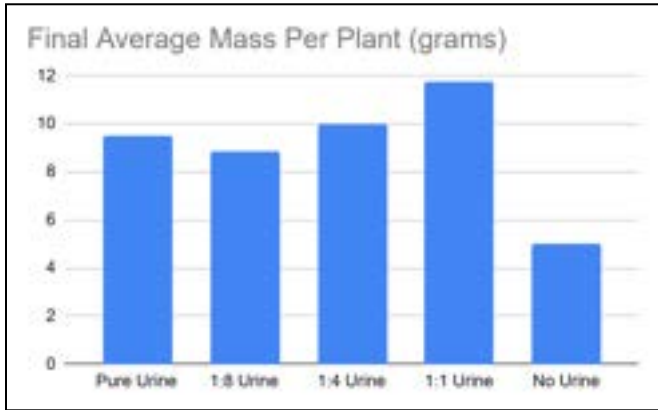
The pure urine and 1:1 dilution showed the tallest plant growth. The plant without any urine was the shortest. Even though the plants were equidistant from the light source, this value wasn't as important to us because leaf growth would offer more nutritional value to astronauts.



We were pleased to see that the control (no urine) had the lowest average of leaves per plant and the 1:1 urine dilution had the most. Interestingly enough the 1:4 dilution (our hypothesis) had the same as no urine at all and the other dilutions ranked at varying degrees. This confused us- and more research would need to be done to explain this data set.



Interestingly, the plant without any fertilizer showed the longest stem growth. This took the students by surprise! They came to understand the descriptive term "legginess" as a plant is striving to reach the light source. The controls were exposed to the exact amount of light as the others, so it was probably due to nutrient deficiency. All dilutions of urine had nearly the same effect on stem length.

 <table border="1"> <caption>Average Longest Leaf (cm)</caption> <thead> <tr> <th>Urine Dilution</th> <th>Average Longest Leaf (cm)</th> </tr> </thead> <tbody> <tr> <td>Pure Urine</td> <td>8.0</td> </tr> <tr> <td>1:8 Urine</td> <td>8.2</td> </tr> <tr> <td>1:4 Urine</td> <td>9.5</td> </tr> <tr> <td>1:1 Urine</td> <td>9.4</td> </tr> <tr> <td>No Urine</td> <td>7.0</td> </tr> </tbody> </table>	Urine Dilution	Average Longest Leaf (cm)	Pure Urine	8.0	1:8 Urine	8.2	1:4 Urine	9.5	1:1 Urine	9.4	No Urine	7.0	We were excited to see that the average longest leaf length belonged to the plants with the 1:4 urine dilution, but shared that place with the 1:1 dilution as well. The plant with straight urine showed second to smallest average leaf length. This we understood as too much nitrogen that can interfere with photosynthetic activity.
Urine Dilution	Average Longest Leaf (cm)												
Pure Urine	8.0												
1:8 Urine	8.2												
1:4 Urine	9.5												
1:1 Urine	9.4												
No Urine	7.0												
 <table border="1"> <caption>Final Average Mass Per Plant (grams)</caption> <thead> <tr> <th>Urine Dilution</th> <th>Final Average Mass Per Plant (grams)</th> </tr> </thead> <tbody> <tr> <td>Pure Urine</td> <td>9.5</td> </tr> <tr> <td>1:8 Urine</td> <td>8.8</td> </tr> <tr> <td>1:4 Urine</td> <td>10.0</td> </tr> <tr> <td>1:1 Urine</td> <td>11.8</td> </tr> <tr> <td>No Urine</td> <td>5.0</td> </tr> </tbody> </table>	Urine Dilution	Final Average Mass Per Plant (grams)	Pure Urine	9.5	1:8 Urine	8.8	1:4 Urine	10.0	1:1 Urine	11.8	No Urine	5.0	The final average plant masses showed that the 1:1 dilution produced more plant mass than any other dilution. We were happy to see that the 1:4 dilution was next, and it was no surprise to see the plant with no urine have the lowest mass.
Urine Dilution	Final Average Mass Per Plant (grams)												
Pure Urine	9.5												
1:8 Urine	8.8												
1:4 Urine	10.0												
1:1 Urine	11.8												
No Urine	5.0												

CONCLUSIONS: Reflect on the season- what did you learn? What's the coolest thing you discovered?

Our data did not support our hypothesis, as the 1:4 urine-to-distilled-water ratio did not have the most significant positive effect on the growth of Swiss chard plants. Instead, the 1:1 urine dilution yielded the best results, producing the highest wet mass, the longest leaves, and the greatest number of leaves.

The students gained valuable insights through this experiment. Many found it fascinating to learn how urine can benefit plant growth, even though using it was initially considered “awkward” for middle school students. One student was particularly impressed by the ability of a simple 50/50 mix of lunar simulant and Earth soil to support plant growth, especially after researching the challenging properties of lunar regolith, such as its pH and soil structure.

During the growing phase, students observed how plants respond to environmental factors like light and temperature. For example, the control plants, which lacked any fertilizer, became “leggier,” indicating they were stretching their stems to move their leaves closer to the light source and improve photosynthetic activity.

FUTURE CONSIDERATIONS: What changes or improvements would you consider if you repeated the experiment?

Since this was the first time conducting an experiment requiring precise measurements over a long period, we honored everyone's request to collect plant measurements. To reduce human error and inconsistencies, we would plan to assign specific persons to handle measurements. We also propose to fertilize the Swiss Chard plants weekly using pooled human urine samples. In this experiment, we only had one sample which allowed us to fertilize twice. Acquiring different samples would accurately reflect the situation astronauts would face. With the understanding that a 1:1 urine-to-water dilution produced the best results for Swiss chard, we are interested in testing its effectiveness on varying lunar simulant-to-Earth soil ratios. For fun, the students want to test the 1:1 dilution on 100% lunar simulant.

Most students were intrigued by the fact that Swiss Chard is a ‘cold hearty’ plant and wondered what would happen if we changed the temperatures while using a 1:1 urine:water dilution. Our experimental setting was on the warm side with temperatures between 69 and 73 degrees F. It was discussed that a lunar capsule would have stable temperatures between 65 and 70 degrees F and the chard might fare better.

The discussion extended beyond growing crops inside a lunar module to the possibility of cultivating crops directly on the moon’s surface in a gardening ‘pod.’ They learned that the day/night cycle on the moon would be about two weeks and proposed another experiment simulating the moon’s light/day cycle. They also considered how to accommodate the great temperature changes a lunar environment would entail..

TEAM & EXPERIMENT PHOTOS



The Major Mooners: Peyton Evans, Roxie Ward, Noah Hunt, Sahaana Vikranth, Nash Hale, Jameson Clark, Owen Wells, Tristan Sherwin, and Alex Parker.



Team Coach: Cristine Casselman

Measuring and preparing simulant mix for pots.	Measuring and preparing simulant mix for pots.	Pots labelled and ready to be planted!	Ready and awaiting germination.

			
Measuring and preparing the diluted urine samples.	The diluted urine samples from left to right, ending with distilled water.	The diluted urine samples from left to right, ending with distilled water.	Applying equal volumes of amendments using a pipette.

	
WEEK 1	WEEK 2
	
WEEK 3	WEEK 4
	
WEEK 5	WEEK 6

	
WEEK 7	WEEK 8



Plant masses (wet) measured in grams.

Work Cited

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