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# Transforming Martian Regolith with Black Soldier Flies, *Hermetia Illucens*

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## Abstract

**The sustainability of long-term Martian colonization hinges on the ability to produce food from the soil of the planet. Current space missions rely on pre-packaged food, a model unsuitable for extended missions to Mars due to mass, supply, and logistical constraints. This study explores the feasibility of Black Soldier Flies, *Diptera:Straitomyidae*; as a soil fertility enhancer via composting organic matter. We designed a 3D printed, compact BSF composter to process Martian soil simulant into arable soil. Additionally, we subjected BSF larvae to G-force testing and demonstrated high survivability under rocket launch conditions and centrifugal forces of up to 40 G's. Composting experiments involved introducing BSF to Martian soil simulant and an organic food source (mung beans) over a one-month composting duration. Soil analyses showed significant nutrient increases, notably in the plant-availability of potassium (+1179%), magnesium (+67%), phosphorus (+803%), and manganese (+7847%), enhancing the soil's suitability for crop cultivation. These findings underscore the resilience and potential of BSF for extraterrestrial agriculture. This study lays the groundwork for BSF-based composting systems as a sustainable solution for soil fertility management and nutritional supplementation on Mars, fostering future research on astro-entomology and in-situ resource utilization.**

***Keywords: Hermetia illucens; Martian regolith; Black Soldier Flies (BSF); Mars colonization; Martian soil***

## Introduction

Yuri Alekseyevich Gagarin was the first human to venture into Space, and on his mission, he brought pureed beef and liver paste (Uri, 2020). He demonstrated eating and swallowing is possible in zero-g environments, despite the food coming from an aluminium tube (Uri, 2020). Subsequently, the 2-weeks-long Gemini 7 mission required more care to be taken with regard to dietary and caloric requirements, while also remaining in an extremely tight mass envelope. This envelope only allowed 0.77kg of food per astronaut per day. Because of this the food taken ended up consisting almost exclusively of dehydrated food that most closely resembled military survival rations. This was acceptable for the Gemini missions, as their primary purpose was to ensure man could survive for an extended duration in a space environment. This envelope; however, was determined to be inadequate for the Apollo missions because it was too small to maintain astronaut weight and there were undesirable physiological and psychological responses to consuming dehydrated food for two weeks straight. Though the Apollo program introduced significant improvements to the astronaut's culinary experience, including the introduction of cheese, sausage, and scrambled eggs, the majority of astronauts did not consume sufficient nutrients. Astronauts aboard the ISS today have much-improved meals, even having specialty food items such as sushi, burgers, turkey, and pizza. Despite this, fresh fruits and vegetables are always in demand as they have limited shelf lives. The ISS is restocked multiple times a year, which allows for the large and varied dietary menu that astronauts enjoy. The ISS can be restocked so often because of its proximity to Earth and the relative ease and low cost of resupply missions. This will not be the case with future Lunar and Martian colonization efforts [1-7].

The large distances and high cost of Lunar and Martian missions mean resupplies must be kept to a minimum. This is coupled with the need to reduce mass wherever possible due to the larger Delta-V requirements for these missions. Extraterrestrial colonization efforts must be as self-sufficient as possible to reduce the need for constant resupplies, and any supplies taken must be lightweight to fit into strict mass envelopes. This means the pre-packaged sustenance model used for all previous missions is no longer acceptable in the new age of space exploration. The ISS food envelope is too large, and the Gemini envelope is both physically and psychologically unsustainable. For future colonization efforts to be a success, astronauts must be capable of growing and producing their own food on whatever celestial body they aim to inhabit [7-22].

The need for agriculture in space has been recognized by NASA, and they have begun the veggie food production system to experiment with growing vegetables aboard the ISS. The project has successfully demonstrated that leafy greens, such as kale and mustard, can be grown and consumed aboard the ISS. While these experiments pave the way for growing crops beyond Earth, they alone are insufficient to begin sustainable agriculture on foreign bodies. The Veggie program grows its crops in soil taken from Earth and uses artificial fertilizers to feed the produce. The use of artificial fertilizers itself is not a bad thing, but if used alone they represent an increase in mass that needs to be transported for the colonization effort. Additionally, it currently takes an average of roughly 5 Hectare, or 50,000 $m^2$ of arable land to feed 100 men. It is unreasonable to expect to take this amount of soil to the Lunar Surface, let alone Mars. This means that astronauts will need a way to convert Lunar and Martian regolith into farmable soil similar to Earth. We believe the fertilization and the soil conversion problems may be alleviated with the usage of Black Soldier Flies, Hermetia illucens [22-38].

Black Soldier Flies (BSF) are acclaimed for their ability to compost organic matter. BSF compost can be used as an organic fertilizer that reduces or eliminates the need for chemical fertilizers. Composting also significantly reduces the health concerns associated with direct application of manure or biological waste [24]. We believe that a lightweight, transportable BSF soil composting system, could be taken to space or celestial bodies to assist with the fertilization of arable soil and convert extraterrestrial regolith into farmland bit by bit. Additionally, regarding astronaut nutrition, BSF can be consumed for additional protein and basic nutrients, especially considering their larvae can yield above 40% protein and ~30% fat. Black Solider Flies have amino acid profiles comparable with common meats such as chicken and could be used as a more feasible alternative to bringing livestock to the Martian surface. Our goal was to compost Martian soil simulant through the usage of 3-D printed BSF composters to increase the nutrient contents of the simulant. This initial system aims to add to the sustainability outlook of future colonization efforts directed towards Mars [38-47].

