Evaluation of Candidate Crop Plant *Lactuca Sativa* in Biologically Enhanced Martian Regolith

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Under a recent NASA EPSCoR grant, the Department of Space Studies at the University of North Dakota, in collaboration with SyNRGE LLC, developed a project to demonstrate the feasibility of biologically processing Martian regolith and inedible biomass through vermicomposting to reduce waste volume, enhance quality of regolith, and recycle and replenish nutrients. *Eisenia fetida* (Red Worms) were fed inedible biomass, consisting of spent growing media, inedible biomass (root balls, leaves, and stems), shredded paper, and other compostable materials, which are produced during simulated planetary missions in the Inflatable Lunar/Mars Habitat (ILMH) analog facility at the University of North Dakota. *In situ* Martian regolith was simulated by adding Martian Global Simulant (MGS-1) into the *E. fetida* feedstock where it was consumed and assimilated by the worms. The biocompatibility of bioprocessed Martian simulant on growth of *L. sativa* was then evaluated using a seedling bioassay system. Lettuce seeds planted on MGS-1 alone germinated but failed to grow. Seeds planted in processed biomass containing from 10 to 25% vermicomposted MGS-1 germinated and grew with no discernable nutritional deficiencies. Fresh weight of the lettuce grown on vermicultured regolith ranged from 70 to 76% of that grown in a commercial potting mix containing controlled release fertilizer. These results suggest that vermicomposting of inedible biomass with *E. fetida* in Martian regolith is a viable technology for use in a closed ecological life support system (CELSS). *Eisenia fetida* can be maintained in dormant condition, consume a wide range of organic material, and require limited volume to be effective. The optimal environmental setpoints are like those of crop growth requirements, and establishing a self-replenishing population eliminates resupply cost. Although these results are promising, several factors were identified that need to be understood before vermiculture can be recommended as a technique for *in situ* processing of Mars regolith.

Nomenclature

BLSS	=	Biological Life Support System
CELSS	=	Controlled Ecological Life Support System
CO_2	=	Carbon dioxide
DCR	=	Digital Color Reader
EPSCoR	=	Established Program to Stimulate Competitive Research
GWC	=	Gravimetric water content
ILMH	=	Inflatable Lunar/Mars Habitat
LED	=	Light Emitting Diode
LLC	=	Limited Liability Corporation
MDV	=	Microbial Diversity Value
MGS-1	=	Mars Global Simulant-1

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NA/NA = Nutrient Agar/Nutrient Agar
NASA = National Aeronautics and Space Administration
NH_3 = Ammonia
NO_3 = Nitrate
RO = Reverse Osmosis
SBD = Soil Bulk Density
SLAN = Solvita Labile amino-N
SP = Saturation percentage
TDS = Total Dissolved Solids
TSA/RB = Tryptic Soy Agar/Rose Bengal Chloramphenicol
VC = Verticompost
VOC = Volatile Organic Compounds
VWC = Volumetric water content
WFPS = Water filled spore space

I. Introduction

A. Plant Growth on Long Duration Missions

A *sustained* presence far from Earth will require a reliable and sustainable bioregenerative life support system (BLSS). To achieve a sustained presence on Mars, plants are a critical component of such a system for maintaining atmosphere, purifying water, and producing food. Effective, efficient bioregenerative technologies to manage waste streams are also required. The BLSS must operate in a closed-loop system that supports food production, reduces resupply demands, achieves compatibility with other physicochemical and engineering technologies, and works seamlessly to maintain steady state conditions capable of supporting human life.

NASA and other space agencies have spent decades researching plant growth systems for space. These efforts have been the subject of several excellent reviews (Wheeler, 2017; Zabel, et al., 2016). The production systems are based on innovative hydroponic nutrient delivery systems, using water soluble inorganic salts to supply the nutrients. These nutrients are either supplied as individual salts, or extracted from inedible biomass (Lunn et al, 2017).

Hydroponic production is a well-established technique suitable for a wide variety of leafy greens, vegetables, herbs and stable crops under space-flight relevant conditions. In addition, a hydroponic system enables optimization of nutrition though autonomous monitoring and control, which results in superior production and quality compared to nearly all other cultivation methods. Several drawbacks, however, should be considered: the requirement for continuous monitoring, control and maintenance of the system, potential for biofouling of water lines and reservoirs, and a high level of sanitation and cleaning to prevent the incidence and transmission of waterborne pathogens.

B. Martian Regolith as Substrate for Plant Growth

There has been interest, but little research, focused on developing biological approaches for *in situ* resource utilization (ISRU) of nutrients available from Martian regolith. Infrastructure, effort, and precision will be needed to produce water soluble plant food using Mars regolith.

Any idea that astronauts will be able to touch down on Mars and plant seeds should be dismissed outright. Successful utilization of Mars regolith to support plant growth will require pH remediation and introduction of copious amounts of organic matter to provide structure, introduce labile-N and C sources, and mobilize nutrients. Processing will also be required to introduce readily available sources of inorganic nitrogen and transform mineral content to bioavailable forms. Improvements in Mars regolith structure to increase water-filled pore space and soil porosity will also need to happen. This processing is necessary so water and oxygen can move freely in the resulting plant substrate and avoid anaerobic conditions (Kaplan et al., 1980; Reinecke & Venter, 1987).

Plant growth studies conducted on Mars regolith simulants have demonstrated that it is possible to grow crops, but with lower harvestable biomass in plants that do survive. The lack of growth is associated with the structure of regolith and the lack of nutrients in available forms to support plant growth (Wamelink, et al., 2014). Some Mars regolith simulants, such as the MGS-1, have been shown to be particularly hostile to germination and growth of plants. (Eichler et al., 2021).

