January 2021

An Evaluation Of Ci Asteroid Regolith As A Plant Growth Medium For Space Crop Production

Steven Jon Russell

AN EVALUATION OF CI ASTEROID REGOLITH AS A PLANT GROWTH MEDIUM FOR SPACE CROP PRODUCTION

by

Steven Jon Russell
Bachelor of Science, University of North Dakota, 2017

A Thesis

Submitted to the Graduate Faculty

of the

University of North Dakota

in partial fulfillment of the requirements

for the degree of

Master of Science

Grand Forks, North Dakota

May
2021

This thesis submitted by Steven Jon Russell in partial fulfillment of the requirements for the Degree of Master of Science from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done and is here by approved.

Dr. Sherry Fieber-Beyer

Dr. Kathryn Yurkonis

Dr. Mike Gaffey

This thesis is being submitted by the appointed advisory committee as having met all of the requirements of the School of Graduate Studies at the University of North Dakota and is hereby approved.

Chris Nelson
Dean of the School of Graduate Studies

Date

PERMISSION

Title	An Evaluation of CI Asteroid Regolith as a Plant Growth Medium for Space Crop Production
Department	Space Studies
Degree	Master of Science

In presenting this thesis in partial fulfillment of the requirements of for a graduate degree from the University of North Dakota, I agree that the library of this University shall make it freely available for inspection. I further agree that permission for extensive copying for scholarly purposes may be granted by the professor who supervised my thesis work, in her absence, the Chairperson of the department or the dean of the School of Graduate Studies. It is understood that any copying or publication of other use of this thesis of part thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and the University of North Dakota in any scholarly use which may be made of any material in my thesis.

Steven Russell

05/05/2021

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

I would like to start this section by thanking Dr. Sherry Fieber-Beyer, Dr. Kathryn Yurkonis, and Dr. Mike Gaffey for their support and guidance throughout this research and during my studies as a graduate student, as well as for serving on my thesis committee. Their assistance has been instrumental to my growth as a student and researcher. Also, I would like to thank the faculty and students of the Space Studies Department at the University of North Dakota for providing me an excellent graduate school experience for the past three years and giving me the opportunity to earn this degree.

Additionally, I would like to thank the North Dakota Space Grant Consortium, especially Marissa Saad and Caitlin Nolby for their support with anything and everything NASA related. Much of my successes in this program are due in part because of them, from graduate research assistantships, a NASA internship, and funding for this project. Providing me with many NASA related opportunities.

Materials and a soil analysis for this research were funded by the North Dakota Space Grant Consortium through a $1000 Mini-Grant. Additionally, CI simulant was purchased through a North Dakota Space Grant by Cedric Ramesh. Lastly, many thanks to the University of North Dakota Department of Biology for providing preparation space and the growth chamber used in this study.

Dedicated to my Family and Friends,

All of whom have supported my ‘out this world’ endeavors,

As well as to my dogs Apollo and Bentley.

ABSTRACT

Asteroids are small, undifferentiated bodies that materialized during the formation epoch of the solar system. With a concerted effort to establish a long-term presence in space, crewed missions to asteroids should not be ignored. A critical aspect of human missions is self-sustainability, primarily growing crops via *in-situ* resource utilization. CI carbonaceous asteroids are a primitive type of asteroid that retain elements found during that early solar system formation. More importantly, the regolith on these asteroids contains soluble elemental nutrients, such as phosphorous and potassium, that crops can use for growth and development. This thesis focuses on the ability of CI carbonaceous asteroid regolith simulant to sustain plant growth and produce edible biomass of lettuce (*Latuca sativa*), radishes (*Raphanus sativus),* and peppers (*Capsicum annuum)*. This study was split into three experiments. Experiment one tested growing the selected crops in increasing mixtures of simulant and peat moss. The second experiment focused on a mixture of simulant and perlite. The final experiment tested the simulant/perlite mixture in simulated microgravity. The results showed clear decreases in germination, plant height, leaf area, and biomass of the crops in the simulant/peat moss mixtures, with no germination growth in pure simulant. Additionally, there was no germination or growth in the simulant/perlite experiments. Subsequent analysis of the simulant showed that the simulant contains plant-usable nutrients, though it has a high pH, low CEC, and is a silt-based soil. These results indicate that the simulant is prone to compaction and crusting, leading to drought stress on the crops. Further investigations are to be needed to assess the effect of plant waste or compost on improving fertility of the simulant conducive for plant growth.

CHAPTER I

BACKGROUND

In 2019, the National Aeronautics and Space Administration (NASA) announced that they would be sending humans back to the Moon by 2024 and on to Mars not long after (Smith et al., 2020). Dubbed the Artemis program, this new directive by NASA has reinvigorated the future of human exploration into space, with a key aspect being long-term settlement. Overshadowed by the prospect of landing humans on Mars, and the relative ease of access to the Moon, asteroids should not be looked past as potential targets for human settlement (O'Neill, 1974). Long-term settlement in space puts forth a myriad of challenges for astronaut crews, scientists, and engineers to overcome. Most importantly is feeding astronauts on these long-term missions. Doing so will require new technologies to grow edible crops, whilst being sustainable without the need of resupply missions from Earth (Wheeler, 2010; Williams, 2002). Accomplishing this goal will require use of small body regoliths as crop soils.

1.1 Space Agriculture

Arabidopsis thaliana was the first plant to undergo a complete growth cycle in space aboard the Salyut 7 spacecraft in their Fiton 3 micro-greenhouse (Merkys et al., 1984). Since then, there has been a significant amount of research conducted in the realm of space agriculture to elucidate how plants react to the space environment. This information has helped to determine how humans can grow plants for food, air recirculation, and water purification for long-duration space missions (Ferl et al., 2002). Due to this, many plant growth systems have been developed to grow a variety of plant species to varying degrees of success (Zabel et al., 2016).

