

## Challenging the agricultural viability of martian regolith simulants

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### ABSTRACT

As human space exploration extends farther from Earth, the cost and risk of resupplying a mission increases substantially. *In situ* resource utilization (ISRU) is the prospecting, extraction, storage, and exploitation of existing resources at the crew location, reducing cost and risk. Crucial to the development of ISRU solutions is the accurate characterization and modeling of regolith for a variety of applications, including as a substrate for food production at lunar or Martian sites. Previous studies have established the capacity of specific regolith simulants to support the growth of a variety of plants in the short term. However, the mineralogical and physical accuracy of these simulants as proxies for the sites on Mars is debatable, and any conclusion drawn from studies with these simulants must be considered with some degree of caution. For the purpose of studying ISRU food production, we present a systematic effort to evaluate the utility of three different Martian Regolith Simulants (MRSs): JSC-Mars-1A, Mars Mojave simulant (MMS), and Mars Global simulant (MGS-1). Our findings confirmed that none of these simulants are capable of supporting plant growth in the absence of nutrient supplementation. However, with the addition of a nutrient supplement, both JSC-Mars-1A and MMS were able to support the growth of both *Arabidopsis thaliana* and *Lactuca sativa*. However, the highly alkaline (pH > 9.0) MGS-1 simulant was unable to support growth even with additional nutrients. Acidification of MGS-1 nearly doubled the longevity of plants grown in this medium, suggesting this simulant may be further modified to eventually support plant growth as well. The addition of calcium perchlorate to each simulant, at concentrations intended to mimic those observed on the surface of Mars, rendered each simulant incapable of supporting plant growth, regardless of nutrient supplementation. These findings underscore that ISRU food solutions are likely at a lower technological readiness level than previously thought. We discuss these findings in the context of future efforts to develop off-world agricultural systems, approaches to developing future MRSs, and the immediate need to address perchlorate contamination as well as nitrogen deficiency for regolith-based food production.

### 1. Introduction

By most projections, the first continually inhabited Martian colony will be established within the next 50 years. Given the challenges associated with the Martian environment, as well as its distance from Earth, nearly all aspects of life on this colony must be considered mission critical, and therefore subject to significant evaluation during mission planning. Success depends on identifying and resolving these issues

prior to the start of such a mission.

An emerging theme in environmental system management and agriculture is sustainability, or the ability to maintain existing production from an ecological system, while ensuring its stability and availability at a future date (Gaffney et al., 2019). While crucial to the success of food security and ecosystem viability on Earth, sustainable systems must be at the heart of every long-term off-world colonization effort. In an off-world system, all of the elements, both biotic and abiotic, should

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be considered in system design. From human waste to inedible plant matter, all components are incorporated into the system to minimize the number, frequency, and amount of external inputs. Sustainability will minimize the need for resupply missions, create a more robust food production system, and support mission success. Indeed, in the absence of sustainable systems, most projected missions have a built-in expiration date, due to costs and/or mission safety issues.

One crucial aspect of off-world sustainability efforts is a dependency on *in-situ* Resource Utilization (ISRU), or the use of existing materials at the site whenever possible. ISRU will allow colonists to introduce new materials into their closed-loop ecosystem without relying solely on resupply missions. On Mars, examples of *in situ* resources include water, light, regolith, and perchlorates for oxygen as well as harnessing the CO<sub>2</sub> in the atmosphere to introduce new biomass for food production, feed-stocks for building materials, pharmaceuticals and more through photosynthesis (Wheeler, 2010).

Both sustainable food production and waste recycling are chief concerns for reducing resupply costs and have been identified as key sustainability investments (Schwartzkopf, 1992). In this context, the potential utility of Martian regolith, water, and captured CO<sub>2</sub> for food production are of particular interest. A number of studies have previously used Mars regolith simulants (MRSs) to evaluate the potential for Martian regolith to support plant growth on site (Guinan et al., 2018; Wamelink et al., 2014, 2019). Such regolith-based plant production systems can assist with life support, food production, the production of pharmaceuticals, and psychological well-being.

One of the biggest challenges with food production on-site is the need to grow as complete and varied a diet as possible with minimal supplementation. As a result, most early models for food production suggested these plants will be used primarily to supplement other dietary sources and limit menu fatigue. Some of these studies have modeled a mix of hydroponically grown crops optimized around nutrition, including rice, corn, sweet potatoes, and bananas (Nelson et al., 2008). However, regolith-based, mixed -crop systems would allow for improved complementary interactions with microorganisms. Plant growth systems, utilizing Martian regolith, could leverage the advances of mixed and sustainable agriculture systems to limit fertilizer, improve disease resistance, and build viable nutrient cycling systems that augment existing life support systems. Indeed, food security alone will demand the presence of multiple sources of food production, including a traditional agriculture approach alongside hydroponics, aquaponics, and others.

The development of regolith-based plant growth systems for Mars is critically dependent on the quality of the MRSs used in these studies. As a result, this process should be iterative, with new simulants being developed as our knowledge of the Martian surface improves. Table 1 provides a summary of the previous elemental and chemical analyses of three of these simulants, as well as the Rocknest landing site on Mars to assist in the evaluation of the work contained herein.

