Martian Alfalfa Experiment

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Introduction

Why Mars:

- The reason Mars is the target planet is due to both Mar's proximity and relative similarity to Earth.
  - On average Mars is about 140 million miles away from the Earth, close enough for current aerospace technology to reach.
  - Unlike Mercy or Venus, Mars contains a relatively habitable surface temperature for most carbon based life.

Atmospheric Elements	Earth	Mars
Nitrogen	78.00%	2.70%
Oxygen	21.00%	0.13%
Argon	1.00%	1.6%
Carbon Dioxide	0.04%	95.32%
Carbon Monoxide	0%	0.80%
Water vapor	>0.04%	>0.04%
Krypton	>0.04%	>0.04%
Xenon	>0.04%	>0.04%
Neon	>0.04%	>0.04%
Helium	Earth specific	0%
Methane	Earth specific	0%
Hydrogen	Earth specific	0%
Nitrous oxide	>0.04%	>0.04%
Ozone	Earth specific	0%
Ammonia	>0.04%	0%
Hydrogen-dueterium-oxygen	0%	Mars specific

Why Plants:

- Growing plants on Mars has the dual purpose of changing the atmospheric composition and providing food for a colony.
  - Plants, through photosynthesis, have the natural ability to filter carbon dioxide from the atmosphere and produce oxygen and other byproducts.

Questions we aim to solve:

- Does Martian soil allow for conditions to foster bacterial infections? This data can be gathered via counting the nodules or calculating the mass of the nodules.
- Based on the Alfalfa's mass, which soil mix cultivates the greatest growth?

Methodology

Required Materials:

- Terran soil: Miracle-Gro from Home Depot
- Terran sand: Quikrete Play Sand from Home Depot
- Martian soil: MMS-1 Mars Regolith Simulant from The Martian Garden
- Seeds: Alfalfa seeds
- Inoculant: Alfalfa/True Clover Inoculant
- 6 plastic potting trays with 72 cells each

Garden Soil (Terran) Composition	Percentage
Compost, Processed forest products, sphagnum peat moss	99.79%
Ammoniacal Nitrogen	0.05%
Nitrate Nitrogen	0.04%
Phosphate	0.05%
Potash	0.07%

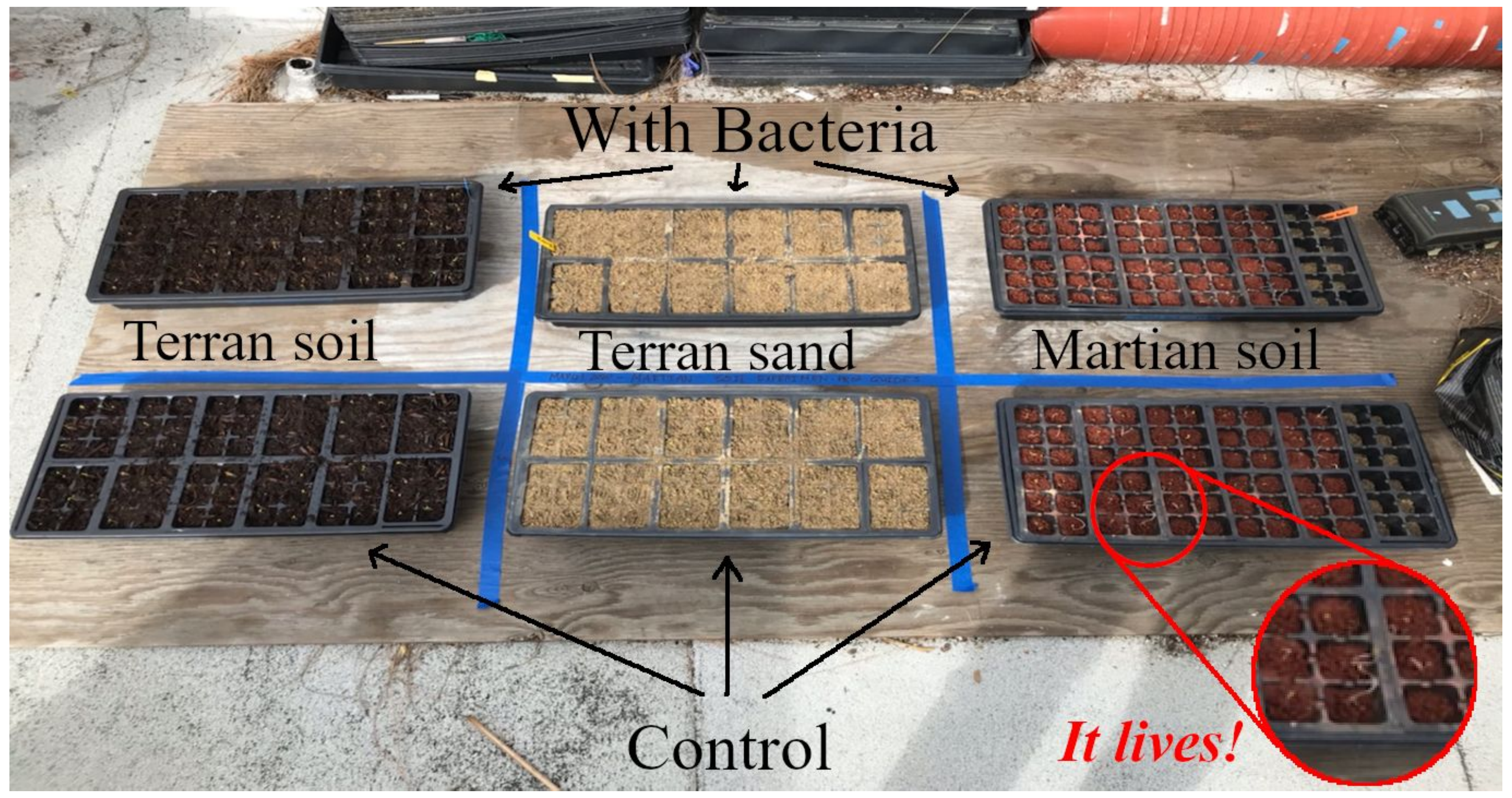
Sand Composition	percentage
Silica Sand (crystialine)	99%
Trace	$\leq$ 1%