The most recent of which is the VEGGIE vegetable production system and the Advanced Plant Habitat (APH). VEGGIE, developed by Orbital Technologies Corporation (ORBITEC), is a small and resource efficient plant growth chamber (Morrow et al., 2005). It was launched to the International Space Station (ISS) in 2014 and was the first plant system to provide edible crops to astronauts that passed NASA microbiological standards for crew food (Massa et al., 2017). APH is a multi-tiered and multi-purpose large volume plant growth chamber. Its intended use is for plant research projects that span longer durations using an automated water and nutrient delivery system (Morrow et al., 2016).

Though plants can grow and reproduce in space, they face unique challenges not experienced on Earth including reduced gravity, reduced solar insolation, reduced CO_2, and the lack of a fluid water supply. For instance, microgravity is the experience of organisms in a constant state of free fall while in orbit around celestial bodies, though it chiefly pertains to low-Earth orbit (LEO). For viable long-duration space missions, organisms will need to adapt to microgravity and plants are no exception. Many studies have indicated that plants can adapt to microgravity through changes to their morphology, physiological processes such as gas exchange and auxin transport, and molecular changes (Morohashi et al., 2017; Paul et al., 2013; Stutte et al., 2006; Vandenbrink & Kiss, 2016). Additionally, plants can change their nutrient uptake when exposed to microgravity. For example, potassium increased in slender goldenweed (*Haplopappus gracilis* now *Machaeranthera gracilis)*, daylily (*Hemerocallis sp.)*, and peas (*Pisum sativum*) and calcium uptake increased in *P. sativum* during spaceflight (Belyavskaya, 1996; Levine & Krikorian, 2008; Nechitailo & Gordeev, 2001). Understanding how plants react to the space environment will be critical in establishing a long-term human population in space.

Briefly, the aforementioned aspects of growing plants in space can also fall under the study of bioregenerative life support systems (BLSS) and closed ecological life support systems (CELSS). BLSS and CELSS refers to a closed system of regenerating food, water, air, and waste management that can sustain human life (Gòdia et al., 2002). Of particular interest is recycling nutrients from both human and plant waste to use as a fertilizer to safely regenerate food (Clauwaert et al., 2017), which has been studied at NASA's Kennedy Space Center with the Biomass Production Chamber (Wheeler et al., 1996). However, this requires a refinery process to extract the nutrients and avoid any potentially hazardous bacteria. This can be mitigated through the incorporation of *in-situ* resource utilization (ISRU) to grow plants.

1.2 In-Situ Resource Utilization

Simply put, *in-situ* resource utilization (ISRU) is "living off the land" and using the resources available without the need of outside input. The identification of such resource materials on other celestial bodies is essential for a sustainable population in space. For instance, these resources can be used to create rocket propellant, infrastructure for habitats, tools, and aid in life support via oxygen and food production (Sacksteder & Sanders, 2007). Much of the focus on *in-situ* resources has focused on the resources and materials on the Moon and Mars. Chemical and mineralogical analyses of the Moon have indicated the presence of oxygen, water, and deuterium in the regolith (Anand et al., 2012). As for Mars, water in hydrated minerals is a viable resource, but it has been proposed that the majority of atmospheric CO_2 could be a source for many applications including carbon and oxygen, refrigerant, and methane production (Ash et al., 1978; Mustard et al., 2008).

While harvesting the Moon for its resources may occur in the near future with Artemis, Mars is still in the distant future. However, one source of resources that should not be overlooked, and could be a steppingstone to Mars, are asteroids. The discourse on using asteroids as a source for materials to sustain a sizable population has been around for some time. Some early work on this topic has postulated that some of the first large colonies in space could use the material in the asteroid belt to sustain themselves, particularly for the use in agriculture and structure building (O'Neill, 1974). Their abundance, proximity to Earth, primarily NEOs, and compositions of carbon, nitrogen, and hydrogen make them ideal targets for crewed missions (O'Leary, 1977). Similar to the Moon and Mars, asteroids have been shown to have hydrated minerals indicating the presence of water in the asteroid's history (Alexander et al., 2012; Feierberg et al., 1981; Milliken & Mustard, 2007). Additionally, C-type asteroids contain material that could help sustain crews via growing edible plants (Mautner, 2014). This will be further discussed in the following sections.

1.3 Asteroids

Asteroids are small bodies that were formed during the early formation of the solar system. Interstellar gas, dust, and ice accreted around our Sun, forming a protoplanetary disk. Gravity induced accretion of material clumped together to form what are known as planetesimals. As these planetesimals accreted more material, denser elements moved to the center of the body, while the less-dense material rises to the surface. The result is a differentiated body that is composed of compositionally distinct layers. However, due to the extensive gravitational force of Jupiter, smaller bodies between Mars and Jupiter could not accrete enough material to differentiate. These undifferentiated bodies are known as asteroids (O'Brien & Sykes, 2011), and are the most numerous bodies in the solar system.