The first of these, JSC-Mars-1, was developed under the direction of NASA's Johnson Space Center in 1997 from material mined from Pu'u Nene, a cinder cone on the island of Hawaii. This material was developed to approximate spectral averages of Mars and used limited observational data from the Viking landing sites (VL-1 and VL-2), rather than a mineralogical analysis. JSC-Mars-1 has been previously characterized in terms of its mineralogical and chemical composition, spectral properties, grain size, and density (Allen et al., 1998).

JSC-Mars-1A has since been developed as a nearly identical simulant obtained from the same source. Wamelink et al., 2014 explored the success of a variety of plant species in JSC-Mars-1A as well as its nutrient composition for the purposes of supporting plant growth. They concluded that the JSC-Mars-1A contained small amounts of nitrate and ammonium with significant residual organics that could support germination as well as initial seedling growth. Meanwhile, other groups have utilized this simulant to observe the viability of various microbes on these substrates, including the cyanobacterium *Nostoc commune* (Arai

**Table 1**

Chemical, mineralogical, and physical properties of Martian Regolith Simulants and the Rocknest eolian deposit in Gale crater, Mars. 'ND' = Not Determined.

Concentration (wt%)	Rocknest Bulk (MSL)	JSC-Mars-1A	MMS-1	MGS-1
SiO <sub>2</sub>	43	43.48	49.4	50.8
TiO <sub>2</sub>	1.2	3.62	1.09	0.3
Al <sub>2</sub> O <sub>3</sub>	9.4	22.09	17.1	8.9
Cr <sub>2</sub> O <sub>3</sub>	0.5	0.03	0.05	0.1
Fe <sub>2</sub> O <sub>3</sub>	–	16.08	10.87	–
FeO	19.2	–	–	13.3
MnO	0.4	0.26	0.17	0.1
MgO	8.7	4.22	6.08	16.7
CaO	7.3	6.05	10.45	3.7
Na <sub>2</sub> O	2.7	2.34	3.28	3.4
K <sub>2</sub> O	0.5	0.7	0.48	0.3
P <sub>2</sub> O <sub>5</sub>	1	0.78	0.17	0.4
SO <sub>3</sub>	5.5	0.31	0.1	2.1
Cl	0.7	–	–	–
Total	100	100	100	100
composition %				
pH	ND	6.74 +/- 0.3	7.28 +/- 0.1	9.45 +/- 0.1
Redox Potential (mV)	ND	211.28	230.8	211.8
Particle Size	<2 mm	<1 mm	<1 mm	<1 mm
Source	Achilles et al., 2017	Morris et al., 2000	Peters et al., 2008	Cannon et al., 2019

et al., 2008; Ramkissoon et al., 2019).

The observed tendency of this simulant to absorb excess water (hygroscopic) compared to the predicted properties of actual Martian regolith drove the development of a second simulant: Mars Mojave Simulant (MMS)(Peters et al., 2008). MMS is based on chemical and mineralogical measurements from various landing sites, such as the Mars Pathfinder and the Mars Exploration Rover (MER) Spirit. Collected from the Saddleback Basalt in the western Mojave Desert, MMS is less hygroscopic than the JSC simulant and was originally intended to better simulate the mechanical properties of Martian regolith. However, there are a number of critical differences between both of these simulants and Martian regolith, such as mineralogical composition and the presence of organic matter (Seiferlin et al., 2008). This simulant is readily available, and there is substantial anecdotal evidence to support the viability of this substrate for plant growth, albeit little peer-reviewed research (Guinan et al., 2018).

More recently, the Mars Global Simulant (MGS-1) was developed based on quantitative mineralogy from the CheMin instrument onboard Curiosity's Mars Science Laboratory (MSL). Unlike JSC and MMS, MGS-1 was synthesized from individual components to match the mineralogy of Martian regolith rather than starting with bulk material from a single location on Earth (Cannon et al., 2019). This makes MGS-1 the most accurate basaltic MRS to date, and it must be considered as a substrate to support plant growth in order to properly evaluate ISRU opportunities on Mars. MGS-1 is based on the Rocknest windblown soil in Gale Crater, which is thought to be representative of basaltic soils at various landing sites and remote sensing locations. Consequently, this simulant represents the average of a substantially larger surface area of the Red Planet than prior simulants (Cannon et al., 2019).

Developing and selecting MRSs is challenging because no space program has successfully returned a regolith sample from Mars, and we are therefore limited to remote surface-based assessments. Additionally, regolith properties vary across the surface of Mars, so any sample returned would only be representative of one potential landing site. A possible problem when evaluating agricultural systems for a future Mars base is that the selection of a MRS may be prioritized for its viability as a substrate for plant growth, rather than ensuring the accuracy in approximating Martian substrates. Rather, any new MRS should be evaluated for its chemical, physical, and mineralogical accuracy to sites on Mars, then evaluated for its ability to support plant growth with or

without pretreatments.