C. Vermicomposting

E. fetida (red worms) have been widely used in composting, bioremediation, and soil ecology studies involving interactions with soil organisms (Koeppel et al., 2013). The work of Edwards and Lofty (1977) and the seminal book *Earthworm Ecology* (Edwards, 2004) are primary sources of literature to which the reader is directed. The role of earthworms in soil microbiomes (Aira et al., 2006); mineralization of nutrients (Alikhani et al., 2017); soil aggregate stability and bioremediation (Bhat, 2018; Fischer & Molnár, 1997); and tolerance to toxic compounds, such as perchlorates, relevant to Mars soils (Landrum et al., 2006) is well documented.

Using vermiculture to compost inedible biomass offers significant advantages compared to traditional composting., Vermicomposting requires no periodic turning, minimal chopping and grinding, no reliance on anaerobic processing that can produce noxious odors, and no complicated recipes or carbon to nitrogen ratios to remember. It is a mesophilic process compatible with crew and plant habit environments (Chandra, 2006) . A small vermireactor can be used in closed environments, or biospheres, where the emissions generated from thermophilically-driven processes are functionally incompatible (Brinton and Evans, 2006; Severinghaus, et al., 1994; Silverstone, et al., 1999).

Vermiculture supports a closed loop system, as it can process organic waste from a variety of sources, including human waste, at ambient temperature and pressure (Atanda, et al. 2018; Mupondi et al., 2011). Vermicomposting can also process waste from secondary systems, such as aquaponic fish culture, (Koub et al., 2018) inedible biomass from plant production, kitchen waste[4], and paper waste. Vermicomposting can reduce volatile organic compound (VOC) emissions during bioprocessing by increasing the concentration of stable organic carbon fractions (humus) and microbial biomass in vermicompost (Bhattacharya et al., 2016). The final result is a fertilizer capable of supporting plant growth with reduced harm potential (Bhat et al., 2018a; Bhat et al., 2018b; Sahariah, et al., 2015).

Vermicomposting can also be a bioaugmentation tool to remediate Mars regolith through the weathering actions of microorganisms that leach minerals, making them available for plant use (Zaets et al., 2011). Regolith contains, in appreciable quantities, the trace minerals that plants need for growth, except for presumably nitrogen, although nitrates have been detected on Mars (Stern et al., 2015). Studies have shown that, in the absence of perchlorates and peroxides[5], Mars regolith simulants are not particularly toxic to microbial growth (Schuerger, et al., 2012; Schuerger, et al., 2017) or to plant growth (Wamelink, et al., 2014). The lack of organic material that is integral to its structure is the problem; without it, Mars regolith has high bulk density and naturally compacts due to gravity.

D. UND ILMH Missions

Plant experiments have been conducted in the University of North Dakota Inflatable Lunar/Mars Habitat (UN ILMH) since Mission IV in 2017, and preliminary testing of vermicomposting using red worms *Eisenia fetida*, and possibly closely-related species, was done during Mission VI in 2019. The Mission VI plant tests evaluated whether inedible biomass can be recycled by worms in the presence of the Martian regolith simulant MMS-1. The resulting vermicompost/regolith mixture was then used to enrich plant growth substrates. The test consisted of six treatments: 1) Mohave Mars Simulant MMS-1 (Martian Garden, Austin, TX), 2) Promix Professional Potting Mix, 3) Inedible biomass without worm castings, 4) Inedible biomass enriched with worm castings, 5.) Vermicompost enriched with MMS-1 (50% w/w) and 6) Vermicompost enriched with MMS-1 (100%w/w).

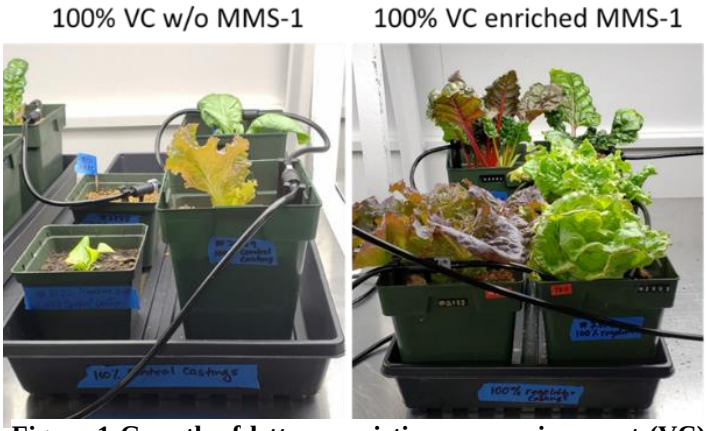

Figure 1 Growth of lettuce varieties on vermicompost (VC) produced during UND ILMH during Mission VI that had been composted without (Left) and with (Right) the addition of MMS-1 regolith to the composter.

The most striking effect of these preliminary tests was that plants failed to thrive when grown solely on vermicompost produced from inedible biomass alone but did thrive on material that had

[4] Vermiculture (in this context) is not suitable for composting meat, dairy, bones, oils, or dressings.

[5] It is assumed throughout this study (unless otherwise specified) that all regolith used as a plant growth medium has been pre-treated by leaching perchlorates with water and are not present in significant quantities.

been enriched with the MM1-regolith in the vermiculture reactor (Figure 1) performed during Mission IV. We then initiated efforts to expand testing to evaluate the feasibility of vermiculture as biological processor of Martian Regolith, identified and obtained a higher fidelity Martian Regolith (MGS), and conducted a series of vermicomposting experiments in reactors specifically designed for that purpose. Those experiments are described below.