## Materials & Methods

Experimentation with BSF comprised primarily two arms of research: the BSF's endurance under high-G stress and BSF ability to effectively compost extraterrestrial regolith within a one-month duration. The high-G testing consisted of several different experiments using centrifuges to induce equivalent conditions to different launches as well as a real-flight Tripoli Level 1 rocket test.

### Black soldier fly colony

BSF colonies were purchased from a commercial insectary. G-force experiments utilized $5^{th}$ instar BSF measuring ~10 mm in size. BSF were purchased and allowed to grow naturally in initial container until approximate $5^{th}$ instar utilization. Composting experiments utilized $1^{st}/2^{nd}$ instar larvae ~5 mm in size. Small larvae ~5 mm in size were selected upon arrival for utilization.

**G-Force centrifuge assessment & tripoli level 1 rocket test**

The high-G testing consisted of several different experiments using centrifuges to induce extraneous G conditions. A Heraeus Multifuge X1R (ThermoFisher Scientific) centrifuge was used for BSF head-up and head-down g-force tests. Centrifuge G-force testing involved placing individual BSF 5$^{th}$ instar larvae ~10 mm and pre-pupa BSF ~15 mL in 1.5 mL conical tubes and spun at either 10 G, 25 G, or 40 G for a 5-minutes spin duration followed by a 10-minutes survivability check. Ten individuals per g-force with three replicates were assessed. The linear G-force assessment utilized a Tripoli Level 1 rocket test. Specifications for the rocket include an AeroTech H283 ammonium perchlorate-based rocket motor with a burn time of 0.7 seconds. The motor produces a peak thrust of 325N, an average thrust of 283 N, and a total impulse of 201 N × s. The airframe tubes of the rocket were constructed from 0.889mm- thick cardboard tubing, the fins were manufactured using 3.175-mm -thick plywood, and the nosecone was 3D printed using PETG filament. The nosecone was printed with a thickness of 6.35-mm, 100% infill in the shoulder and tip, 25% infill in the rest of the nosecone, and 3 outer walls. The launch was conducted at the South East Alabama Rocketry Society (SEARS) launch site in Samson, Alabama. The parachute used had a diameter of 0.762 meters and was constructed from ripstop nylon. Contained within the payload section, eight biological replicates for head-down and nine biological replicates for head-up were used each oriented in their own individual holding 1.5 mL angled tubes. Additionally, three replicates of five larvae were combined in 2 mL tubes. The BSF tubes were secured in a specially 3d-printed and designed payload module called the Biological Unit for Gravitational Study (BUGS). It consists of four sections, can hold twenty-four individual 2 mL centrifuge tubes, as well as the Altus Metrum EasyMini computer. The four sections of BUGS are the battery container, the Sample and Electronics Enclosure, the Upper Sample Enclosure, and the Sample Enclosure Cap, and each was 3D printed using PLA filament. The Battery Container housed the 9 V battery used to power the EasyMini, and the Sample and Electronics Enclosure contained 12 mL tube slots and the EasyMini computer. Above that, the Upper Sample Enclosure housed an additional 12 mL tube slots, and the Sample Enclosure Cap retained these tubes. This system was secured using a ¼”-20 steel threaded rod through the center of each of the four sections. For the test flight, BUGS was placed within the payload tube of the Tripoli Level 1 rocket. The Altus Metrum EasyMini computer took real flight data during the launch through landing.

**Composter cad design and function**

The BSF composter consists of several compartment drawers contained by a main chassis **FIG. 1**. Access from one compartment to another is restricted by several slides that can be inserted or removed when it is desirable for access to be denied or granted. The BSF larvae begin in the compost tray, which contains soil and beansprouts to sustain the BSF through their larval stage **FIG. 1A**. The rear of the compost tray contains a ramp which the larva will climb up and out of the compost tray once they have reached older instar stages and the slots are pulled open **FIG. 1E**. The tray also contains a removable bottom, which can be pulled out to drop the contents of the composter tray into the tray below it for extra storage **FIG. 1C**. Once the larvae have developed to an appropriate stage (prepupa), they will move up the ramp at the rear of the compost tray and into one of two slot channels. Access to these channels is restricted by slides that may be inserted or removed if desired to limit the larvae from progressing until they have sufficiently developed. The larger drop section leads directly to the larval collection tray **FIG. 1D**, which contains no soil or nutrients and from which the larvae cannot escape, to be held until they are collected for harvest or set for re-use with new soil. The smaller slot leads to the ‘recycling track’ in which the larvae will remain in the composter and develop into adults as the angular, funnel shaped bottom is designed to make escape difficult. Following pupation, the winged adults can fly up into the top breeding chamber where they may reproduce allowing for continued colony propagation within the composter. Once an adult has reached the adult breeding chamber, the design allows mating and the laying of eggs in one of the egg laying cartridges fitted inside the breeding compartment. These cartridges contain many small holes designed for the flies to deposit eggs into and may be removed for ease of cleaning or to be replaced with a cartridge of a different design if desired. The bottom of the breeding compartment is perforated with a number of holes ~2 mm for a new larva to fall through but too small for an adult to fall through. The design is such that new larvae may exit the breeding compartment and fall though into the composting compartment, where they may begin the cycle again. Access to the composting compartment through these holes is controlled by a slide contained by the chassis underneath the breeding compartment. The breeding compartment is covered by another slide, which may be made out of a clear material in order to provide a view into the compartment for inspection and also allow light to enter the breeding chamber. This aids in manual visualization of adults within the top bug breeder compartment.