The aforementioned analyses used for the development of all three of these MRSs confirms the presence of a variety of important micronutrients for plants, including molybdenum, chloride, and iron in accessible forms for plants. Similarly, macronutrients such as potassium ( $K_2O$ ), phosphorus ( $P_2O_5$ ), magnesium ( $MgO$ ), and calcium ( $CaO$ ) have also been observed and incorporated into existing MRSs. Notably absent, however, are detectable concentrations of the micronutrients copper and boron, as well as, and perhaps most importantly for plant growth, nitrogen. Nitrogen is a rate-limiting nutrient for biomass accumulation, and its conspicuous absence strongly argues against the viability of any of these substrates to support growth in the absence of additional nutrients. Therefore, the presence of trace nitrogen compounds, as well as some of the organics, identified in the JSC-Mars-1A, are artifacts resulting from Earth's biome and not likely to be representative of Mars (Wamelink et al., 2014). Indeed, Wamelink et al., confirmed the necessity of the addition of supplemental nutrients ( $NH_4$ ,  $NO_3$ , P, K, Ca, Mg, and  $SO_4$ ) to JSC-Mars-1A for the successful growth of fruit-bearing plants (Wamelink et al., 2019).

In addition to the notable absence of specific micro- and macronutrients, a variety of studies have confirmed the widespread presence of calcium perchlorate ( $Ca(ClO_4)_2$ ) at relatively high concentrations (0.5%–2% w/v) and at multiple latitudes on the Martian surface (Hecht et al., 2009; Navarro-González et al., 2010). Perchlorates at these concentrations pose a direct threat to potential colonists (Lawrence et al., 2000) but may also adversely influence plant growth in pathways distinct from sodium or aluminum salts (Hamissou, 2011; Lee et al., 2010; Tollenaar, 1972; Weaver, 1942). In addition, bioaccumulation of perchlorates in plant tissues could further impact food safety and availability (Sanchez et al., 2005). Yet the potential effects of perchlorates on plants grown in MRSs have yet to be evaluated.

In the present study, we have evaluated three different MRSs: JSC-Mars-1A, MMS, and MGS-1 for their ability to support plant growth with and without nutrient supplementation. We have also evaluated the impact of Mars-relevant concentrations of perchlorates in these simulants on plant growth as well. We investigated chemical properties, such as pH, and redox potential, and measured chlorophyll and carotenoid content of the plants grown in the various simulants. We discuss our findings within the context of the importance of site selection for ISRU support of research as well as models for food production systems for any off-world base.

## 2. Materials and methods

### 2.1. Martian regolith simulants

The Martian regolith simulants used in this study were: (i) JSC-Mars-1A (Orbitec, now owned by Sierra Nevada), (ii) Mars Mojave Simulant (MMS-1, the Martian Garden), or (iii) the Mars Global Simulant-1 (MGS-1, Center for Lunar and Asteroid Surface Science). MGS-1 samples with (MGS-1p) or without (MGS-1) calcium perchlorate (2% w/v) were utilized in this study. MRSs were sieved in-house into large ( $>0.5$  mm) or small ( $<0.5$  mm) particle sizes and utilized 'as is' or mixed in equal volumes. Calcium perchlorate tetrahydrate ( $Ca(ClO_4)_2 \cdot 4H_2O$ ) (2% w/v) was added to mixed particle size samples of JSC-Mars-1A and MMS-1 then mixed in a vertical shaker (overnight) for the relevant perchlorate studies. These perchlorate-treated samples are labeled as JSC-Mars-1Ap and MMS-1p herein.

### 2.2. Verifying regolith composition and properties

High-resolution images of the mixed-size MRSs were collected via Scanning Electron Microscopy (SEM). Energy-Dispersive Spectroscopy (EDS) using SEM backscatter images were used to verify previously published elemental characterization of the simulants (Hapca et al., 2015). Chemical properties including redox potential and pH of mixed

particle size samples were verified using ASTM procedures G200 and D4972 respectively (ASTM International, 2014; ASTM International, 2013).

### 2.3. Plant selection

For the present study we focused on two plant species: *Lactuca sativa* var. *Longifolia* (Outredgeous Romaine Lettuce, Territorial Seed Company) and the model angiosperm, *Arabidopsis thaliana* ecotype: Columbia-0 (Lehle seeds). *L. sativa*, was evaluated due to its existing space-flight history with NASA's Veggie program on the International Space Station (Massa et al., 2017) and simplicity as a pick-and-eat food. Meanwhile, *A. thaliana* is arguably the most prominent model research plant system in the world with a vast assortment of genetic resources that can be utilized to determine optimal plant features for growth in MRSs.

### 2.4. Construction of a chamber for short term evaluation of plant growth in regolith

Four  $\approx 0.16$  cm diameter holes were drilled into the base of 50 ml conical tubes to allow for drainage of excess liquid. The tubes were then cut into two pieces so that the bottom portion could hold 20 ml of material. The upper portion of the tubes were discarded. The bottom portion of the tubes were filled with 20 ml of one of the MRS described above. The tubes could then be transferred to conical tube racks for ease of transport and placed in a secondary container to collect runoff.

### 2.5. Seed germination and Plant growth

30 seeds of *A. thaliana* or *L. sativa* were germinated directly on one of the four MRSs described above with or without supplemental nutrients, in the chambers described above. Unless specified otherwise, mixed particle size substrates were utilized for all germination and growth studies. Nutrient supplementation was provided by watering plants as needed with a 1xHoagland's No. 2 solution (Arnon, 1950; Smith et al., 1983), prepared using 18 M $\Omega$  ultrapure water. Germination was evaluated at 5 (*A. thaliana*) or 14 (*L. sativa*) days and seedlings were allowed to grow for an additional 15 or 28 days, respectively. All plants were grown at  $22 \pm 1$  °C, with a relative humidity range between 65 and 75%, illuminated with a 16:8 day:night cycle ( $\approx 10,000$  LUX).