Asteroids have specific characteristics that can be studied to separate them into subclasses. These characteristics include size, rotation, mineral composition, and albedo. For the purposes of this study, the focus will be on the albedo and composition using the Tholen taxonomy. The Tholen taxonomy consists of 14 types of asteroids that can be separated into three main groups: C-type (chondrite), S-type (stony), and M-type (metallic). Asteroids are classified into these groups based on mineralogical data from meteorite analogs and spectral characterization (Gaffey et al., 2002; Tholen, 1989). Along with mineralogical classification, albedo assists in inferring the composition of asteroids. For instance, a low albedo is indicative of darker C-type and vice versa for S-type (Zellner & Gradie, 1976). The chondrites, or C-type, are distinctive from the other taxonomic types because they contain some of the oldest material known to have accreted during the early solar system formation epoch (molten mineral grain droplets, known as chondrules) (Alexander et al., 2008). The abundance and composition of chondrules can vary; likely due to the degree of aqueous alteration the chondrite has experienced (Section 1.4). Furthermore, chondrites can be considered some of the oldest material in the solar system (Connelly et al., 2012), and can be further divided into subcategories based on their mineralogy and chemical composition: enstatite, ordinary, and carbonaceous (Van Schmus & Wood, 1967; Weisberg et al., 2006)

There are currently over 1 million asteroids in our solar system, a majority of which are found in the main belt between Mars and Jupiter, with a subset of asteroids, known as the Trojans, that share an orbit with Jupiter on both of the Lagrange points. Another subset of asteroids that enter Earth's orbit are called Near-Earth Objects (NEOs). To be considered an NEO, its closest approach must be less than 1.3 astronomical units (AU). These asteroids are then further broken down into subcategories known as the Apollo (cross Earth's orbit and reside mostly outside Earth's orbit), Amor (do not cross Earth's orbit), and Aten (cross Earth's orbit and reside mostly within Earth's orbit) asteroids. Figure 1 shows a conceptualization of the location of asteroids. NEOs are of particular interest to researchers due to potential impacts to Earth (Chapman, 2004), some of which can be classified as potentially hazardous asteroids (PHA). However, NEOs have also been considered targets for potential mid- to long-duration crewed missions because of their close proximity to Earth and resources for ISRU implementation (Abell et al., 2009; Jones et al., 2002).The primary rationales for crewed missions to NEOs are based around being a steppingstone to Mars or resource mining (Binzel, 2014; O'Leary, 1977; Ross, 2001). The C-type carbonaceous asteroids are of considerable interest for crewed missions as they have been postulated to contain volatiles such as water bound in minerals, rare-earth elements (albeit in low abundance), and bioavailable nutrients that could sustain sizeable populations (Jewitt et al., 2007; Martínez-Jiménez et al., 2017; Mautner, 2014).

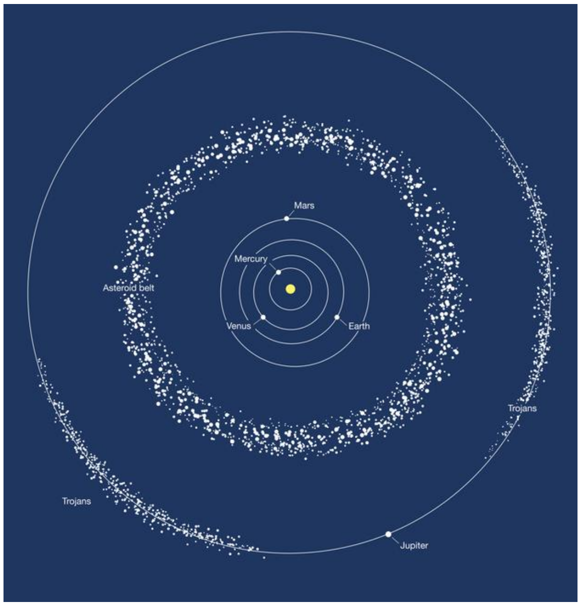


Figure 1. Conceptualization of the location of asteroids. Points in an orbit between Mars and Jupiter indicate main belt asteroids. Points on either side of Jupiter indicates the Trojan asteroids. Photo: ESA under a Creative Commons Attribution 4.0 International License.

1.4 Carbonaceous Chondrites

Carbonaceous chondrites are a unique class of meteorites that have been the source of much scientific inquiry. They are considered remnants of the early solar nebula, and are known as the oldest and most primitive celestial bodies in the solar system (Buseck & Hua, 1993). Because of this, they are important for studying the environment of the early solar system. Carbonaceous chondrites can be further sub-divided into CI, CM, CV, and CO chondrites based on mineral composition and degree of aqueous alteration (Cruikshank, 1997; Wasson & Kallemeyn, 1988). Table 1 is a list of the carbonaceous meteorite classes. What makes carbonaceous chondrites unique is the abundance of carbon. For example, carbon can range between 1.5-6% CI and CM meteorites (Scott & Krot, 2007). Because of the carbon content, researchers have considered the carbonaceous asteroids as a source of early organic material on Earth dating as far back as 3.5 billion years. These impacts have been suggested as contributing approximately 20 g/cm^2 of organic matter that may have aided the beginning of life (Anders, 1989; Chyba & Sagan, 1992; Cruikshank, 1997). Additionally, the presence of amino acids and sugars found in the Murchison CM meteorite have supported that claim (Cooper et al., 2001; Cronin & Moore, 1971).

Table 1. Carbonaceous Chondrite Classes[a] with their respective meteorite analog and current knowledge of aqueous alteration.

Classification	Meteorite Analog	Aqueous Alteration
CI (C1)	Ivuna Orgueil	Primitive, 18-22% H_2O Alteration to hydrous silicates. Organics also altered.
CM (C2)	Mighei Murchison	6-16% H_2O. Alterations to silicate minerals and organics
CV (C3)	Vigarano Allende	Least altered. with minimal H_2O
CO (C3)	Ornans Lance	
Others CH, CB, CK, CR, Ungrouped		

[a]Table was derived from a figure presented in Cruikshank 1997.