**Manufacture**

Fused Deposition Modeling (FDM) 3-D printing was chosen as the method of manufacture for the composters. This provided a low cost, ease of manufacturing, rapid prototyping and iteration, and allowed the creation of complex internal geometries not possible with other manufacturing methods. The multiple internal channels and passageways in the main chassis of the composter would have been unreasonably difficult to machine in a single frame using traditional subtractive manufacturing. The large chambers on the front top and bottom rear edges of the otherwise cubic chassis are so that it may be printed at a 45° angle. This minimized the amount of bridging and support material that needed to be generated by the printer, reducing production time and improving build quality. Polyethylene Terephthalate Glycol (PETG) filament was chosen as the material for the composter to be made from. This material was chosen for its low cost, wide availability, ease of printing and resistance to degradation. Compared to other common 3-D printing filaments, namely Polylactic Acid (PLA), PETG exhibits much greater resistance to degradation from both moisture and UV light. This was critical as the composter will contain moist soil and bio-matter, and it is desirable for it to be able to be operated in direct sunlight. In addition, PETG is more heat resistant, and would be less likely to deform if exposed to high temperatures and sunlight for extended periods of time.

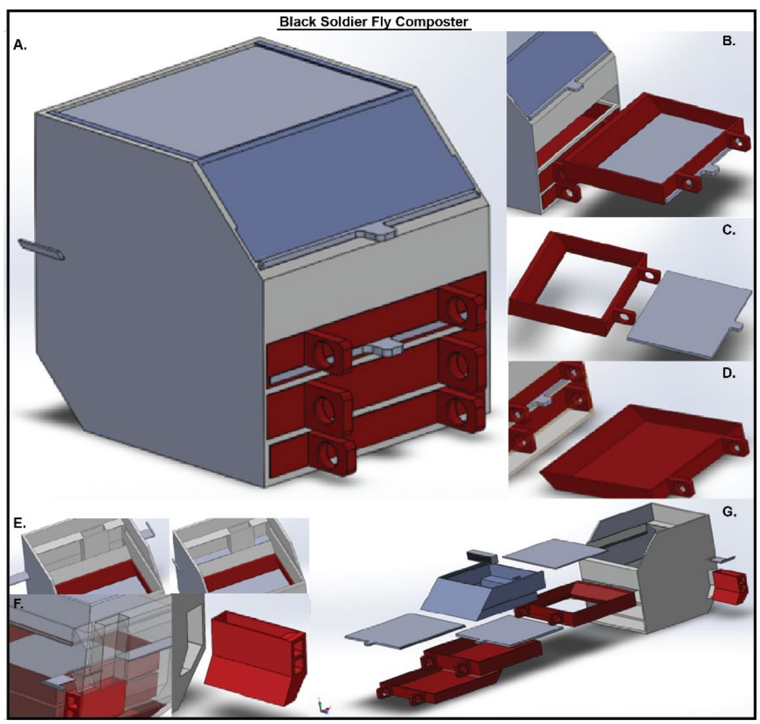



FIG 1. **Black Soldier Fly Composter. This figure depicts the SolidWorks design for the BSF composter. A. Enclosed total cube-sat composter. B. Primary soil draw for soil, BSF larvae, and food source (bean sprouts). C. Removable slot to allow soil to drop down to drawer 2 for soil storage. D. This is the bottom pupa collection drawer where larvae fall to pupate following slot opening. E. Inside view of composter where two closable slots lead to either bottom pupa collection draw**

**(left), or recycling track. F. Visual of recycling track internally and removed. This compartment traps larvae with inverted funnel design preventing escape until pupation where adult flies can fly up to top section. G. Visualization of opened composter with top of composter (breeding chamber) visible. The breeding chamber is the angular top compartment which houses adult flies for breeding and egg deposition into rectangular egg deposition cartridges. The top of the breeding chamber is outfitted with clear PETG lid for visualization of adult flies.**