As seeds failed to germinate on either MGS-1 or MGS-1p, the toxicity of this substrate to plant growth was further evaluated by germinating and growing 30 seedlings of *A. thaliana* or *L. sativa* for 5 or 14 days, respectively on rockwool (1 cm<sup>3</sup>). These samples were maintained hydroponically with a solution of 1xHoagland's No. 2 under the same light, temperature, and humidity conditions for seedlings grown in MRSs (see above). Seedlings were then transferred, still in rockwool, to the MGS-1 or MGS-1p simulants or JSC-Mars-1A as a control for transfer. Growth and viability were evaluated daily in these samples. MGS-1 pH was adjusted by treating 20 g of simulant with 1 ml of 10%  $H_2SO_4$  (Khor-sandi, 1994).

### 2.6. Evaluating toxicity of MGS-1 runoff

20 ml of MGS-1 were transferred to 5 of the growth chambers described above and watered with sufficient 1xHoagland's No. 2 solution to yield approximately 5 ml of runoff. The runoff was collected and 100  $\mu$ l was directly applied to ungerminated seeds of *A. thaliana* on sterile filter paper in a petri dish. Alternatively, 4 ml of the runoff was concentrated by evaporation (45 °C) to  $\approx 1$  ml, and 100  $\mu$ l of this solution added to 30 ungerminated seeds of *A. thaliana* on sterile pre-moistened (18 M $\Omega$  ultrapure water) filter paper (10 mm diameter) in a petri dish. Petri dishes were then sealed with parafilm to prevent evaporation and stored under ambient temperature conditions under cool white fluorescent light (16:8 h day:night cycle) Germination was evaluated 5 days

after treatment. Similar experiments were performed with seeds treated with only 100  $\mu\text{l}$  of Hoagland's No. 2 or a 100  $\mu\text{l}$  solution of 10  $\mu\text{M}$   $\text{AlCl}_3$  in Hoagland's No. 2, a known toxicant of *A. thaliana* (Lee et al., 2010).

### 2.7. Growth stage identification of *Arabidopsis thaliana*

Phenotypic analysis of relative *A. thaliana* growth rates was determined by the previously established growth stage method (Boyes et al., 2001).

### 2.8. Chlorophyll and carotenoid content

Leaves were harvested at 15 (*A. thaliana*) or 28 (*L. sativa*) days post-germination and their mass determined with an analytical balance. Approximately, 500 mg of plant material (wet weight) was then powdered by freezing in liquid nitrogen and grinding with a mortar and pestle. Powdered leaf material was used immediately or stored at  $-80^\circ\text{C}$ . To determine chlorophyll and carotenoid content, 100 mg of powdered leaf material was transferred to microcentrifuge tubes with 50  $\mu\text{l}$  of glass microbeads and 1 ml of methanol ( $-20^\circ\text{C}$ ). Samples were vortexed for 15 s then centrifuged for 10 min in a microcentrifuge (16,873  $\times g$ , max speed). The resulting supernatant was transferred in 200  $\mu\text{l}$  aliquots to a 96-well plate ( $N = 3$  for each sample) and absorbance values measured on a SpectraMax i3 plate reader (Molecular Devices). Absorbance readings at 430, 440, 470, 650, and 665 nm were used to confirm carotenoid (470 nm), chlorophyll a (430 nm and 665 nm) and chlorophyll b (440 nm and 650 nm) content respectively, based on known equations for methanol as a solvent (Ritchie, 2008; Sumanta et al., 2014).

## 3. Results

### 3.1. Physical and chemical verification of MRSs utilized in this study

Scanning electron micrographs of mixed size samples of all three regolith simulants are shown in Fig. 1. Energy dispersive spectroscopy, while not quantitative, did confirm that the elemental composition of all three simulants were consistent with previously published values (Allen et al., 1998; Cannon et al., 2019; Peters et al., 2008) (data not shown) and contained a high concentration of basalt. We then considered the pH and redox potential of these simulants, important factors for plant growth and microbial associations (Fuller et al., 2017; Hussion, 2013). As shown in Table 2, the redox potentials for all three simulants were similar, ranging from approximately 210–230 mV, within an acceptable range for sustaining plant growth. However, while JSC-Mars-1A and MMS-1 were generally neutral in pH ( $\approx 6.5$ – $7.3$ ), MGS-1 was considerably more alkaline with a pH  $\approx 9.5$ . This was inconsistent with the findings of the Phoenix Mars Lander, which while alkaline, had a pH  $\approx 7.7$  (Hecht et al., 2009). The higher pH of MGS-1 is likely due to the presence of silicates such as olivine, plagioclase, and pyroxene present in this simulant (Cannon et al., 2019).

**Table 2**

Determined pH and redox values for mixed particle size MRSs utilized in this study.

Simulant	pH	Redox potential (mV)
JSC-Mars-1A	6.7 $\pm$ 0.3	211.3
MMS-1	7.3 $\pm$ 0.1	230.8
MGS-1	9.4 $\pm$ 0.1	211.8

### 3.2. Seed germination in MRSs

We performed all of our initial growth and germination studies on MRSs of mixed particle size. Seeds of *L. sativa* and *A. thaliana* were successfully germinated ( $\geq 90\%$ ) in both JSC-Mars-1A and MMS-1 with or without the addition of any nutrient supplement (1xHoagland's No2 solution, Fig. 2). Perchlorate-treated JSC-Mars-1Ap and MMS-1p both showed equivalent germination to their perchlorate-free controls as well (Fig. 2).