Understanding the origin of these meteorites involves understanding their parent body asteroid. A majority of carbonaceous meteorites have been determined to originate from C-type asteroids due to the presence of chondrules, though the CM chondrites have similar spectral characteristics to G-type asteroids, which are similar to C-type (Burbine et al., 2002). A primary insight into the parent bodies of carbonaceous chondrites is the degree of aqueous alteration of their meteorite analog. Aqueous alteration of carbonaceous chondrites is indicative of water processes on asteroids that may have occurred during the early formation of the solar system and was a critical component of the geological evolution of carbonaceous asteroids (Brearley, 2006; McAdam et al., 2015). This has implications for future human missions to asteroids as water is crucial to space-based populations and ISRU applications. Determining which asteroids have water bearing minerals will be a key criterion. Spectrally, hydrated minerals in C-type asteroids are characterized by a near-infrared absorption features that span from 0.9 – 3.0 microns (Gaffey et al., 1993; Jones et al., 1990; Merényi et al., 1997).

Thus far, there have only been two missions that have sent spacecraft to carbonaceous asteroids. Those being the Hayabusa 2 and the OSIRIS-REx missions, both of which are sample return missions. After the success of the first Hayabusa mission that returned a sample of the S-type asteroid 25143 Itokawa, a second Hayabusa mission was launched for a sample return mission to the asteroid 162173 Ryugu. Ryugu has a perihelion of 0.963 AU, an aphelion of 1.416, and a rotational period of 7.625 h (Wada et al., 2018), categorizing it as a NEO. Near-infrared data of Ryugu yields a narrow band at 2.72 microns, suggesting the presence of hydroxyl-bearing minerals, similar to that of the analog meteorite Ivuna (Kitazato et al., 2019). This indicates the presence of water volatiles in its surface regolith. Sample collection occurred in February 2019 and returned to Earth in December 2020. The second mission to a carbonaceous asteroid, and the most recent, is the OSIRIS-REx mission to 101955 Bennu. Bennu has a perihelion of 0.90 AU, an aphelion of 1.36 AU, and a rotational period of 4.297 h (Hergenrother et al., 2020; Nolan et al., 2013), categorizing it as a NEO. Similar to Ryugu, near-infrared data from the OSIRIS-REx spacecraft yielded an hydration absorption feature at 2.7 microns (Hamilton et al., 2019). In October 2020, the OSIRIS-REx spacecraft successfully touched down on Bennu, collected a sample, and is currently on return back to Earth.

The study of these asteroids, and their meteorite analogs, have proven to be useful in determining which asteroids should be targeted for future missions. Both Ryugu’s and Bennu’s proximity to Earth and evidence of hydrated minerals could be a jumping-off point for a crewed mission with astronauts that could utilize the resources within. Additionally, the organic content of these types of asteroids, and by proxy, meteorites, particularly in the regolith, has implications and potential for space agriculture, which is further discussed in section 1.6 below.

1.5 Plant-Soil Interactions

Soil on Earth is highly complex, being an interconnected substance of inorganic material, organic material, and living organisms. The Soil Survey Staff, of the United States Department of Agriculture, define soil as:

> "*a natural body comprised of solids (minerals and organic matter), liquid, and gases that occurs on the land surface, occupies space, and is characterized by one or both of the following: horizons, or layers, that are distinguishable from the initial material as a result of additions, losses, transfers, and transformations of energy and matter or the ability to support rooted plants in a natural environment*." (Soil Survey Staff, 2014)

This definition sets the precedent that soil is the primary medium that supports plants through the movement of energy and matter throughout. Soil nutrients and atmospheric CO_2 are the food that, along with water and sunlight, give plants energy and the building blocks that they use to grow and develop. Moreover, soils have specific characteristics that help support plant life.

Soil nutrients play a critical role in the growth and survival of plants, in that some nutrients are used for structural growth and some are used for metabolic processes (Gurevitch *et al*., 2018, Morgan and Connolly, 2013). These nutrients can be subdivided into macronutrients and micronutrients. Macronutrients, such as nitrogen, phosphorus, and potassium, are needed in large quantities. Conversely, micronutrients, such as zinc, iron, and copper, are needed in smaller amounts (Nathan, 2009). Though most nutrients are absorbed through the roots via the soil, the way in which those nutrients get into the soil is different. For instance, most come from the minerals within the soil via geological processes and decomposition of organic matter. However, essential nitrogen is added to the soil via nitrification. Nitrogen fixing bacteria and decomposed plants take in atmospheric nitrogen, convert it to ammonium (NH_4^+), which nitrifying bacteria convert ammonium into nitrites (NO_2^-) and subsequently nitrates (NO_3^-) that plants can use as nutrition (Alexander, 1965). A summary of nutrients and their functions in plants can be found in Table 2. Additionally, water is essential for nutrients to be released from the soil and be taken up into the roots.

In addition to nutrients, the pH of the soil can either enhance or inhibit the growth of plants. In acidic soils, toxic nutrients such as Al and Mg become more available to plants. Conversely, in more alkaline soils, P and other micronutrients become less available (Kleupfel and Lippert, 2012). Vegetables tend to prefer slightly acidic soil. Another important characteristic of soils in regard to plant life is cation exchange capacity (CEC). CEC is the ability of the soil to absorb cations such as Ca, Mg, K, and some others and supply them for plant uptake. Along with this, CEC varies with pH where CEC tends to increase as pH increases and can contribute to more alkaline soils (Sonon *et al*., 2017). Lastly, soil texture and the presence of organic matter are more crucial characteristics of soil. Soil texture is the percentage of sand, silt, and clay minerals where loam is a mixture of the three, adding porosity, volume, and chemical properties, respectively, to soil (Nathan, 2009). Figure 2 shows a soil texture triangle that illustrates how certain soils are classified. Soil organic matter is all organic material (plants, microorganisms, decomposed residues). Organic matter can improve soil structure, promote water and air movement, supply nutrients, and improve CEC (Nathan, 2009).