II. Materials and Methods

A. Production and Collection of Inedible Biomass`

Plant growth experiments (Batches 1 and 2) were performed inside the ND ILMH plant production module during analog Mission VIII. As in previous studies performed in the ILMH, plants were exposed to the environmental conditions and variables necessary to support human habitation in a closed environment. It is a mesocosm that cannot be accurately reproduced in the lab. For the study, control plants were inoculated with beneficial bacterial consortiums and were grown during Mission VIII in October 2019, without vermicompost treatments. The inedible biomass (potting soil, root pellets, and inedible stems and leaves) was collected, frozen, and later used as substrate and bedding material to initiate Batches 3-4 in March 2020 in the UN ILMH).

During Mission VIII, only Batch 1 was ready to be harvested at 60 days, and it was necessary to produce more vermicompost to initiate plant growth experiments with Mars candidate crops (lettuce, wheat, and tomato) *and* to provide sampling for analysis. Due to time constraints during Mission VIII, the crew did not harvest the castings, but they did initiate Batch 2.

B. Mars Regolith Simulant

The Mars regolith simulant used during this study period was the Mars Global Standard-1 (MGS-1) provided by Exolith labs at the University of Central Florida. The MGS-1 formulation was not enriched with nitrates or perchlorates (Cannon, et al., 2019). Regolith was sterilized using a Bioclave pressure and steam autoclave (Ramsey, MN).. A laboratory incubator was used to oven-dry compost samples at 60^{o}C.

C. Vermicomposter

A commercial "Hot Frog Living Composter" (Uncle Jim's Worm Farm, Spring Grove, PA) was used to process the inedible biomass and Martian regolith simulant. The vermicomposter consists of three 15" x 15" x 22" (38 cm x 38 cm x 55 cm) trays with capacity of ~ 3 gallons (11.3 L). Each tray is designed with 40 angled tunnels that extend into the bedding material of the tray below, enabling movement between the different sections. A valve on the bottom of the unit allows for collection of leachates from the vermicomposter without disturbing the worms. Red worms (*Eisenia fetida*) were also obtained from Uncle Jim's Worm Farm (Spring Grove, PA).

The vermicompost was initiated following the instructions provided by the supplier. Briefly, substrate was layered followed by bedding containing the regolith simulant in approximately equal proportions by volume. The substrate included inedible biomass, packing supplies, and other inorganic material. For Batches 1-2, we used bedding material provided by the supplier (coconut coir and shredded paper). For Batches 3-4, we used sifted growing substrate collected at the conclusion of Mission VIII as the bedding material. The supplier-recommended additives were also added to the vermicomposter at this time. Additives included Jobe's compost starter, biochar, and zeolite. Each layer was sprayed thoroughly with reverse osmosis (RO) water as the layer was applied, and the process repeated until the tray was full. Substrate was allowed to cure for at least 10 days between substrate additions and substrate harvesting.

D. Compost Maturity Index

Compost maturity and stability were measured: Solvita (Woods End Laboratory, Mount Vernon, ME) High CO_2 test probes to measure compost maturing and NH_3 test probes to measure compost stability. Microbial respiration rates at field (basal) and burst (re-wetted) were evaluated with Low CO_2 probes. Soil labile amino nitrogen (SLAN) was detected using the Solvita NH_3 probes to assess compost maturity/stability but following SLAN protocol. The Sovita test strips provide a semi-quantitative measure of biological activity in the compost by using colorimetric reactions for CO_2 and NH_3. These balance between the C and N breakdown in the compost. The composite of these two values has been modelled to provide a "Compost Maturity Index". For the analysis, a Solvita Digital Color Reader (DCR)- Multi-Mode portable colorimeter (Rev. 701.2) was used to quantify the color results.

E. Harvest of Worm Castings from Vermicomposter

Finished vermicompost was air-dried, sifted through 10 mm mesh screen, and worms and large pieces of biomass set aside. The compost was then resifted through 6 mm mesh screen; worms and compost greater than 6mm were removed as before. The sifted worms and biomass were used to initiate the next batch. Vermicompost was sifted a final time through 2mm mesh screen to remove zeolite and biochar >2 mm before transfer and storage in food grade containers at a minimum of 35% moisture content. At 35% moisture content, the biological activity of the compost stabilizes, with minimal changes in composition during storage. At moisture content >35%, there is continued decomposition of the organic material; at lower moisture values, stratification of material occurs due to setting of the regolith. The harvested worm casting was using as inoculant in Batch 3 and Batch 4.

F. Nutrient Analysis

Qualitative colorimetric soil fertility tests were performed with Lamott's (Chestertown, MD) Professional Series Model STH-14 Soil Testing Outfit (STH Series 5010), Hydroponics 4-Way Test Kit (No. 3561), and Aquaponics Test Kit (No.3637). The testing protocols described in Lamott's STH-32 and AM-41 testing kits (www.lamotte.com) were followed.

In the STH-14 Soil Testing Outfit, tests for calcium, sulfate and chlorides are based on turbidity measurements. Potassium analysis also employs a turbidity measurement, using a proprietary reading that presents data directly in pounds per acre. A single extraction procedure, using Morgan Universal Extraction Solution, provides the liquid soil extract for all the nutrient tests except for chloride, which is extracted with demineralized water. Soil pH is determined calorimetrically, using a series of pH indicators and color charts covering the range of pH 3.8 to 9.6.

The Hydroponics 4-Way Test Kit enables colorimetric estimation of solution pH and concentration of nitrogen, phosphorus, and potassium in the solution. The Aquaponics Test Kit enables colorimetric estimation of pH, nitrogen, and iron content of the solution.