Notably, neither species successfully germinated on either the MGS-1 or MGS-1p (with perchlorate) substrates, even with the addition of supplemental nutrients. Ungerminated seeds, recovered from these MGS-1 treatment conditions (treated/untreated or with/without perchlorate) failed to show any germination after 14 days on filter paper, supplemented with 1xHoagland's No2 solution. The inability of these seeds to germinate upon recovery from the MGS-1 suggested this simulant was potentially toxic, even in the absence of perchlorates.

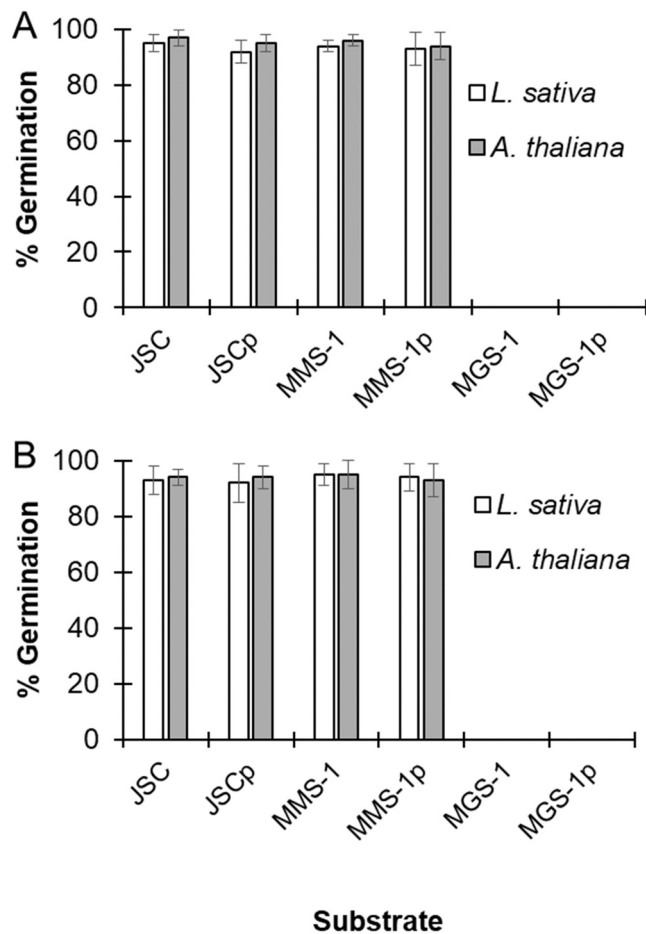
### 3.3. Plant growth in MRSs

*A. thaliana* and *L. sativa* seedlings were allowed to grow for a total of 15 or 28 days post-germination, respectively. However, neither substrate was able to support growth beyond 7 days after germination in the absence of additional nutrients (Representative data for *A. thaliana* shown in Fig. 3). As shown in Fig. 4, germinated seedlings successfully grew on both the JSC-Mars-1A and MMS-1 substrates, in the presence of additional nutrients. *Arabidopsis* plants grown in either simulant reached comparable phenotypic growth stages, based on rosette size and leaf number (Boyes et al., 2001), and were statistically indistinguishable from one another after 15 days (Table 3). These stages are consistent with previously observed growth over the same duration in a variety of substrates (Boyes et al., 2001). Similarly, the edible biomass of *L. sativa* samples grown in the two MRSs were statistically indistinguishable after 28 days (Table 4). Based on these findings, we decided to perform our remaining experiments exclusively with nutrient supplemented plant samples.

Next we considered the effects of perchlorate on plant growth. *A. thaliana* seedlings grown in perchlorate containing JSC-Mars-1Ap and MMS-1p all perished within 5 days of germination. *L. sativa* seedlings perished after 9 days. The addition of 1xHoagland's to any of these samples was unable to extend viability (Data not shown). In conjunction with the germination assays, this suggests that perchlorate toxicity occurs post-germination, likely once the seedling has exhausted internal



**Fig. 1.** Scanning Electron Microscopy (SEM) images of the MRS utilized in this study. Left to Right: JSC-Mars-1A, MGS-1 and MMS-1. These images were collected from mixed particle size samples.



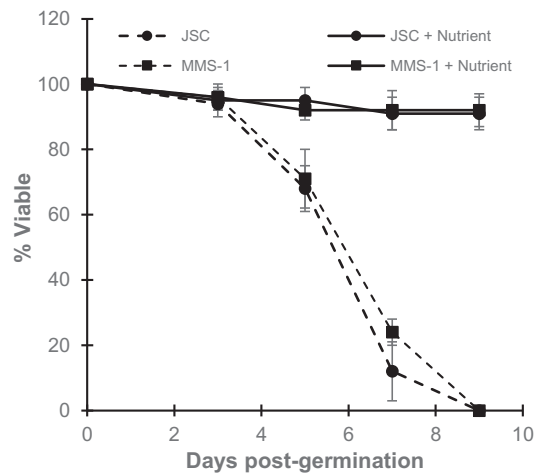
**Fig. 2.** Evaluating MRS effects on germination. 30 seeds of *L. sativa* or *A. thaliana* were placed on the surface of the indicated substrate either without (A) or (B) with the addition of Hoagland’s No. 2. Samples were evaluated for germination after 5 (*A. thaliana*) or 14 (*L. sativa*) days. Studies were performed in triplicate and the results are expressed as the average with error bars representing standard error. JSC-Mars-1A and JSC-Mars-1Ap are abbreviated as JSC and JSCp, respectively.

reserves.