Table 2. Nutrients essential for plant growth[a].

Elemental Nutrient	Essential Function(s)
Carbon (C)[b]	Photosynthesis energy transport, carbohydrates, cellulose
Oxygen (O)	Cellular Respiration
Hydrogen (H)	Biochemical reactions
Nitrogen (N)	Nucleic acids, proteins, amino acids
Potassium (K)	Enzyme activator, stomatal action, ion and pH balance
Calcium (Ca)	Cell wall strength, cell division, structural, membrane permeability
Magnesium (Mg)	pH regulation, chlorophyll molecule.
Phosphorous (P)	Energy transfer, structural component for nucleic acids, ATP, and proteins
Sulfur (S)	Amino acid component
Chlorine (Cl)	Stomatal regulation, splitting water molecules
Iron (Fe)	Heme proteins
Manganese (Mn)	Activation of enzymes
Boron (B)	Cell wall synthesis
Copper (Cu)	Pollen formation and ovule fertilization
Nickel (Ni)	Nitrogen metabolism
Molybdenum (Mo)	Pollen formation, seed dormancy
Sodium (Na)*	Ion balance
Cobalt (Co)*	Nitrogen fixation
Silicon (Si)*	Disease resistance

*Indicates plant specific nutrients.
[a]Table derived from Gurevitch et al. 2018.
[b]Atmospheric CO_2

The definition of soil mentioned earlier can be applied to material of extraterrestrial origin. Though this section primarily focused on plant-soil interaction, it should be noted that from the aforementioned definition, a soil may not strictly be of biotic origin or have the need for plant life. Some would argue that soil is ‘information’ recorded about the geologic history of a particular celestial body and would conclude that surface deposits on rocky bodies in the solar systems could be classified as soils (Certini et al., 2009).

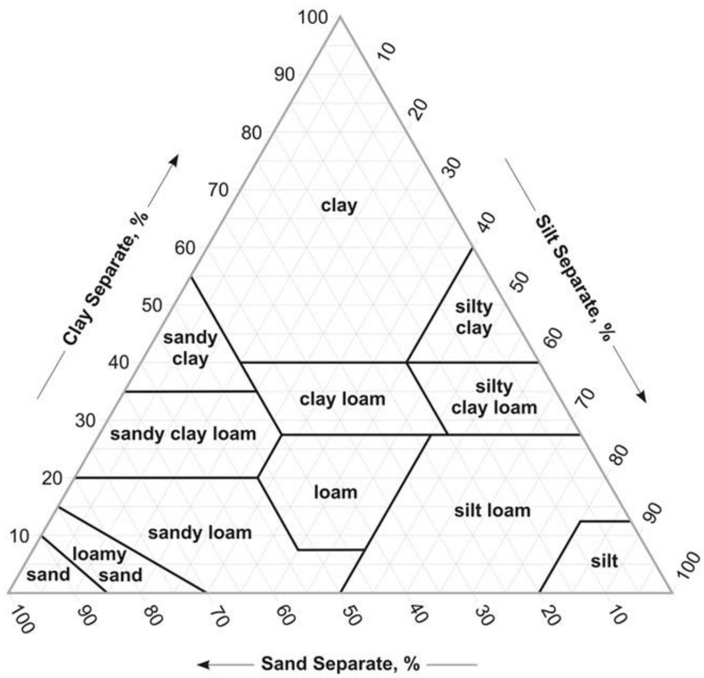


Figure 2. Soil Texture Pyramid. This pyramid aids in classifying the type of soil being used to grow crops by separating particles between % sand, % silt, and % clay. Loam is considered the 'ideal' soil type. Image credit to the United States Department of Agriculture.

1.6 Plants and Carbonaceous Chondrites

The literature pertaining to plants and carbonaceous chondrites, specifically, is minimal. Previous soil analysis of hand-grinded portions of the Murchison and Allende meteorites indicated plant available nutrients, both essential and trace, were present and that these materials have CEC levels similar to that of Earth based soils (Mautner, 1997). However, in that same analysis, the plant available P was quite low. Plant available nutrient concentrations in these meteorites are listed in Table 3 below. In CI meteorites; however, soluble plant-available nutrient concentrations have not been assessed, though it has been shown that they contain major and trace elements necessary for plant growth (Barrat et al., 2012).

Table 3. Plant available nutrients (mg/kg) and CEC (meq/100g) from meteorite extracts[a].

Meteorite	N[b]	S	P	Ca	Mg	Na	K	Fe	Al	Cl	CEC
Murchison	<10	4500	6	4000	1700	570	650	126000	3000	200	5.8
Allende	2	180	160	130	130	60	30	43000	1600	100	0.4

[a]Table was derived from values from meteorite extracts in Mautner 1997.
[b]Plant available nitrogen in this context refers to NO_3.

Nutrient extracts of the Murchison meteorite also had some effect on the growth of plants in plant assays. Potato (*Solanum tuberosum)* tissue cultures exposed to Murchison or Allende nutrient extract with deionized H_2O showed an 18% increase in fresh weight and an increase in green pigmentation compared to cultures exposed to pure deionized H_2O (Mautner 1997). In a second experiment, plant tissue cultures of asparagus (*Asparagus officinalis)* and potatoes (*Solanum tuberosum)* were either exposed to grounded Murchison meteorite mixed with deionized H_2O, a standard growth medium, sucrose, inorganic salts (NH_4NO_3, $NH_4H_2PO_4$), mixtures of sucrose and inorganic salts or without extract of the mediums. There were different patterns of growth between the two species. *A. officinalis* tended to have enhanced growth in mediums with more concentrated Murchison extract, whereas *S. tuberosum* tended to have enhanced growth with diluted extract, with a similar effect on the fresh weight of the plants (Mautner et al., 1997).