G. Environmental Monitoring

An Apera Instruments (Columbus, OH) ZenTest PH60-Z Smart Multi-Parameter Pocket Tester was used to measure pH, conductivity, TDS, salinity, resistivity, and temperature of the compost leachate. We are reporting only pH and conductivity. Moisture content of the compost inside the reactor was measured using an ECOWITT WH0291 moisture meter (www.ecowitt.com).

H. Phytotoxicity Testing-Cress Seedling Bioassay

Filtrate from each vermicomposting batch was collected and stored. The pH, EC, Total Dissolved Solids (TDS), salinity, and resistance of the filtrates were measured. Filtrates were stored at 4^{o} C until used.

Prior to starting the bioassay, the filtrate was diluted with DI water through filter paper. The saturated filter-paper disk was placed into a Petri Dish, and seeds of either Cress (*Lepidium sativum*) or dwarf wheat (*Triticum aestivum L.* cv. USU Perigee) were placed on the filter paper. Petri dishes were sealed with Parafilm and allowed to germinate for 3-5 days. Germination percentage and radical length was then determined.

Seed germination was observed visually, and emerging radicals were then measured. The germination index, which incorporates both percent germination and radical growth, was calculated using the formula adapted from Majlesse et al. (2012). Seeds with emerging radicles less than 2mm were excluded from calculations.

I. Phytotoxicity Testing-Lettuce Growth Assay Treatments

The Martian Global Simulant MGS-1 and vermicomposted MGS-1samples from 4 different batches (harvests) were shipped from University of North Dakota, (Grand Fork, ND) to SyNRGE, LLC, (Space Life Science Laboratory, Exploration Park, FL) where biocompatibility testing was performed. The composted MGS-1 samples consisted of one batch of immature compost and three batches of mature compost. Commercial Potting Mix (Miracle Gro, Marysville, OH) was used as the control.

There was a total of six treatments used in this bioassay. Each treatment was replicated three times.

- Martian Global Simulant (MGS-1) without amendment.
- Batch 1 of vermicomposted MGS-1 (immature compost)
- Batch 2 of vermicomposted MGS-1 (mature compost)
- Batch 3 of vermicomposted MGS-1 (mature compost)
- Batch 4 of vermicomposted MGS-1 (mature compost)
- MiracleGro™ Potting Mix (0.21-0.11-0.16) with nutrients (Control)

The composition of each of the MGS-1 vermicomposted batches is shown in Table 1. Batches 1 and 2 were prepared using only organic material derived from the habit experiments. Batch 3 and 4 contained 20-23% of sifted compost from either Batch 2 or 3 produced during UND ILMH missions. The percentage of MGS-1 varied among treatment, ranging from a low of 10.8% (Batch 3) to high of 25.9% (Batch 2). Th

Table 1 Composition of Vermicompost Batches (% total)

Biomass Type	Batch 1	Batch 2	Batch 3	Batch 4
Bedding	21%	8%	8%	24%
Newsprint	5%	4%	2%	3%
Biochar	5%	9%	0%	2%
Zeolite	5%	4%	1%	0%
Grains	5%	6%	1%	2%
Leafy Veg	13%	0%	4%	24%
Mun bean	5%	0%	0%	0%
Mixed Veg	23%	36%	0%	0%
Fruit Waste	0%	0%	33%	1%
Sifted Contents *	0%	0%	19%	24%
Bioactivator	5%	8%	3%	2%
MGS-1	15%	26%	11%	17%
Total	**100%**	**100%**	**100%**	**100%**
*Sifted contents from prior UND ILMH missions.				

MGS-1 Mars Global Simulant is a mineralogical standard for basaltic soils on Mars that was developed based on the mineralogy obtained from the Martian Science Laboratory (MSL) Curiosity rover. MGS-1 is made by sourcing individual minerals but does NOT include perchlorates which are thought to compose up to 2% of the total mass of the regolith. The particles are all less than 1 mm in diameter, with a mean particle size of 105 µm. Composition is reported by Cannon et al. (2019).

The control treatment is a commercial potting mix (Miracle Gro, Marysville, OH) consisting of processed forest products, sphagnum peat moss, perlite, and compost and is supplemented with a polymer-coated slow-release fertilizer derived from ammonium nitrate, ammonium phosphate, calcium phosphate, potassium sulfate, ammonium nitrate, ammonium phosphate, calcium phosphate and potassium sulfate.

*1. **Conical Tube Growth System:***

A custom-made growing system was prepared from 15 ml polypropylene conical centrifuge tubes (Falcon™, Fisher Scientific, Waltham, MA). Four 3 mm holes were drilled approximately 2.5 cm from the tip to allow water flow into the tube. A rockwool plug (Grodan, The Netherlands) was placed into the tube and extended ~2 cm from the access holes, functioned acted as the primary 'buffer' for water in the tube. A circle of germination paper was placed on top of the rockwool, and then approximately 10 ml of substrate was added to the growth tube (Figure 2).

Lettuce seeds (*Lactuca sativa* L. Grand Rapids, Ferry-Morris Seed, Norton, MA) were used for these tests with two seeds being planted per growth tube. The seeds were watered until media was wet and a translucent germination cover for three days was placed over the tops to ensure high humidity during the germination process.