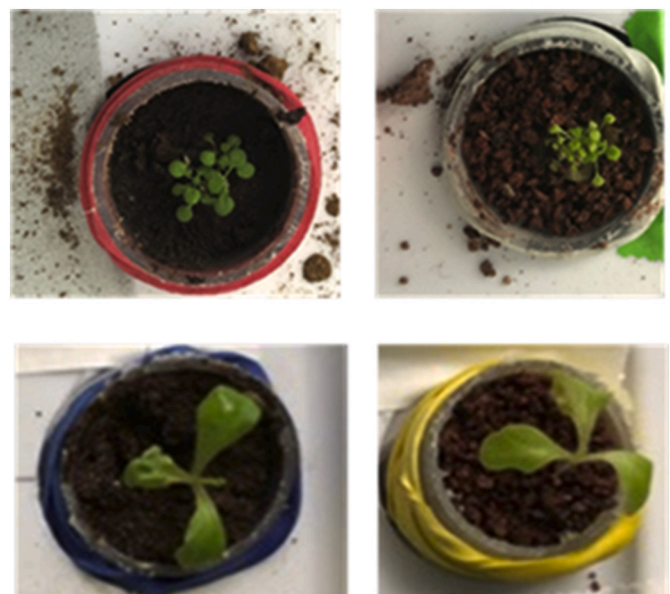
As neither species showed viable germination on MGS-1 or MGS-1p, we first germinated 30 seeds of *A. thaliana* on rockwool beds and allowed them to grow for 5 days. 30 seedlings were then transferred from rockwool to MGS-1 or MGS-1p. In every attempt, regardless of the presence or absence of nutrient supplementation, all samples perished within 5 days of transfer. A similar attempt with one-month old *A. thaliana* plants grown in rockwool also resulted in rapid death of all samples transferred ( $\leq 7$  days). This suggests that even in the absence of perchlorates, the MGS-1 simulant does not support plant growth ‘as is’.

### 3.4. Photopigment concentration in MRS

Next, we determined the photopigment concentration (chlorophyll *a*, chlorophyll *b*, and carotenoids) of both *A. thaliana* and *L. sativa* in JSC-Mars-1A and MMS-1, at 15 or 28 days, respectively (Fig. 5). In both species, photopigment concentrations were statistically similar in both simulants. Furthermore, these photopigment concentrations were comparable to previous studies for wildtype plants of these species (Su et al., 2015; Urbonavičiūtė et al., 2007). While preliminary, these studies do suggest that with appropriate nutrient supplementation these plants can mimic growth in traditional plant growth substrates.



**Fig. 3.** Evaluating viability of *A. thaliana* post germination. Seeds were scored for germination after 5 days and all viable seeds were counted at the indicated intervals after this time point. Seeds were germinated and grown in either JSC-Mars-1A or MMS, with or without nutrient supplementation (Hoagland’s No. 2). Studies were performed in triplicate and the results are expressed as the average with error bars representing standard error. JSC-Mars-1A is abbreviated as JSC here.



**Fig. 4.** Growth of plants on different Martian regolith simulants. Representative *A. thaliana* (top) and *L. sativa* (bottom) seedlings grown on JSC (left) or MMS-1 (right). Seedlings grown in the specialized growth chambers used in this study and supplemented with Hoagland’s No. 2 (See methods).

**Table 3**

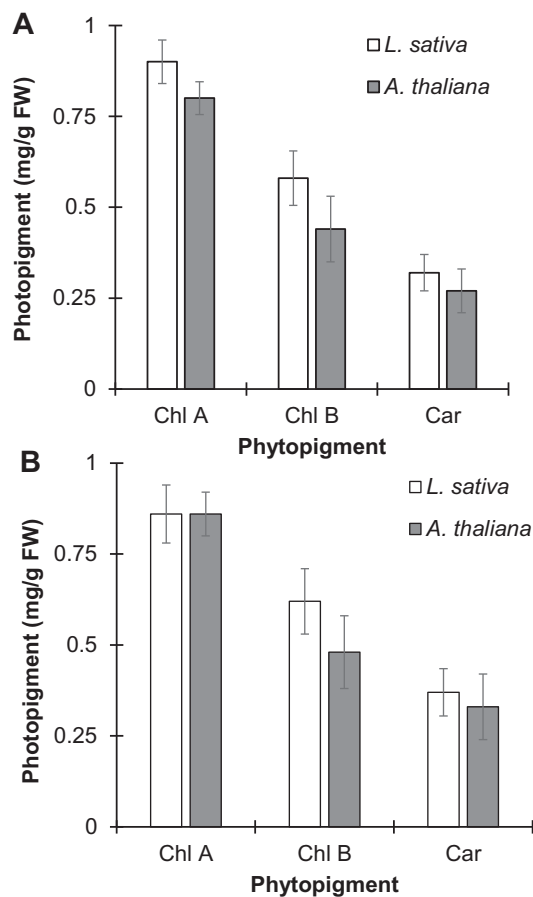
*A. thaliana* growth stages after 15 days of growth on Hoagland’s No. 2 supplemented MRS (mixed particle size).

