Investigating Eisenia *fetida* Survival in High Fidelity Lunar and Martian Regolith Vermiculture

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Introduction: For long-term habitation of Lunar and Martian bases, it is necessary to utilize in situ resources to develop economic and ecologically sustainable agriculture. However, both Lunar and Martian regolith are dusty, nutrient-poor, and contain phytotoxic minerals. To grow crops in these environments, the regolith will need to be heavily amended for nutrients, texture, and remediated to remove phytotoxins.[1] Worms are commonly used for soil enrichment processes on Earth to sequester heavy metals, increase nutrient availability, aerate soils, improve soil texture, and therefore have great potential for in situ regolith remediation. [2] Additionally, worms consume composted animal and food waste to produce worm castings, further decreasing Earth resources necessary, and increasing the bioavailability of nutrients in the compost. The proposed research seeks to refine our understanding of the survival and reproduction of the worm species Eisenia *Fetida (*E. *fetida)*, the quality of castings produced, and efficacy of E. *fetida* to remediate lunar highland (LHS-1) [3] and martian global (MGS-1) [4] regolith simulants. These regolith simulants are mineralogically accurate representations of the regolith found on both the Moon and Mars. This is important, as minerology determines much of the vermicultures chemical and physical characteristics.

Methods: In a preliminary study, fifty worms were added into each vermiculture, utilizing LHS-1, MGS-1, and Desert Silver Sand. As a proxy for human waste, horse manure was successfully hot composted for 2 months and used as a feeding source for E. *fetida*.[5] All worms were quarantined in peat moss for three days to prevent interference with gut material that hasn't yet been excreted. [6] Both regolith simulants and desert sand were mixed with an even volume of hot composted horse manure for worm consumption, along with approximately 25 mL of distilled water. Each container vermicompost utilized a two-tier method to drain excess leachate and maintain moisture levels. [7] E. *fetida* worms were mostly left undisturbed to acclimate but were aerated manually followed by surface feeding each week. 150 grams of composted horse manure and 20 mL of distilled water was added to the surface weekly for 12 periodic weeks. If the soil retained enough water, then none is added for that week. Each vermicompost is made up of two 1.5-gallon plastic containers with $\frac{5}{32}$" holes through the lid to ensure proper oxygenation and drilled at the bottom of the top container for proper drainage to the bottom tier. [8]

__Figure 1:__ Initial setup of each vermiculture bedding with a 1:1 ratio by volume of desert sand, MGS-1, or LHS-1 (left to right), and composted horse manure.

Results: After 12 weeks, E. *fetida* in LHS-1 regolith thrived, reproducing approximately every 60 days. The length of time it took for reproduction to occur indicates a tolerant environment for E. *fetida* LHS-1 worms. The reproduced LHS-1 worms were observed to be very active, and a majority resided at the top of the containers. As the offspring matured, more interaction between the regolith mixture was observed.

Reproduction in the earth-controlled desert silver sand vermicompost transpired shortly after. However, very few worms survived in the martian regolith.

After 12 weeks, the regolith in LHS-1 and MGS-1 turned into conditioned regolith, resembling a less dense and fluffy texture. Fungi growth was observed in MGS-1 due to the composted horse product. Soil samples were collected and are currently under review for Nitrogen, Phosphorus, and Potassium (NPK) analysis and general fertilizer compound testing.

__Figure 2:__ Earth-controlled desert sand, MGS-1, LHS-1 (left to right) activity taken fourteen days into initial trial.

Discussion: In the proposed work, we will pursue further analysis of the quality of fertilized regolith and increase our understanding of vermiremediation of nutrient-deprived lunar and martian soil. In particular, we will investigate if the high salt concentration in MGS-1 compared to LHS-1 contributed to the difference in worm survival observed during the preliminary study. Enumeration of gut bacteria population and changes of gut microorganisms in E. *fetida* between LHS-1 and DSS as well as MGS-1 and DSS is planned to be analyzed in future studies. Experiments involving plant growth with conditioned regolith are currently being conducted, and have found germination of Cherry Belle radishes, Raphanus *sativus* in all three vermicultures thus far.

Although compost worms in Lunar and Martian analogs haven't been studied in great abundance, and no research with high-fidelity regolith involving earthworms, there is still great potential involving vermiculture to improve nutrients in desolate regolith. This 12-week study suggests E. *fetida* reproduction in LHS-1 under correct conditions, as well as a possible chance of E. *fetida* in MGS-1.

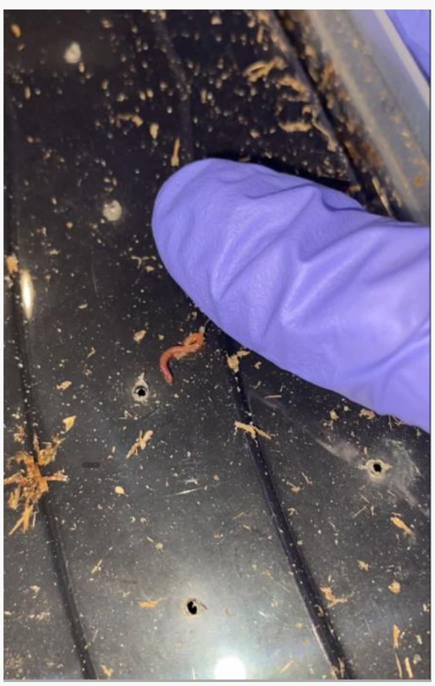

Figure 3: *LHS-1 offspring taken approximately 60 days into the initial trial.*

References: [1] Caporale, Antonio G., et al. "How to make the Lunar and Martian soils suitable for food production-Assessing the changes after manure addition and implications for plant growth." *Journal of Environmental Management* 325 (2023): 116455. [2] Edwards, Clive A., and Norman Q. Arancon. "The science of vermiculture: the use of earthworms in organic waste management." *Vermi technologies for developing countries. Proceedings of the international symposium–workshop on vermi technologies for developing countries, Philippine Fisheries Association Inc, Los Baños, Laguna, Philippines*. 2005. [3] Exolith Lab, LHS-1 Spec Sheet (Nov. 2021) [4] Exolith Lab, MGS-1 Spec Sheet (Nov. 2021) [5] Desta, Kefyalew Girma, and Muaid S. Ali. *Compost Turning: the key to quick composting*. Oklahoma Cooperative Extension Service, 2009 [6] Wamelink, Gerrit Willem Wieger, et al. "Growth of Rucola on Mars soil simulant under the influence of pig slurry and earthworms." *Open Agriculture* 7.1 (2022): 238-248. [7] Abbasi, S. A., et al. "A novel flippable units vermireactor train system─FLUVTS─ for rapidly vermicomposting paper waste to an organic fertilizer." *Journal of Cleaner Production* 198 (2018): 917-930 [8] Bolong, Nurmin, and Ismail Saad. "Characterization of university residential and canteen solid waste for composting and vermicomposting development." *Green engineering for campus sustainability*. Springer, Singapore, 2020. 193-206