The results of the above studies are noteworthy as they could contribute to the sustainability of a large space-based human population. Nutrient concentrations of carbon, nitrogen, potassium, and phosphorus derived from the Murchison soil analysis is on a small scale; however, it can be extrapolated to a much larger object. Upscaling nutrient availability to a

100 km radius, 10^9 kg asteroid and using a designed model, it was estimated that an asteroid of this size could sustain 10^8 kg of biomass and a significantly sized human population (Mautner 2014). These estimates lend credence to the idea of sustaining a large population using asteroid material.

Some considerations to be aware of when growing plants in carbonaceous regolith/simulants or other asteroids for that matter, is the surrounding environment. For instance, asteroids have an extraordinarily minimal gravitational force, indicating that plants will most likely be grown in a microgravity environment. As stated in an earlier section, many studies have indicated that plants can adapt to microgravity. Though the interaction asteroid regolith and microgravity remains to be unseen. Another issue that should be addressed is the availability of light. Take again, for example, Ryugu, which has a perihelion of 0.963 AU, an aphelion of 1.416, and a rotational period of 7.625 h. Because of these parameters, it is unlikely that there would be enough sustained natural light for plant growth, even at perihelion, as plants have evolved to have a photoperiod of approximately 16 hours of daylight and 8 hours of night. For sustained human presence around an asteroid, artificial lighting should be used. Additionally, if directly planting in the regolith or used as a fertilizer, the presence of toxic polycyclic aromatic hydrocarbons (PAHs) and phenols, which could affect astronaut health, should be studied further (Patel et al., 2020; Singleton & Kratzer, 1969). For instance, in the Murchison meteorite, it was found that PAHs were a major component of the organic material and contain several phenolic acids (Giese et al., 2019; Hayatsu et al., 1980). It has also been investigated that non-carbonaceous asteroids could be used to avoid these health risks (Marcano et al., 2005). Lastly, plants require CO_2; since asteroids do not have an atmosphere, growing crops on a mission to an asteroid will need to obtain CO_2 from the ambient environment of a spacecraft in a growth unit such as VEGGIE or APH (Massa et al., 2016).

1.7 Plants and Regolith Simulants

Similar to that of plants and carbonaceous chondrites, the literature on the usage of regolith simulants to study plant growth is minimal and a relatively new field of investigation. The need for regolith simulants arises as the amount of regolith directly from the Moon, Mars, or asteroids available to be studied is virtually non-existent, with the exception of the Apollo lunar and asteroid (2514) Itokawa samples (Tsuchiyama et al., 2011).

A set of studies have investigated the viability of Martian and lunar simulants to sustain plant growth. One study concluded that three groups of plants (natural, nitrogen fixing, and crops) (n = 14) were able to germinate and grow in Martian and lunar simulants for 50 days without supplemental nutrients (Wamelink et al., 2014). All but one of the species tested, the nitrogen fixing legume common vetch (*Vicia sativa*), had seeds that germinated across all soil types (Mars, Moon, and Earth control). The Mars simulant tended to have more biomass production, where the lunar simulant tended to have lower biomass production. In both cases, *V. sativa* had less biomass than the control or did not germinate, respectively. Also, in this study, the researchers conducted nutrient and pH analyses, a summary of those findings can be found in Table 4. Secondly, a follow-up study found that by adding organic material, mimicking remains from prior harvests, had increased biomass production in their simulants compared to the previous study (Wamelink et al., 2019). This study raises interesting points about constructing a complex soil from bare-inorganic regoliths, suggesting that more organic matter is needed to produce more biomass as it provides a source of N and P mwhich the JSC-1A simulant is deficient. This was made more evident in a similar study where Salanova lettuce (*Latuca sativa* var ‘capitata’) yielded more fresh weight in a Mars simulant/organic compost mixture (Duri et al., 2020). They indicate that the compost and organic matter aids in soil fertility by supplying the key nutrients of N, P, and S, as well as providing better exchange of nutrients, water, and oxygen. In that study; however, they used MMS-1 Mojave Mars Simulant, which was different than Wamelink et al. (2014) and Wamelink et al. (2019). These three studies showed clearly that self-pollinating naturally occurring plants, nitrogen fixing plants, and crops can germinate, grow, produce edible biomass, flower, and produce seeds in regolith simulants with the added caveats of the need for additional nutrient supplementation, as well as the simulants not being entirely representative of actual regoliths.

More recently, a study looked into the viability of the JSC-1A, MMS-1, and MGS-1 Martian simulants to grow both *A. thaliana* and ‘outredgeous’ lettuce (*Latuca sativa*) in the presence or absence of nutrient supplementation (Eichler et al., 2021). They were able to determine that the seeds of both species would germinate in both JSC-1A and MMS-1 with and without nutrient supplementation, there was zero germination in the MGS-1 because of the high pH (>9). However, the two successful simulants were unable to support growth after a week post-germination. Additionally, with nutrient supplementation, the JSC-1A yielded more edible biomass of *L. sativa* than the MMS-1, though the difference was not significant. They concluded that these Martian simulants would need additional nutrient supplementation, particularly nitrogen, and the composition of fine particles could be an obstacle for root-based crops such as potatoes.

Table 4. Plant available nutrients (mg/kg) and pH from the JSC-1A Lunar and Mars-1A simulants[a].