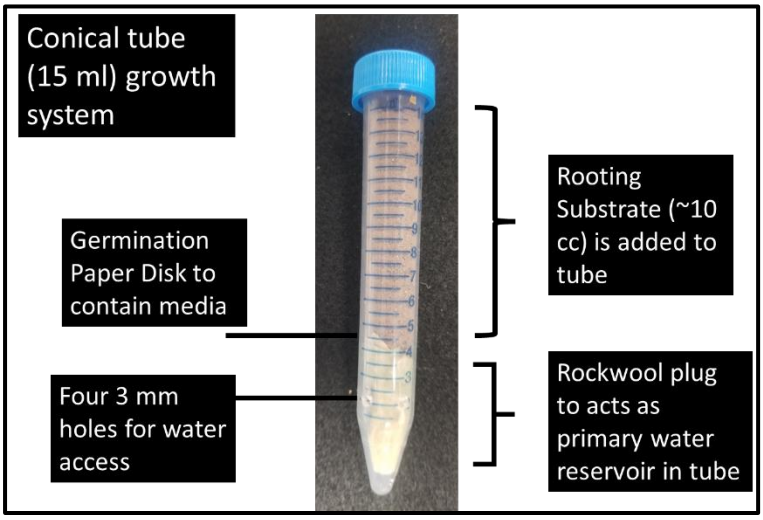

Figure 2 Components of the conical plant growth tube used in the whole plant lettuce bioassay.

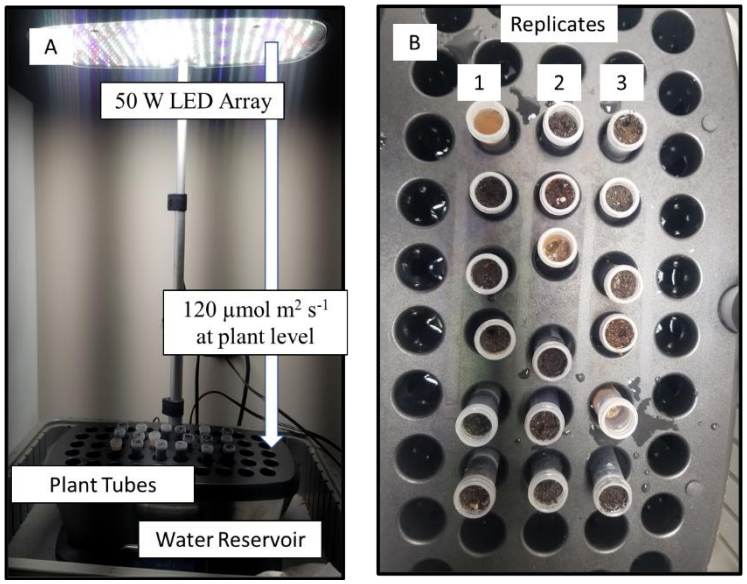

Figure 3 A: Test stand as configured with three replicates of conical growth tubes, 50-Watt broad spectrum LED lighting and common 5 Liter water reservoir of the AeroGarden™ test stand. B. Conical tubes with media were randomized in the

J. Environmental Test Stand

The environmental control test stand consisted of a commercial counter-top hydroponic plant growth system (Aerogarden Bounty Model, MiracleGro, Marysville, OH) with a seed starter insert on the hydroponic reservoir. The conical tube growth systems were placed into the test stand, with the holes of the tube ~1 cm into the reservoir. All tubes shared the continuously aerated water supply for the duration of the test.

Lighting was provided with a 50W LED array of blue, white and red LEDS on a 16-hr. light/ 8 hr. dark cycle at 120 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) at the plant surface to provide 6.9 Moles of light per day. This is a photoperiod and light intensity that correspond to spectral conditions utilized in lettuce testing for space missions (Stutte et al. 2009; Massa et al., 2015)

The spectra consisted of 26.6% blue (400-500 nm), 37.4% green (500-600 nm), and 36.0% red (600-700 nm) wavelengths of photosynthetically active radiation (PAR) and ~.1% of ultraviolet (380-400 nm) and 2% of far-red (700-780 nm).

The conical plant growth tubes containing the various substrates were place into the center section of the planting module to ensure uniform lighting across the treatments. (Figure 3). The test stand was maintained under ambient temperature, relative humidity and CO_2 concentration in the SyNRGE, LLC laboratory in the Space Life Science Laboratory in Exploration Park, Florida. The nominal conditions were 20°C (+/- 1°C) and 60% Relative Humidity (55% +/ 5%).

The nutrient reservoir was filled with distilled water and maintained at a constant level throughout the tests.

III. Results

A. Vermicomposting

The culture conditions enabled the growth and maturity of the worms in all cases, and both horizonal and vertical migration of the worms in search of food was observed (Figure 4). The vertical migration generally occurs when competition increases for food (available organic material) and is a biological indicator that the bioconversion of biomass is occurring.

A segregation of habitation of the adult worms and wormlings (young worms) within the vermicomposter was observed. The basis and characteristics of why this is occurring is not known but will affect the overall management strategy of composter to ensure that both mature and juvenile worms are maintained in the system.

1. Vermicompost Composition.

Nutrient composition of the each of the batches was determined. and data from Batch 1 and Batch 2 are given in Table 2 and compared to the MGS-1 and commercially available castings. The overall changes in composition suggest that vermicomposting with the MSG-1 results in a final product that is comparable to commercially produced worm castings.

The stored MGS-1 vermicompost (Batch 1) had higher Phosphorus, Chlorine, and Magnesium levels but lower Ca levels than the control treatment. The stored MGS-1 Vermicompost (Batch 1) had a Ca level comparable to that of the MGS-1. The colorimetric reading suggests that this product is of relatively high quality, but these values need to be validated with more precise quantitative analysis.

Figure 4 Red Worms (E. fetida) reproduced and migrated freely through the vermicomposting reactor.