Martian soil Composition	Percentage
Silica	49%
Iron Oxide	11%
Aluminum Oxide	17%
Calcium Oxide	10%
Magnesium Oxide	6%
Sulfate	< 1%
Trace	6%

Overview:

This experiment directly compared the growth of alfalfa plants in Terran soil, No Nutrient sand, and Martian soil, both with and without the presence of inoculant. Six potting trays with 72 cells each were sanitized with isopropanol and allowed to dry. Of these trays, two were filled with Terran soil, two with No Nutrient sand, and two with Martian soil. We only had a limited amount of Martian soil, so only 60 of the 72 cells in each Martian soil tray were filled. All of the trays were then thoroughly watered. The alfalfa seeds were given several washes in bleach and DI water to eliminate any contaminants that may alter the results of the experiment. For each soil type, one tray received control seeds and the other received seeds dusted with inoculant. The trays were then placed on the roof to receive ample sunshine.

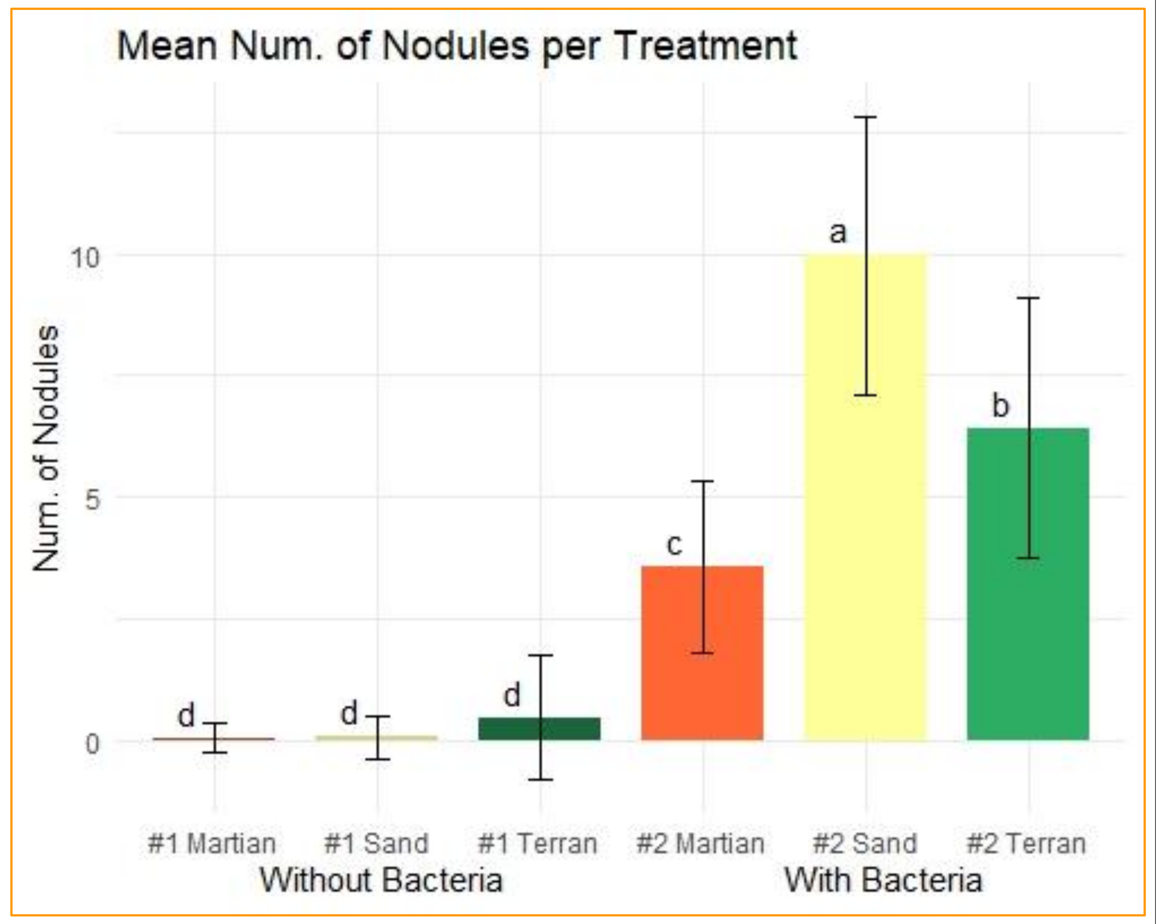
<u>But due to the COVID-19 outbreak, our experiment was cut short, and our instructor, Dr. Kenjiro Quides, provided mock data for us to use.</u>



Data & Analysis

For this section, an R program was written to automatically sort the data given in an Excel Sheet and conduct statistical tests. It then visualized the results by producing these graphs.

Figure 1. Mean Number of Nodules across all treatments. The Sand treatment with bacteria had the highest letter group while all three treatments without bacteria had the lowest. The connecting letter report indicates significant difference between treatments based on a One-Way ANOVA and Tukey's Pairwise Comparison.