Simulant	Growth Stage
JSC-Mars1A	1.02 +/- 0.02
MMS-1	1.03 +/- 0.03
MGS-1	DEAD

**Table 4**

*L. sativa* edible biomass after 28 days of growth on Hoagland's No. 2 supplemented MRSs (mixed particle size).

Simulant	Edible biomass
JSC-Mars1A	21 g +/- 4 g
MMS-1	18 g +/- 5 g
MGS-1	DEAD



**Fig. 5.** Phytopigment distribution in plants grown on MRS. Plants were grown in either JSC-Mars-1A (A) or MMS-1 (B) and supplemented with Hoagland's No. 2 solution. Samples were grown for 15 (*A. thaliana*) or 28 (*L. sativa*) days post-germination. Samples were then harvested and prepared for phytopigment determination (see methods). Measurements of chlorophyll A (Chl A) and B (Chl B) as well as carotenoids (Car) were determined spectrophotometrically (See methods). Studies were performed in triplicate, and results expressed as the average with error bars expressing standard error.

### 3.5. Can plant growth in MGS-1 be improved?

The observation that MGS-1 was unable to support plant growth in our studies, could suggest a major setback for ISRU food production efforts. Based on our observations of this simulant, we considered two features of the regolith we could easily modify: particle size and pH.

All three MRSs are hygroscopic and have a tendency to aggregate upon watering. In the case of the MGS-1, this effect was particularly dramatic, leading us to hypothesize that this effect might suffocate the roots of plants, explaining the rapid death of plants on this substrate. In order to test this hypothesis, samples of JSC-Mars-1A, MMS-1, and MGS-1 were sieved into large (>0.5 mm) and small (<0.5 mm) particle sizes then re-evaluated for their ability to germinate or support *A. thaliana* plant growth. No statistical difference in germination or growth between

the two different particle sizes and the mixed particle size samples were observed for JSC-Mars-1A or MMS-1 with nutrient supplementation (Data not shown). MGS-1 was unable to support seed germination regardless of particle size, and transferred seedlings died within 7 days of introduction to any of the three size categories of particles.

Next we considered the pH of the MGS-1 (pH > 9.0) which is likely to render several critical nutrients, such as iron, phosphorous, and zinc, inaccessible to plants. A number of methods exist to lower soil pH, and direct treatment with acid is viable for the relatively small volumes we are using in this experiment. Using sulfuric acid we artificially adjusted the MGS-1 pH to  $\approx 7.2$  (See Methods) and then evaluated the ability of this modified MRS to support *A. thaliana* growth. These treatments increased seedling viability by an additional week, but all samples on MGS ultimately perished (Data not shown). While preliminary, these findings suggest that the MGS-1 is capable of supporting plant growth with some additional remediation.

Finally we considered the possibility that the MGS-1 simulant may contain an undetected toxicant responsible for killing the plants. To test this hypothesis, we collected runoff from watering our individualized growth chambers containing MGS-1 with the same nutrient solution used above, and applied this directly to seeds of *A. thaliana* on filter paper discs (100  $\mu$ l/30 seeds). We also prepared concentrations of the runoff by evaporation and applied these to the seedlings as well (100  $\mu$ l/30 seeds). The addition of runoff had no effect on germination compared with the direct addition of nutrient solution (Hoagland's No2). Runoff concentrate showed only a moderate (93% vs 84%,  $p$ -value < .03) reduction in germination relative to controls (Table 5). Treatment with aluminum trichloride ( $AlCl_3$ ), a known toxicant in *A. thaliana*, was applied in a similar manner as a control and successfully inhibited germination (Lee et al., 2010).

## 4. Discussion & Conclusion

ISRU is a crucial element of plans to develop sustainable off-world communities with the potential to drastically reduce mission costs and improve outcomes. As with all predictive efforts, successful modeling of sustainable systems is critically dependent on the accuracy of the elements comprising these models. In other words, a poor foundation results in a poor model.

As the number of MRSs grows, it becomes increasingly important to consider what aspect of Martian regolith these simulants are attempting to model. MRSs are important for a host of chemical, mechanical, and biological applications. Bulk simulants that mimic mechanical properties may be cheaper and more readily available but may not be suitable for biological studies as their mineralogical accuracy may be incorrect. While early regolith simulants, based on indirect measurements of the Martian surface, remain useful for certain applications, it is clear that these simulants may be less accurate as models for biological applications, which are often highly sensitive to changes in redox potential and/or pH.

In the present study, we have evaluated the potential for three different MRSs to support plant growth in the presence or absence of nutrient supplementation as well as with or without the addition of perchlorates. In addition to the chemical composition of these simulants, we also evaluated the effects of particle size on growth. With regards to the latter, we determined that particle size had little effect on growth. Future studies will investigate these effects on plants with longer growth

**Table 5**

*A. thaliana* germination in response to MGS-1 runoff or concentrate.