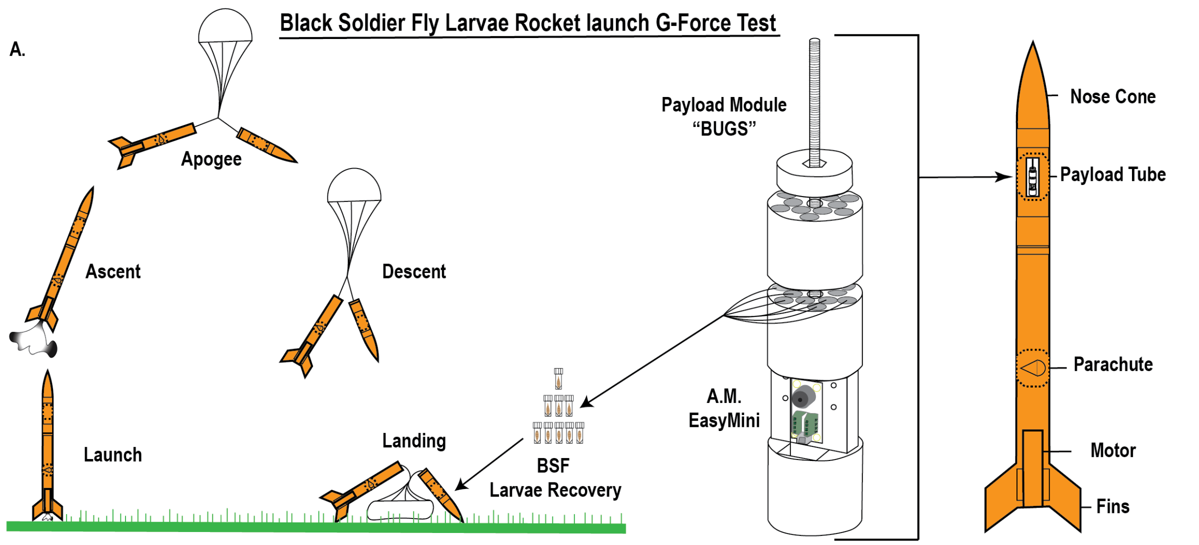
### Composting and soil data acquisition

Composting experiments consisted of 12 total BSF composters. Composters were introduced to 300 larvae each with 100g of beansprouts (LEASA) initially and 100g more bean sprouts on the beginning of the 2nd week of the experiment. Six composters utilized Alabama Control Soil (ACS) and six utilized MMS2 purchased from The Martian Garden. ACS was collected from (32.59410°N, 85.48267°W) then baked for 24 hrs at (204°C) for sterilization and total drying. ACS was then sieved finely and stored in a sealed tub until use. Fifty g of each soil type was given to Auburn University Soil Testing Laboratory (AUSTL) for Mehlich 1 and total element extraction. Each composter was introduced to 150 g of soil type, 100 g of crushed bean sprouts (LEASA), 300 1st/2nd instar larvae ~5 mm in length, and 50 ml of initial DIW. Each composter was misted once weekly during the duration of the experiment with 3 mL of water. Composters were stored at (28°C) and (70%) for 4 weeks. On day 14, 100 more grams of bean sprouts were added to each composter for BSF consumption. At the start of the 3rd week of composting both slits **FIG 1C** were opened to allow BSF self-sorting. Upon one-month composter completion, soil was removed from the composters and weighed. Larvae were allowed to remain in the composter with no additional food or water until total death. Carcasses were then counted and assessed. Fifty g of soil from each composter was sent to AUSTL for Mehlich 1 and Total Element Extraction. Following soil extraction one-way t-test analysis were performed comparing initial soil reading values to the six replicate soil values per soil type.

## Results

### BSF G-force testing

G-force testing BSF is crucial to preliminary feasibility of composting on Mars, as BSF must first be able to survive the G-force of a rocket. Initial G-force testing of 5th instar BSF resulted in both head-up and head-down surviving at 100% after a 5-minute centrifuge time at 10, 25, and 40. Only 2 pre-pupa individuals out of the total 90 pre-pupa tested did not respond following testing. Following the centrifugal G-force assessment the linear vertical G-force test was conducted **FIG 2A**. Due to size limitations of the level 1 rocket and payload size constraints, eight biological replicates for head-down and nine biological replicates for head-up were used each oriented in their own individual holding 1.5 mL angled tubes. Additionally, three replicates of five larvae were combined in 2 mL tubes. The rocket launch lasted 36.4 seconds from launch to landing and resulted in a maximum height of 585.0 meters (1919 feet), a maximum speed of 124.5 $m/s^2$ (408 fps), and a peak g-force of 6.74 G (**FIG 2B** and **2C**). Upon descent it was noted our parachute become tangled leading to a faster descent than anticipated, with the final 10 seconds of decent resulting in ~225 m loss in altitude. As a result, the nose cone penetrated the soil ~7cm into the sod field topsoil. Upon BSF recovery, all tubes and larvae were recovered. One 2 mL containing 5 larvae had a cracked lid from the impact resulting in a singular larva to be ejected through the payload tube and into the nose cone. That larva and all other larvae were successfully recovered and were alive. All larvae survived initially and were still alive even one week post launch. Both centrifugal 10 G to 40 G and linear vertical 6.74 maximum G testing demonstrated total survivorship of all BSF larvae following g-force exposure.