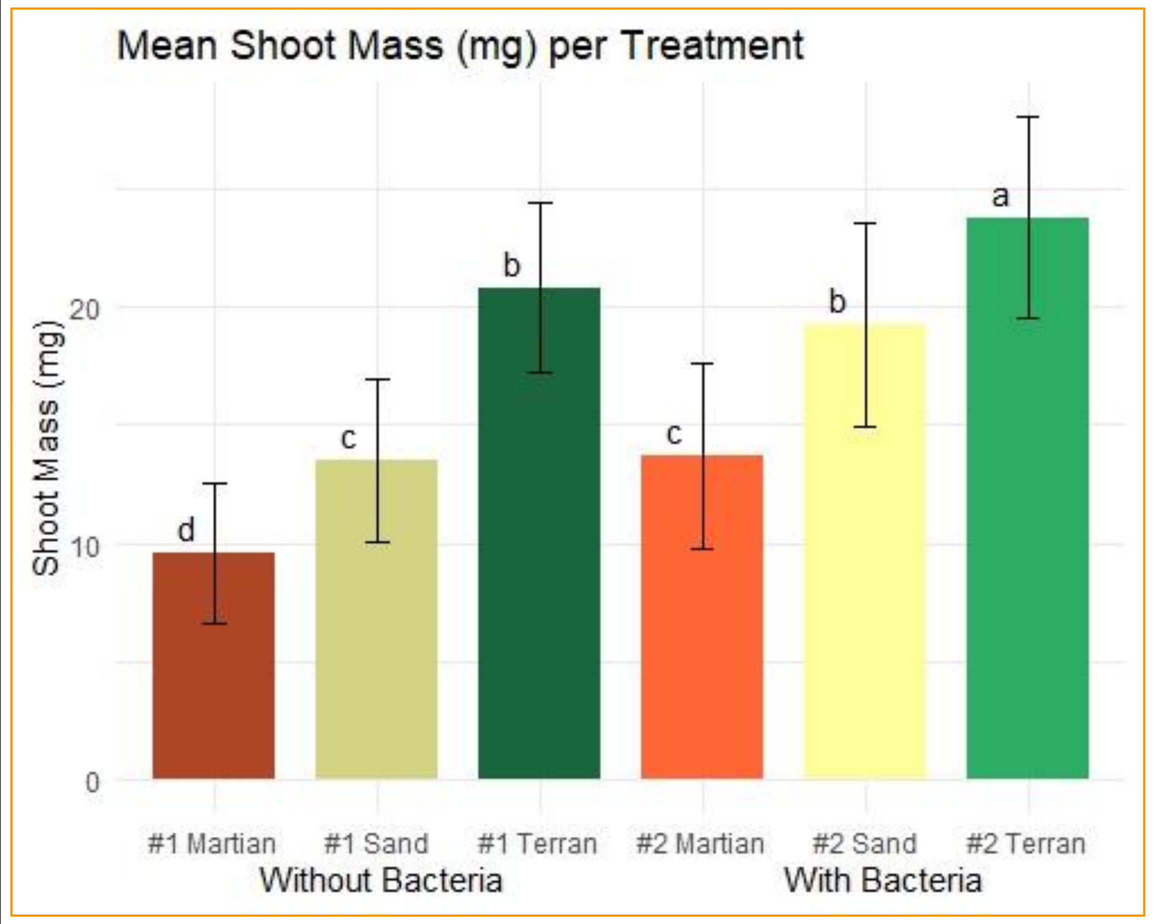


Figure 2. Mean Shoot Mass across all treatments. The Terran treatment with bacteria had the highest letter group the Martian treatment without bacteria had the lowest. The connecting letter report indicates significant difference between treatments based on a One-Way ANOVA and Tukey's Pairwise Comparison.

Author Contributions

NC, CT, LW, conceived the experiment. NC, CT, LW, conducted the experiment. CT, analysed the data. NC, wrote the introduction section, and the author contributions. LW, wrote the methodology. CT, wrote the acknowledgements section, data and analysis, results and discussion, and conclusion. DH, wrote the future directions section. NC, CT, LW, DH, editors.

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References

1. Kiers ET, Rousseau RA, Denison RF. Measured sanctions: legume hosts detect quantitative variation in rhizobium cooperation and punish accordingly. Evolutionary Ecology Research. 2006.
2. Peters GH, Abbey W, Bearman GH, Mungas GS, Smith JA, Anderson RC, Douglas S, Beegle LW. Mojave Mars simulant—Characterization of a new geologic Mars analog. Icarus. 2008;197(2):470–479. doi:10.1016/j.icarus.2008.05.004
3. Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. 7th Edition. Cengage Learning. 2018.
4. Foley CN, Economou TE, Clayton RN, Dietrich W. Calibration of the Mars Pathfinder alpha proton X-ray spectrometer. Journal of Geophysical Research: Planets. 2003 [accessed 2020 May 1];108(E12). https://agupubs.onlinelibrary.wiley.com/doi/abs/10.1029/2002JE002018. doi:10.1029/2002JE002018
5. Guan J, Liu A, Xie K, Shi Z, Kubikova B. Preparation and characterization of Martian soil simulant NEU Mars-1. Transactions of Nonferrous Metals Society of China. 2020;30(1):212–222. doi:10.1016/S1003-6326(19)65193-9
6. October 13 TS, 2017. Earth's Atmosphere: Composition, Climate & Weather. Space.com. [accessed 2020 May 8]. https://www.space.com/17683-earth-atmosphere.html
7. September 12 TS, 2017. Mars' Atmosphere: Composition, Climate & Weather. Space.com. [accessed 2020 May 8]. https://www.space.com/16903-mars-atmosphere-climate-weather.html

Results & Discussion

Figure 1

The data from Figure 1 indicates that there were residual, native bacteria that still stimulated nodule production in the treatments without bacteria, which are different from the foreign bacteria expected in the treatments with bacteria. This foreign bacteria was introduced via the inoculant and accounts for the greater number of nodules developed in the treatments with bacteria. The One-Way ANOVA test establishes that the Martian soil had the lowest average amongst the treatments with bacteria suggesting that, although it can foster bacterial infections, it would be limited compared to the soils here on Earth.