	Plant Available Nutrients							
Simulant	**Al**	**Fe**	**K**	**Cr**	**NH_4**	**NO_3+NO_2**	**PO_4**	**pH**
JSC-1A Lunar	0.5	0.0	27.0	0.0	0.3	4.2	0.2	9.6
JSC-1A Mars-1A	0.0	0.0	138	0.0	3.9	2.1	0.0	7.3

[a]Table was derived from values determined by soil analyses conducted by Wamelink *et al.* 2014

Many of the produced high-quality simulants are either lunar or Martian regoliths. JSC-1 lunar simulant (McKay et al., 1993), JSC Mars-1 Martian simulant (Allen et al., 1998), Mojave Mars Simulant (Peters et al., 2008) and Mars Global Simulant MGS-1 (Cannon et al., 2019) being the simulants used in the above studies. However, there have been many simulants developed, to varying degrees of accuracy and fidelity and are rated using a figure of merit system (Schrader et al., 2009) though all are used for different purposes. More recently, the Center for Lunar & Asteroid Surface Science (CLASS) Exolith Lab at the University of Central Florida (UCF) has developed multiple asteroid regolith simulants. Three types of simulants were developed: CI, CM, and CR. The mineralogy of each simulant was based on the carbonaceous chondrite analogs of Orguiel, Murchison, and an average of five CR samples, respectively (Britt et al., 2019). The simulants were constructed using terrestrial sources and suppliers based on meteorite compositional data from previous studies. Along with the meteorite data, the developers also constructed a model of regolith formation to use as a guide for development and to be evaluated on the figure of merit system (Metzger et al., 2019; Metzger & Britt, 2020).

For the purposes of this study, there will be a focus on the CI simulant, where a summary of the bulk chemistry and composition can be found in Table 6 in section 2.1.2 of Chapter 2.

1.8 Problem Statement

There has been a considerable amount of completed work with edible crops on the ISS and in the general space environment. However, with the push to establish a permanent human presence in space, there will need to be significantly more research conducted on *in-situ* resources when related to space agriculture. Using the nearest and most abundant resources available is a logical step in this direction. This being the case, asteroids are an abundant source of raw materials that have a relative ease of access, in the case of NEOs. Most importantly of which for space agriculture, are the carbonaceous asteroids which contain both bioavailable nutrients and organic matter that are essential for plant growth. Given this, a major question that needs be addressed is: Can carbonaceous asteroid regolith support the growth and development for edible crops?

The few studies that have been conducted on this relationship have been small in scale on CM meteorites and had promising results. Similarly, few studies have addressed plant growth in extraterrestrial regolith using simulants, zero of which have used asteroid simulant. There is a considerable need for experiments and data pertaining to asteroid-plant interactions and the usage of planetary simulants. By studying the plant growth properties of germination rate, plant height, leaf area, and biomass as well as the soil characteristics; the experiments in this pilot study will test CI carbonaceous asteroid regolith as potential *in-situ* resource for plant growth, using a CI regolith simulant.

CHAPTER II

METHODOLOGY

2.1. Materials

2.1.1. Crop Selection

Crops were chosen based on variety, the edible portion of the crop, and applicability to human missions to the ISS and the Moon (Massa et al., 2013; Perchonok & Bourland, 2002). A summary of each crop can be found in Table 5. Food type is based on the edible portions of the crops. The edible portion of lettuce is the leaves, the taproot is the edible portion of radishes, and the edible portion of peppers are the fruits. Lettuce and radish seeds were purchased commercially online from Johnny's Selected Seeds (Johnny's Selected Seeds Co., Winslow, ME, USA), and pepper seeds were purchased commercially online from the Sandia Seed Company (Sandia Seed Co., Albuquerque, NM, USA).

Table 5. Crop species selected.

Scientific Name	Common Name	Cultivar	Edible Portion
Latuca sativa	Lettuce	'Outredgeous'	Leaf
Raphanus sativus	Radish	'Pink Celebration'	Taproot
Capsicum annuum	Pepper	'Chimayo'	Fruit (berry)

2.1.2. Regolith and Soil

CI asteroid regolith simulant was purchased from CLASS Exolith Lab at the University of Central Florida (UCF, Orlando, FL., USA). Mineralogy and bulk chemistry composition of the simulant is based on the Orgueil CI carbonaceous meteorite (Britt *et al* 2019). Composition of the simulant can be found in Table 7. Based on the stated composition, the simulant is deficient in nitrogen; though it is sufficient in plant available phosphorous (P_2O_5), potassium (K_2O), and trace micronutrients. However, as stated by Britt *et al.* (2019), the simulants bulk chemistry is not a perfect representation of the analog CI carbonaceous meteorite. For instance, due to the use of terrestrial minerals, there is a significantly higher concentration of K_2O in the simulant compared to actual reference Orgueil meteorite.

Earth-based soils included locally purchased SunShine sphagnum peat moss and topsoil with added vermiculite as a standard control. Peat moss is a source of organic matter, but is low in plant available nutrients. Vermiculite is a common garden additive for water retention and soil aeration. Nine weight percent vermiculite was added to the topsoil for added water retention, aeration, and its similar use in the regolith simulant. In a second and third experiment, the simulant was mixed with perlite. Perlite is a chemically inert soil amendment that aids in both water retention and aeration. These soil amendments were chosen based on the use of the Fafard #2 plant medium used in plant growth and microbial studies at NASA's Kennedy Space Center, which is a peat moss and perlite soil mix (Hummerick et al., 2012; Massa et al., 2013).

Table 6. CI Asteroid Simulant Minerology and Bulk Chemistry[a].