Table 2: Colorimetric nutrient analysis of MGS-1, Commercial castings, and MGS-1 vermiculture batches.

	MGS-1	Commercial (Control)	Batch 1 (stored)	Batch 2 (Active)	Batch 2 (finished)
NO_3-N (ppm)	5	75	75	75	75
P (ppm)	.5	50	100	75	75
K (ppm)	18	90	70	110	200
Al (ppm)	54	5	5	5	5
NH3-N (ppm)	286.7	5	0	5	1
NO2-N (ppm)	110	1	1	1	1
Ca (ppm)	2083.3	3500	2800	100	1400
Cl (ppm)	---	25	100	50	0
Fe2+ (ppm)	7.5	0	0	2.5	0
Mg (ppm)	43.3	12.5	5	5	5
Mn (ppm)	---	5	5	0	0
S (ppm)	2000	50	50	1000	1000

Analyses of Batch 1 and Batch 2 show the transition and bioweathering of MGS-1 during the vermicomposting cycle. In the transition from Batch 2 active to Batch 2 finished, the N and P concentrations are unchanged (at upper limit of assay), but there is doubling of the K content of the media. The most significant modification of composition is the increase in Ca from 100 ppm in Batch 2 active to 1400 ppm in Batch 2 finished.

2. MGS-1 Vermicompost Maturation

Basil respiration rates of soil and availability of labile amino-N are measures of soil health. This value was estimated using the Sovita CO_2 Burst and SLAN protocols. The characteristics of the four batches of MGS-1 Vermicompost are compared to the commercial control and MGS-1 in Table 3. The data are presented in both Solvita numbers and the appropriate units. In addition, the physical properties of the vermicompost such as bulk density, gravimetric and volumetric water content are presented. As expected, there is no respiratory activity detected in the MGS-1 regolith since there is no organic matter present. In addition, there is no free water associated with dried regolith.

The results indicate that while the basal respiration rates of the four MGS-1 verticompost batches are comparable to commercial worm castings, there is a significant variation in the SLAN values. This is associated primarily with Batch 1, which is just 1/3 of the control although the SLAN Color value was 3 times higher. CO_2 emissions and maturity index of all four MGS-1 verticompost batches were higher than the commercial control. The bulk density of the compost ranged from 0.22 to 0.41 gm/cc. This variation is dependent on the compression factor of the media, but are clearly less dense than the MGS-1 itself, which has a density of 1.7 gm/cc. The overall assessment is that the vermicomposting of the MGS-1 using the inedible biomass from the ILMH resulted in a stable, high-quality compost that is equal, or better, in quality than the commercial control.

Table 3: Solvita color number results for soil respiration, compost maturity, and labile amino-nitrogen of the four batches of MGS-1 verticompost, a commercial control product, and MGS-1 regolith.

Compost Health Parameter	Batch 1	Batch 2	Batch 3	Batch 4	Control	MGS-1
CO_2-Basal (#)	4.76	4.72	4.81	4.89	4.64	NT
Basal Respiration (ppm)		41.3	44.4	48	38.6	NT
CO_2-Burst (#)	3.55	3.93	4.6	4.68	4.35	3.68
CO^2 Respiration (ppm)	4.76	63.9	115	123	92.6	51.4
SLAN color #	3.54	1.25	2.18	2.09	1.09	4.57
SLAN- labile amino-N, mg/L	167.5	447.5	335	345	467.5	20
Compost emissions: CO_2-hi (#)	7.76	7.64	7.15	7.15	5.86	NT
NH_3 color (#)	5	4.68	4.6	4.62	4.65	NT
Maturity index (#)	7.4	7.1	6.6	6.6	5.6	NT
SBD _dry (g/cc)	0.35	0.41	0.27	0.22	NT	1.73
Field density (g/cc)	0.6	0.7	0.76	0.72	NT	2.04
GWC(g/g)	1.86	1.43	2.55	2.26	NT	NT
VWC (g/cc)	0.65	0.59	0.69	0.5	NT	NT
WFPS(%)	74.9	69.8	76.8	54.4	NT	NT
SP (g/cc)	0.87	0.85	0.9	0.92	NT	NT

Abbreviations: CO_2-Carbon dioxide; NH3- ammonia; SLAN- Solvita Labile amino-N; SBD- Soil Bulk Density ; GWC-Gravimetric water content ; VWC-volumetric water content ; WFPS-Water filled spore space ; SP-saturation percentage.

These results suggest that vermicomposting is a viable technique for the bioconversion and nutrient enrich of regolith and holds promise as tool to enable ISRU for crop production.

B. Plant Biocompatibility

1. Phytotoxicity of Regolith Simulant MGS-1 leachate

Two separate lots of MGS-1 regolith were received and phytotoxicity determined using the Cress seedling bioassay with 20 seeds per treatment. Treatment consisted of Lots 1A, 1B, 1C and 2 were assessed different dilutions and effect of sterilization. The unprocessed concentrated MGS-1 prevented germination, irrespective of treatment.

Although variation was high, there was a clear reduction in radical length unless the concentration of leachate was reduced an order of magnitude (data not shown).

It was concluded that the leachate concentrates exhibited phytotoxicity, but that it did not completely inhibit germination and that the effect could be overcome with dilution.

2. Germination/Seedling Establishment

No germination of seeds occurred in the MGS-1 treatment after 7 days. Excellent germination occurred in the remaining treatments after 10 days. Germinated seeds in Batch 1 (immature compost) were severely stunted and failed to thrive at 10 days after harvest, and had died, with no survivors, at harvest (Figure 5).