Figure 2

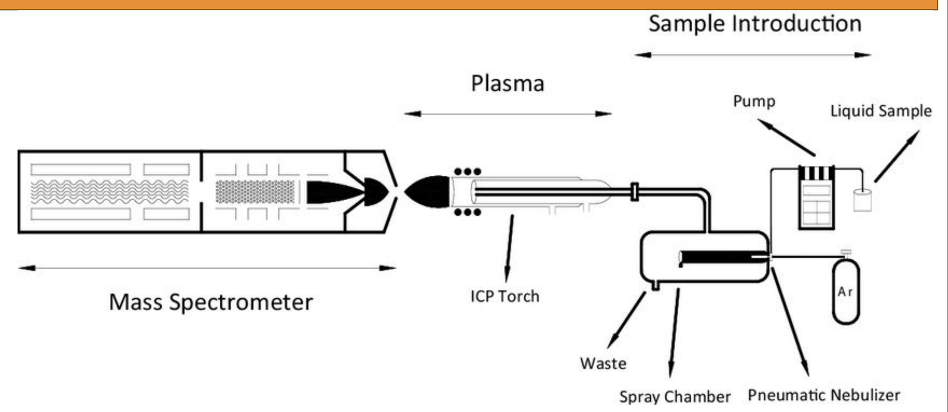
In Figure 2, a trend can be seen where average shoot mass increased for each of the treatments with bacteria when compared to their non-bacteria treatment counterparts. Therefore it can be concluded that a larger population of bacteria correlate to a larger shoot mass since all of the treatments with bacteria had higher averages. Figure 2 also visualizes the poor growth of the Alfalfa in the Martian soils when compared to the Sand and Terran soils. And it should be noted that the Martian soil with the bacteria shares similar growth to the Sand treatment without bacteria. So this could be a potential method for testing expected crop growth for colonists in a Martian environment while still on Earth.

Conclusion

Based on a paper analyzing nodule mass in fluctuating atmospheric nitrogen levels, it was determined that nodule mass was lower when atmospheric nitrogen levels are lower (Kiers et al. 2006). Since Mars only has 3% of the nitrogen that Earth's atmosphere contains, our experiment overestimated the number of nodules that would likely form in a Martian environment due to not accounting for the atmospheric consistency. And another factor to consider was that heavy metals may have inhibited Alfalfa growth in the Martian soil. This reduction might be due to high concentrations of cobalt and nickel which are also in high concentrations in serpentine soil (Peters et al. 2008). Serpentine soil is dangerous ground for cultivating crops because the crops will retain the heavy metals which are toxic to ingest.

Future Direction

Initial Plan:



- Analyze heavy metals in initial soil samples by performing soil-acid digest followed by inductively coupled plasma-mass spectrometry or ICP-MS (analysis to be done in Keck Center).
- ICP-MS uses inductively coupled plasma to ionize the sample, the sample is then separated into its components based on their mass to charge ratio (Skoog et. al. 2018).
- Analysis of the soil would gives us quantitative data of what components made up each type of soil used, this data would be compared to similar data collected in the leaves of the plants.

Other Methods of Chemical Analysis:

- Future work may include other analytical techniques to quantitatively determine different components of both the soil and the plants themselves.
- X-ray spectroscopy (specifically alpha-decay) is commonly used to analyze elemental components of soil (Guan et. al. 2020), it has previously analyzed carbon, oxygen, silicon, calcium, iron, sodium, magnesium, aluminum, sulfur, and nitrogen in soil samples taken on mars (Foley et. al. 2003).

Future Experimentation:

- In future experiments, other variables could be manipulated in order to determine their effect on plant growth:
  - Composition of atmosphere (specifically CO_2,O_2, and N_2), gravity, radiation