B.

| | | | |
|---|---|---|---|
| **Device** | EasyMini-v3.0 | Version 1.9.17 | Serial 10032 |
| **Flight** | 5 | | |
| **Maximum Height** | 585.0 m | 1919 ft | |
| **Maximum Speed** | 124.5 m/s | 408 fps | Mach 0.4 |
| **Maximum boost acceleration** | 65.9 m/s² | 216 m/s² | 6.72 G |
| **Average boost acceleration** | -7.4 m/s² | -24 ft/s² | -0.75 G |
| **Ascent time** | 1.9 s boost | 8.4 s coast | |
| **Drogue descent rate** | 21.8 m/s² | 71 ft/s² | |
| **Main Descent time** | 22.5 m/s² | 74 ft/s² | |
| **Descent time** | 16.3 s drouge | 9.9 s main | |
| **Flight time** | 36.4 s | | |

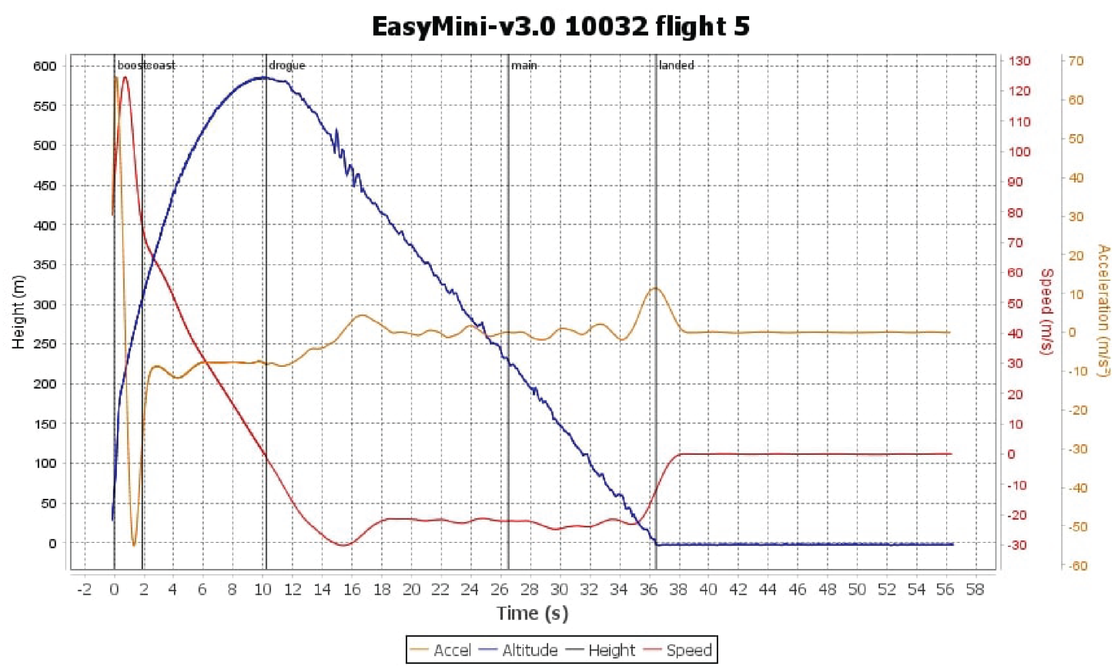



FIG 2. **Black Soldier Fly Larvae Rocket launch G-force Test. A. Visual representation of the rocket launch containing the custom 3d-printed BSF Payload Module which housed our experimental BSF larvae for testing within the payload tube. B. EasyMini-v3.0 real time date collected during the rocket launch through landing. C. EasyMini-v3.0 visualized flight data rocket launch.**

### Composting soil analysis

Two soil analysis were conducted, Mehlich 1 extraction and Total Element Digestion. Mehlich 1 extraction is useful for assessing how nutrient content that be up taken by a plant/crop, while Total Element Digestion assesses the total composition of elements within the soil. When comparing the Mehlich 1 for Alabama Control Soil (ACS) to the 6 ACS composted replicates, the one-month BSF composted ACS revealed statistically significant differences in Calcium, Potassium, Phosphorus, Aluminium, Boron, Magnesium, Manganese, Sodium, Zinc, Nitrogen, pH, and organic matter **FIG 3A**. Only Copper and Iron from the composted ACS did not statistically differ. Calcium, Phosphorus, Aluminium, Boron, Iron, Manganese, and Zinc went down, while Potassium, Magnesium, Copper, Sodium, Nitrogen, pH, and organic matter went up. When comparing the Mehlich 1 for MMS2 (MMS) to the 6 MMS composted replicates, one-month composted MMS revealed statistical significance regarding Calcium, Potassium, Phosphorus, Aluminium, Boron, Magnesium, Manganese, Copper, Iron, Zinc, Nitrogen, pH, and organic matter, with the only non-significant element change being Sodium. MMS composting resulted in an increase Mehlich 1 change in Potassium, Magnesium, Phosphorus, Copper, Manganese, Nitrogen, and organic matter **FIG 3A**. When comparing the composted Mehlich 1 differences among ACS to those of MMS the composting process resulted in MMS having an increase in Mehlich 1 of phosphorus and Manganese compared to MMS control, while ACS composting resulted in the opposite with Phosphorus and Manganese decreases compared to ACS control. The primary take away from MMS Mehlich 1 testing is Potassium change in Potassium by 1179%, Phosphorus by 803%, Magnesium by 67%, and Manganese by 7847%. Regarding the Total Element Digest, both ACS and MMS saw statistical significance in post composting soil composition compared to their pre-composting soil composition in Calcium, Potassium, Magnesium, Phosphorus, Aluminium, Arsenic, Boron, Barium, Cadmium, Chromium, Iron, Manganese, Molybdenum, Sodium, Nickel, Lead, and Zinc. The only non-statistical total element digest change for both was in the element Copper (**FIG 3B).** Both ACS and MMS post composting shared increases in Potassium, Phosphorus, Arsenic, Boron, Cadmium, Copper, Iron, Molybdenum, and Nickel. Both ACS and MMS shared decreases in Calcium, Magnesium, Barium, and Manganese. MMS post composting saw increases in Aluminium, Chromium, Lead, and Zinc, while ACS post composting saw a decrease. Only Sodium increased in ACS post composting compared to MMS decrease post composting. Key take aways from total element digestion of MMS2 post composting revealed a 132% increase in Potassium, 82% increase in Magnesium, and 26% in Zinc. Both soil data types reveal successful one-month composting as soil compositional change occurred statistically throughout both soil composting types.