Figure 5 Germination and seedling growth of L. sativa cv. Grand Rapids at 3 (Left)and 10 (Right) days after planting.

3. Plant Growth

At harvest, there were no plants in the MGS1 or Batch 1 treatments. There was normal growth of lettuce in the vermicomposted Batches 2, 3 and 4 as well as in the control treatment (Figure 6).

Although there was good germination and growth of the vermicompost system with Batch 2, 3, and 4, the growth was 24 to 30% shorter than the Commercial Potting Mix control (Figure 7) and 37 to 50% less fresh mass than the commercial potting mix (Control) treatment (Figure 7).

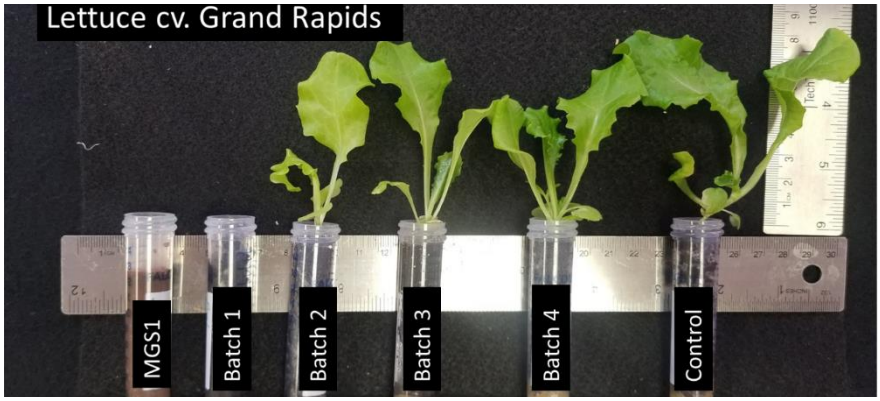

Figure 6: Growth of Lettuce cv. Grand Rapids on MGS-1, MGS-1 vermicompost and commercial potting mix. MGS-1=Martian Global Regolith-1 without supplementation; Batch 1 Immature vermicompost with 15% MGS1; Batch 2= Mature regolith with 26% MGS 1; Batch 3= Mature regolith with 11 % MGS 1; Batch 4= Mature regolith with 17% MGS 1; Control= Miracle Gro Promix without MGS-1.

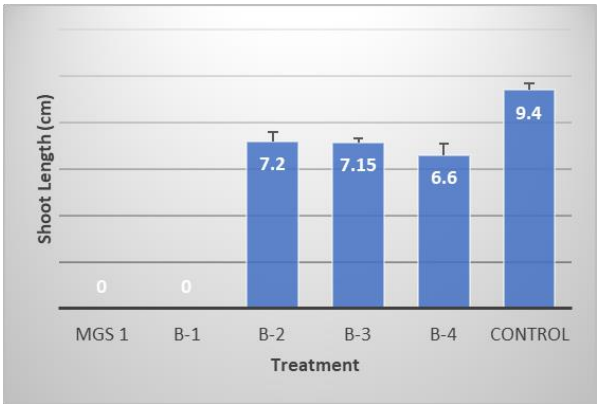

Figure 8 Shoot length (cm) of lettuce cv. Grand Rapids on MGS-1, vermicompost from UND ILMN and commercial potting mix.

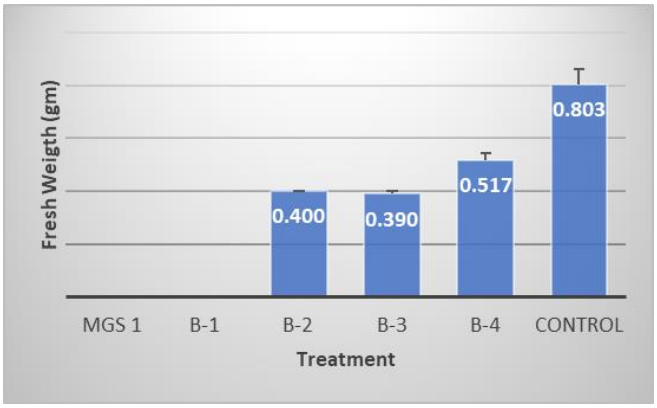

Figure 7 Fresh Weight (gm) of lettuce cv. Grand Rapids on MGS-1, vermicompost from UND ILMN and commercial potting mix

4. Root Growth

Because of the high level of loss in recovering and removing roots from the rooting media, no data were taken on root growth *per se*. However, visual inspection of the root mat showed no obvious morphological or developmental differences in the root structure within the tubes (Figure 9).

Figure 9 Root mat development in the Control (MiracleGro™ Potting mix) and vermicomposted media containing 17% MGS-1 Martian Simulant.

IV. Discussion

These experiments suggest that bioprocessing Martian regolith though a vermicomposter can enable its use as an *in-situ* resource to support plant growth. The addition of MGS-1 into the vermicompost, at concentrations ranging from 10% to 25% total mass, did not appear to affect the maturity or stability of the resulting compost material. The resulting material had comparable water holding capacity and bulk density as commercial grade worm castings.

These promising results demonstrate that vermicomposting is a viable means of bioprocessing MGS-1, which supports plant growth poorly if at all, into a viable component of a plant substrate. Passing Mars regolith through the gut of the worms may be means of extracting inorganic elements and converting them into useful form for plant growth. This process is compatible with nutrient recovery approaches from inedible biomass produced by the crew. The resulting nutrients can either be extracted and used in hydroponic food production systems or incorporated into a substrate-based production.