Solution (100 $\mu$ l)	% Germination
MGS-1 runoff	91 $\pm$ 6
MGS-1 runoff conc.	84 $\pm$ 5
Hoagland's No. 2	93 $\pm$ 4
Hoagland's No. 2 + $AlCl_3$	34 $\pm$ 9

times and more elaborate root systems, which would likely be significantly impacted by particle size. A high density of fine particles could limit root development in these plants, especially for the production of root vegetables, such as potatoes, which can be nutrient dense and are therefore high value targets. Particle size can be easily controlled by sieving bulk sand or by milling rocks to size, making this an easy to control feature of these simulants. Future work will further explore the potential impacts of particle size on the success of plant growth.

Our studies confirmed the absolute need for the addition of nutrient supplements, especially nitrogen, for survival under these conditions. We now consider the potential implications of our studies to the future of ISRU-based food production systems on Mars. As previously mentioned, these simulants, like much of the surface of Mars, by current estimates, are deficient in copper, zinc, and boron, all of which are critical micronutrients for plant growth. While not originally detected in the studies on which these simulants were designed, all three of these micronutrients have been detected on the surface of Mars at various locations, suggesting they may be mined to enrich regolith on-site to facilitate food production directly (Gasda et al., 2017; Lasue et al., 2016; Payré et al., 2019).

While the discovery of several micronutrients increases the relative ISRU potential for food production on a Mars colony, it is the low abundance of nitrogen which is the larger concern for plant growth. Indeed, further studies of the Martian surface have detected only trace concentrations of biologically available nitrogen, well below what would be necessary to support sufficient biomass accumulation for food production (Stern et al., 2015). Moreover, nitrogen gas (N<sub>2</sub>) only composes ≈2% of the Martian atmosphere, which is considerably less dense than that of Earth, making this element a challenging resource to acquire *in situ*. Nitrogen may well be an element that must be shipped to Mars in abundance for incorporation into the colony and could significantly impact mission startup and maintenance. Nitrogen availability will therefore be a vital consideration in the habitat water recycling/purification system, and nitrifying bacteria will almost certainly be an important component in making this element available to plants in viable forms. Such strategies are being extensively explored by multiple space agencies and independent researchers (Clauwaert et al., 2017; Hao et al., 2018; Nelson et al., 2008; Schwartzkopf, 1992; Shimura et al., 1999; Wheeler, 2010).

While both JSC-Mars-1A and MMS were capable of supporting plant growth with additional nutrient supplements, the MGS-1 simulant proved remarkably toxic to both *A. thaliana* and *L. sativa*. Yet, based on the known composition of MGS-1 (Cannon et al., 2019), there is no immediately apparent toxic component absent from either of the two other simulants evaluated in this study. Runoff liquid collected from the MGS-1 substrate failed to significantly impact the germination of *A. thaliana* to which it was added. However, concentrated runoff produced a moderate reduction (≈10%) in germination suggesting a water soluble unidentified toxicant as a potential source. Obviously, these studies are preliminary, and we are currently investigating the chemical composition of each simulant's runoff, both organic and inorganic, in an effort to resolve such questions.

An alternate explanation for the toxicity of the MGS-1 substrate lies in its pH, which was significantly more alkaline (pH > 9) than the other two substrates, which tended to be much closer to neutral (pH: 7). In alkaline soils, crucial macro- and micronutrients like nitrogen, iron, zinc, manganese, and copper have reduced availability to plants potentially explaining the inability of plants to survive on the MGS-1 substrate. This is supported by the observation that the rapid acidification of the substrate extended the viability of plants on the substrate. Ongoing studies are further evaluating the source of MGS-1 toxicity and the potential for improving viability through acidification. However, the obvious inability of the most accurate simulant currently available to support plant growth raises serious questions about the role of regolith in ISRU-based food production on Mars.

Of equal concern to the absence of nitrogen is the presence of

perchlorate (ClO<sub>4</sub><sup>-</sup>) at multiple sites on the Martian surface, at levels that would be dangerous to colonists. While high concentrations of other salts may adversely impact growth, perchlorate concentrations are of particular concern given their potential to accumulate in plant tissue and threaten human health (Sanchez et al., 2005). The addition of perchlorates to each of the simulants rendered them incapable of supporting viable plant growth, underscoring the need for perchlorate removal from these samples. The potential for more perchlorate-tolerant plants, or perhaps bacteria, to engage in bioremediation of regolith on the Martian surface can be evaluated using MRSs. Chemical solutions to perchlorate removal could similarly be evaluated using MRSs.

Our findings highlight the growing need for more accurate models across a wide range of sites on Mars. Agricultural solutions on Earth are not one-size-fits-all, but rather adapt to the terrain and soil composition available. It is extremely likely that the viability of Martian regolith for food production will be equally site dependent. More accurate simulants for biological assays will allow us to explore soil remediation efforts and/or the selection of mutant plants capable of surviving these soils. However, it cannot, and must not, be ignored that the variability between these simulants raises questions as to the viability of food production in regolith and underscores the importance of ongoing food production research through multiple strategies. Finally, it is clear that regardless of the simulant or the ultimate landing site both the absence of nitrogen and the presence of perchlorate are glaring concerns, which will need to be addressed in mission planning.

## Declaration of Competing Interest

None.

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