Additionally, upon soil recovery, the mean weight for ACS was 157.8 g and 158 g for MMS2. Adding a total of 8 g more soil mass than the starting 150 g of each soil type. Following the experimentation period and allowance for total death, a mean of 261.17 larvae from ACS and 267 larvae from MMS2 were recovered. Surprisingly despite the removal of food and soil after the 4 weeks, 40 adult carcasses from ACS and 7 from MMS2 composters were later recovered. This is of note as it typically takes a black soldier fly ~45 days to reach adulthood. Larvae only fed for 4 weeks with some being able to reach adulthood demonstrates the resilience of BSF and warrant for future longer-term composting testing to assess BSF maturation with a continuous food source.

## Mehlich 1 & Total Element Digenstion Post-Composting Data

### A. Post-Composting Mehlich 1 Soil Data

**Mehlich Soil Change Comparisons**

| | ppm | ppm | ppm | ppm | ppm | ppm | ppm | ppm | ppm | ppm | ppm | % | pH | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sample ID** | **Calcium** | **Potassium** | **Magnesium** | **Phosphorus** | **Aluminum** | **Boron** | **Copper** | **Iron** | **Manganese** | **Sodium** | **Zinc** | **Nitrogen** | **pH** | **Organic Matter** |
| **Alabama Control Soil (ACS)** | 1054 | 64 | 171 | 76 | 371 | 0.8 | 0.5 | 227 | 86 | 59 | 15 | 0.203 | 5.3 | 2.8 |
| ACS (1-6) Rep Average | 711.50 | 741.83 | 209.00 | 50.25 | 234.83 | 0.63 | 0.58 | 167.67 | 67.92 | 98.35 | 12.95 | 0.27 | 6.73 | 4.00 |
| ACS Average Change | -342.50 | 677.83 | 38.00 | -25.75 | -136.17 | -0.17 | 0.08 | -59.33 | -18.08 | 39.35 | -2.05 | 0.07 | 1.43 | 1.20 |
| ACS Percent Change | -32% | 1059% | 22% | -34% | -37% | -21% | 17% | -26% | -21% | 67% | -14% | 32% | 27% | 43% |
| Alabama Composting Change | - | + | + | - | - | - | + | - | - | + | - | + | + | + |
| **Sample ID** | **Calcium** | **Potassium** | **Magnesium** | **Phosphorus** | **Aluminum** | **Boron** | **Copper** | **Iron** | **Manganese** | **Sodium** | **Zinc** | **Nitrogen** | **pH** | **Organic Matter** |
| **MMS-2 Control** | 5010 | 37 | 2014 | 4.2 | 3.2 | 15 | 0.2 | 3.8 | 0.5 | 397 | 1.9 | Below Det | 9.9 | 1.1 |
| MMS2 (1-6) Rep Average | 4330.50 | 695.33 | 3357.67 | 37.92 | 1.28 | 7.08 | 0.28 | 1.73 | 39.73 | 364.83 | 0.77 | 0.11 | 8.40 | 2.57 |
| MMS2 Average Change | -679.50 | 658.33 | 1343.67 | 33.72 | -1.92 | -7.92 | 0.08 | -2.07 | 39.23 | -32.17 | -1.13 | 0.11 | -1.50 | 1.47 |
| MMS2 Percent Change | -14% | 1779% | 67% | 803% | -60% | -53% | 42% | -54% | 7847% | -8% | -60% | Large | -15% | 133% |
| MMS2 Composting Change | - | + | + | + | - | - | + | - | + | - | - | + | - | + |