The resulting composted material is biologically diverse, containing a variety of bacteria, fungi, yeast, and protozoa, in addition to the worm population. This microbial and biological diversity is part of the 'food-web' that exists within biological waste processing systems. Any attempt to maintain a completely sterile environment in systems that interact with humans, is futile and, in many cases, can be counter-productive. Inoculation of a growing media, or vermicomposting bed, with microbes that are beneficial to plant growth helps the soil outcompete human associated pathogens. Preliminary results from the ILMH Mission VIII suggests that, under conditions of low microbial diversity in the media, it becomes enriched with organisms that survive the worm's gut.

While these results are promising, several caveats need to be considered. First, the microbial and biological diversity within the vermicomposter, which is responsible for the bioconversion of the MGS-1 regolith, is not well defined. In these studies, we intentionally inoculated the media with commercially available products. In preliminary studies, we had utilized a commercial potting soil that had been inoculated with endomycorrhiza as a bedding material and the resultant population was unknown. During subsequent experiments, we prepared our own media and inoculated with a variety of beneficial microbes. Growing media used during UND ILMH Mission VIII was prepared inhouse form sterile materials, and then inoculated with a host of beneficial microbes. The commercial microbial inoculants contained ectomycorrhiza (e.g., *Rhizopogon* sp., *Scheroderma* sp.) endomycorrhiza (e.g., *Glomus* sp., *Paragomus* sp., *Sclerocystis* sp.) and beneficial bacteria (e.g *Azotbacter* sp., *Bacillis* sp. *Paenibacillus* sp., *Pseudomonas* sp,). These inoculants, which are essential components of a soil-based media, were included to simulate 'soil-like' conditions. However, the introduction of these organisms makes it difficult to differentiate the role of specific microbes in the soil from the role of the passage of regolith though gut of the *E. fideta* on nutrient mobilization. We were unable to complete a thorough assessment of the microbial load throughout the production process due to COVID-19 constraints.

However, it is anticipated that any biological processing of organic waste material on a Mars base, with or without vermicomposting, will be microbially based, and thus any vermicomposting system must be compatible with a microbially diverse rhizosphere. Future studies of soil, human, and surface microbiomes would help isolate worm species that could thrive and benefit the development of intentional consortiums designed for agriculture and human health.

In addition to microbial inoculants added to the media, we added biochar and zeolite to the matrix to minimize potential odors in the habitat module. We also added a compost starter material to the initial batch to speed up compost maturation. Further effort is needed to define whether these additives played a significant role, positive or negative, in the vermicomposting process and the bioprocessing of the MGS-1 regolith.

These experiments are focused on the biological processing of Martian regolith to provide nutrients for plant growth. However, there are other ancillary biological processes where vermiculture can play an important role in the establishment of a sustainable life support system. These could include the utilization of the regolith enrich media as component substrate for mushroom culture which will facilitate the breakdown organic material with a high lignin content. Similarly, effluent from an aquaculture system can passed through a vermicomposter to convert NH_3 to NO_3. These components are all compatible with a biological life support system.

V. Conclusions

Results indicate that vermicomposting of inedible biomass with *E. fetida* in Martian regolith is a viable technology for use in a closed ecological life support system (CELSS). *Eisenia fetida* can be maintained in dormant condition,

consume a wide range of organic material, and require limited volume to be effective. Optimal environmental setpoints are like those of crop growth requirements, and establishing a self-replenishing population eliminates resupply cost. Vermicomposting for bioremediation of Mars regolith has enormous potential for future Martian colonists through reduction in payload mass and a regolith-based agricultural system. Identifying a technology gap, this project has demonstrated the feasibility of using biological processing of inedible biomass through vermicomposting to reduce waste volume, enhance quality of regolith and recycle and replenish nutrients. There are several key take-aways from the biocompatibility testing performed at SLSL;

- Lettuce seeds did not germinate in the MGS-1 regolith simulant media alone.
- Lettuce seeds germinated in the Batch-1, which contained ~15% MGS-1, but the seedlings failed to develop. This is most likely due to incomplete composting of the organic material since the SLAN color number was nearly 2X that of Batches 2, 3 and 4, and the SLAN-labile amino-N concentrations (mg/L) were less than ½ the later batches.
- Lettuce seeds were able to germinate and grow in Batches 2, 3 and 4 from the UND ILMH missions that contained from 10 to 25% MGS-1. There were no obvious nutrient deficiencies associated with the plants grown on biologically processed MGS-1 media in these batches
- Fresh weight of the harvested plants was ~70% of that achieved with a commercial potting mix that was supplemented with slow-release fertilizer.

Overall, these results are promising. The organic material that provided food for the vermiculture was obtained from waste material produced by the UND ILMH crews, and a relatively high concentration of MGS-1 (25%) could be added without having an apparent negative impact on growth. There was also a rich microbial community that developed within the rhizosphere that has potential to increase the bioavailability of nutrients from the Martian Soil over longer periods of processing.

Still, several factors need to be understood before vermiculture can be recommended as technique for *in situ* processing of Mars regolith. These include, but not limited to, the following:

- Establish the percentage of regolith that can be added to vermiculture feed stock.
- Determine the stability of the resulting vermicompost during storage.
- Identify interactions of regolith with different sources of organic feedstock.
- Determine biocompatibility with wider range for Mars candidate crops.
- Assess the stability of worm eggs to conditions of long duration space flight.
- Quantify nutrients mobilized by the worms during the passage through the gut.
- Develop techniques for assessing maturity of compost.
- Optimize composting duration and conditions.
- Determine operational range of environmental conditions within habitat.
- Develop techniques for processing, harvesting, and drying vermicompost.

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