### B. Post-Composting Total Element Digestion Soil Data

**Total Element Digestion Soil Change Comparison**

| | ppm | ppm | ppm | ppm | ppm | ppm | ppm | ppm | ppm | ppm | ppm | ppm | ppm | ppm | ppm | ppm | ppm | ppm |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sample ID** | **Calcium** | **Potassium** | **Magnesium** | **Phosphorus** | **Aluminum** | **Arsenic** | **Boron** | **Barium** | **Cadmium** | **Chromium** | **Copper** | **Iron** | **Manganese** | **Molybdenum** | **Sodium** | **Nickel** | **Lead** | **Zinc** |
| **Alabama Control (ACS)** | 1319 | 229 | 466 | 335 | 7854 | <0.1 | 7 | 260 | <0.1 | 15 | 11 | 19356 | 135 | <0.1 | 27 | <0.1 | 30 | 45 |
| ACS (1-6) Rep Average | 775.50 | 978.50 | 279.17 | 640.33 | 7004.17 | 3.93 | 10.15 | 27.57 | 0.37 | 14.12 | 11.28 | 21025.50 | 95.50 | 0.83 | 33.35 | 1.45 | 20.18 | 39.37 |
| ACS Average Change | -543.50 | 749.50 | -186.83 | 305.33 | -849.83 | 3.93 | 3.15 | -232.43 | 0.37 | -0.88 | 0.28 | 1669.50 | -39.50 | 0.83 | 6.35 | 1.45 | -9.82 | -5.63 |
| ACS Percent Change | -41% | 327% | -40% | 91% | -11% | Large | 45% | -89% | Large | -6% | 3% | 9% | -29% | Large | 24% | Large | -33% | -13% |
| Alabama Composting Change | - | + | - | + | - | + | + | - | + | - | + | + | - | + | + | + | - | - |
| **Sample ID** | **Calcium** | **Potassium** | **Magnesium** | **Phosphorus** | **Aluminum** | **Arsenic** | **Boron** | **Barium** | **Cadmium** | **Chromium** | **Copper** | **Iron** | **Manganese** | **Molybdenum** | **Sodium** | **Nickel** | **Lead** | **Zinc** |
| **MMS-2 Control** | 28798 | 876 | 22521 | 559 | 6670 | <0.1 | 42 | 122 | <0.1 | 14 | 17 | 31369 | 613 | <0.1 | 1211 | 40 | <0.1 | 34 |
| MMS2 (1-6) Rep Average | 25897.83 | 2028.67 | 19108.67 | 1015.67 | 7640.83 | 9.92 | 51.93 | 75.53 | 0.40 | 15.98 | 17.07 | 36180.00 | 585.83 | 0.44 | 867.72 | 40.53 | 0.98 | 42.72 |
| MMS2 Average Change | -2900.17 | 1152.67 | -3412.33 | 456.67 | 970.83 | 9.92 | 9.93 | -46.47 | 0.40 | 1.98 | 0.07 | 4811.00 | -27.17 | 0.44 | -343.28 | 0.53 | 0.98 | 8.72 |
| MMS2 Percent Change | -10% | 132% | -15% | 82% | 15% | Large | 24% | -38% | Large | 14% | 0% | 15% | -4% | Large | -28% | 1% | Large | 26% |
| MMS2 Composting Change | - | + | - | + | + | + | + | - | + | + | + | + | - | + | - | + | + | + |

FIG 3. **Mehlich 1 Extraction and Total Element Digestion Soil Data. A. Mehlich 1 soil data of Alabama Control Soil (ACS) and MMS2 (MMS) pre and post composting. B. Total Element Digest soil data of Alabama Control Soil (ACS) and MMS2 (MMS) pre and post composting.**

## Discussion

Humanity'sinnate desire to explore the cosmos is a quest comprised of incremental small steps which add up to major accomplishments. Before humans can become a multi-planet species and colonize Mars, we must be able to solve the steps of logistics and practicality regarding terraforming and human survival on the red planet. Our goal revolved around the potential of improving soil fertility of Martian regolith, through the usage of Martian soil simulant, with the assistance Black Soldier Flies (BSF) composting. Prior to BSF soil composting, we evaluated BSF under high G-forces to assess initial transport feasibility regarding rocket G-force exposure, which would be experienced during an actual launch. We conducted high G-force centrifugal testing as well as a vertical G-force actual rocket launch using a Tripoli Level 1 rocket with success. Both Centrifugal and linear G-force assessments yielded ~100% survivorship. We purposefully tested far beyond what would be experienced by any assent or re-entry to indicate that they would be viable in any reasonable scenario. Most currently used human-rated governmental and commercial launch vehicles only experience 2-3Gs of sustained acceleration and 5Gs of maximum acceleration during ascent [39]. Some launch vehicles, such as Starship, which is developed for both human and cargo missions, can experience max acceleration above 5Gs, but even this is well within what we have demonstrated BSF can handle (SpaceX, 2020). Additionally, it is also possible to cryofreeze BSF, which may be pertinent to long term transit (Giliad et al.) 2023. This may be important when considering the potential regarding travel time to Mars as frozen embryos could be sent.

The composter design was successful in such a way in that BSF were able to compost the Martian soil simulant during the one-month duration in a contained all-inclusive unit. The composters require minimal training to operate and little to no oversight when being used outside of misting. This minimizes the potential for user error, allowing consistent results from even untrained individuals. The innovation regarding the design and total self-containment of the composting unit can save astronauts time which can then be devoted to other experiments. The composters were also designed to fit into a standard cube sat format. This means they can be efficiently packed and stacked with both each other and any other item that fits these standard dimensions. This will allow for easy transport of the boxes from Earth to whatever celestial body they are being employed on. Additionally, the boxes are easily scalable. If a larger BSF colony and more composted soil is desired, larger boxes can be assembled by scaling the dimensions of the design. This will allow the composters to continue to fit the needs of a growing extraterrestrial colony. Regarding the composting itself, we were successfully able to compost organic material on Martian soil simulant to discern statistically significant differences of one-month composted soil in 17 key soil nutrients. Of note regarding the plant available nutrients, provided by Mehlich 1 testing, Potassium, Magnesium, Phosphorus, and Manganese showed the greatest percent change in availability post composting. Total Element Digestion revealed the highest MMS2 post composting increases in soil concentrations of Potassium, Phosphorus, and Zinc. Potassium is a vital mineral for plants assisting in cytosol in plant cells, osmotic potential relating to turgor pressure, $K+$ channels, enzyme activation, phloem transport of sucrose, and much more (White and Karley, 2010; Marschner, 2011; Prajapati, 2012). Magnesium also plays a vital role in plants regarding photosynthesis, phosphorylation, enzymes, growth and cellular signalling and functions (Verbruggen and Hermans, 2013; Kleczkowski and Igamberdiev, 2021; Cakmak and Yazeie). Deficiencies of magnesium in plants have been shown to be of concern (Guo et al., 2016). Phosphorus is another essential soil mineral vital to root development (Shen et al., 2011; Lambers, 2022). The ability of a plant or crop to acquire phosphorus is vital to that plant's establishment within the soil and growth. Phosphorus is also scarce as it is often lacking macronutrient relating plant development (Schachtman et al., 1998). Mehlich 1 testing revealed a mean increase in plant availability of phosphorus by 803% after only one-month of composting. Another macronutrient of note is Manganese. Initially Mehlich 1, MMS2 soil was low in Manganese at 0.5 ppm. Following composting, availability went up a staggering mean of 7,847% to 39.73ppm. Manganese is a vital to plant kinetics, photosynthesis, hydrolyzation, as well as oxygen utilization within cells (Burnell, 1988; Schmidt and Husted, 2019). The increase of Zinc in the MMS2 soil post composting is also beneficial. Zinc is important in plants for its role regarding shoot growth, root growth, and primarily enzymatic functioning (Lindsay, 1972; Brown et al., 1993; Broadley et al., 2007). The Zinc concentration in the MMS2 composted soil experienced a 26% increase.

## Conclusion

Following the successful composting of Martian soil simulant (MMS2), further work could investigate improving composted soil and plant growth assays. To enhance crop growth, the addition of Plant Growth Promoting Rhizobacteria (PGPR) should be explored. Utilizing PGPRs for composted regolith inoculation, or seed inoculation may provide continued crop benefits, especially due to their beneficial secondary metabolites and stress-mitigating capabilities which can provide a promising strategy for enhancing plant resilience and growth in such challenging substrates (Nelson, 2004; Nadeem et al., 2014; Prasad et al., 2015; Khatoon et al., 2020). Future testing could explore inoculation of seeds with a PGPR consortium comprised of *Bacillus megaterium*, *Bacillus velenzensis, Variovorax paradoxus,* and *Enterobacter asburiae*. This bacterial mixture for example may provide a capacity to improve root architecture, plant growth, ion homeostasis, cytokinin signalling, antioxidant levels, and other stress response mechanisms (Mahdi et al., 2020; Toukabri et al., 2021; Chen et al., 2022; Lee et al., 2024). These traits may enhance plant viability in the composted Martian soil simulant, promoting adaptation to otherwise adverse soil conditions. These tests could involve comparing plant growth, nutrient content, and stress responses in PGPR-treated composted Martian soil versus non-treated controls. Key metrics to assess could include root and shoot biomass, nutrient uptake efficiency, and indicators of stress tolerance such as peroxidase and catalase activity. By systematically evaluating these parameters, the efficacy of PGPR consortia as an amendment in Martian soil, may increase the viability of in-situ agricultural methods for future Martian terraforming ventures.

The goal of this study was to compost Martian soil simulant for a one-month duration with BSF. In doing so we assessed the high G-force exposure of BSF while also designing an all-inclusive, compact, confined, and easy to use composter. Our findings reasonably demonstrate BSF should be able to withstand any G-forces experienced by a typical government or commercial rocket. We also showed BSF have the ability to compost Martian soil simulant in a one-month duration and significantly alter the mineral content of the soil. Both astro-entomological assessments suggest further testing and research should be done to assess the continued feasibility of utilizing black soldier flies as a potential tool in Martian colonization and soil reclamation.

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## Declarations

**Conflict of interest:** The authors declare no competing interests.

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