Minerology	Wt%	Bulk Chemistry	Wt%
Mg-serpentine	48.0	SiO_2	25.0
Magnetite	13.5	TiO_2	0.5
Vermiculite	9.0	Al_2O_3	3.1
Olivine	7.5	Cr_2O_3	0.2
Pyrite	6.5	FeO_T	25.8
Epsomite	6.0	MgO	30.2
Sub-bituminous coal	5.0	CaO	3.0
Attapulgite	5.0	Na_2O	6.4
		K_2O	0.4
		P_2O_5	0.4
		SO_3	4.9

[a]Values were derived from Britt et al. 2019 and Exolith Lab.

2.1.3. Environment

The environment for the crops was controlled in a Percival AR-66L (Percival Scientifics, Inc.) environmental growth chamber provided by the University of North Dakota Department of Biology. Environmental parameters that were controlled are humidity and temperature. Humidity was controlled to around 50-60%, while temperature was controlled from19.8^{o} C to 23.4^{o} C. CO_2 was kept at ambient levels of the surrounding chamber. Temperature was programed to ramp up and down throughout the day to mimic outside temperature changes. Figure 3A shows ramping sequence. Both temperature and humidity were monitored live using a raspberry pi fixed with a DHT22 temperature and humidity sensor.

An array of 32 watt (W), 14 W cool white fluorescent and 25 W incandescent lights were used to give the crops optimal light for growth and development by providing the plant with the full visible spectrum of light (white light). The photoperiod was 16-hours of daylight and an 8-hour nighttime cycle. Similar to that of temperature, lighting also was ramped throughout the day to mimic sunrise, mid-day, and sunset (Fig. 3B).

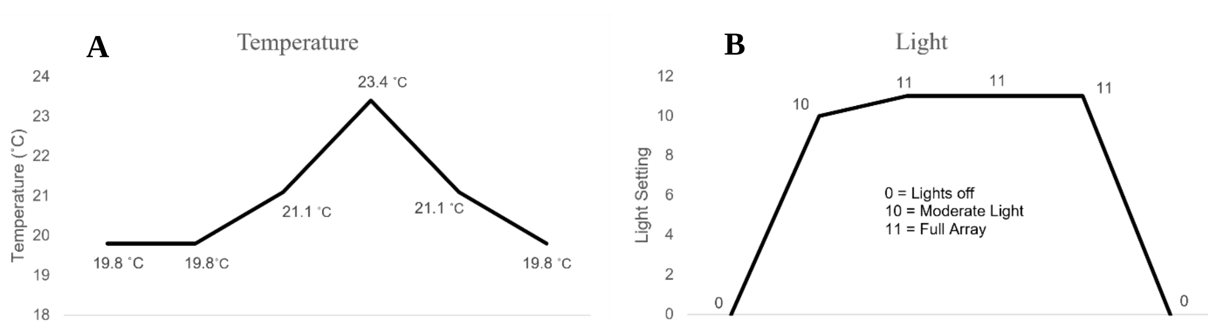


Figure 3 Ramping sequences for temperature and light in the Percival AR-66L. **A.** Ramping sequence of the temperature. The temperature begins below 20^{o}C in the morning but increases throughout the day, then back down at night. This is to mimic increasing temperatures with increasing sunlight. **B.** Ramping sequence of light array. Number signifies setting where 0 = off, 10 = Moderate/Half of light array, 11 = Intense/Full array. This is also to mimic increasing/decreasing sunlight.

2.1.4. Microgravity Simulation

For microgravity simulation, 2D clinostats with plant pot adapters developed by Eisco Labs were used. Clinostats simulate microgravity by rotating the object to be studied at a 90° angle to the surface at a constant rate, which mimics "free-fall" as there is no constant force of gravity 'pulling' the object down. When rotated parallel to the horizon, this reintroduces gravity to the system.

2.2 Experimental Design

2.2.1 Plant Set-Up and Maintenance

In all experiments, small (10.2 cm depth and width) plastic pots were filled with an equal volume, as each soil media had different densities. In each pot, five seeds were sown ~2.54 cm deep. Additionally, Pots were watered every other day to field capacity with distilled water. For experiment A, the three crops (lettuce, radish, and pepper) were grown in the CI asteroid regolith simulant, peat moss, and increasing combinations thereof (100% peat moss, 75% peat moss/ 25% simulant … 100% simulant). Crops were also grown in topsoil with added vermiculite as a standard/quality control. Pots were arranged in a randomized complete block design with 3 blocks. Each block contained 18 pots (3 plant species × 6 soil treatments), (Fig. 4A). Appendix A shows images of the filled pots and the pots in the growth chamber. For experiment B, pots were arranged in a randomized complete block design with 3 blocks. Each block contained 9 pots (3 species × 3 soil treatments) with 50% simulant/ 50% perlite, pure perlite, and topsoil with 25% vermiculite/ 25% perlite mixture (Fig. 4B). Lastly, for experiment C, two pots with radish seeds were planted into 50% simulant/ 50% perlite mixture soil then placed into two 2D clinostats. One clinostat was rotated 360^o once per day upright as the control, the second was continuously rotated 360^o once per day (24 h rotation) 90^o parallel to the ground to simulate microgravity (Fig. 4C).

2.3 Analyses

2.3.1. Harvest and Analysis

Harvesting of the crops occurred at 55 days after planting (DAP). This ensured that the edible portions of the lettuce and radishes had fully grown, and so that the peppers begin to flower based on time of harvest information from the seed packets. Data for germination rate, plant height, leaf area, and total biomass were collected. At 55 DAP crops were removed from their pots and were washed of any remaining planting medium. After washing, plant height was measured by measuring the crop from the bottom of the stem just above the roots to the leaf canopy. The crops were then dried at 60^{o}C for 64 hours. Once removed, biomass of above ground greens and some roots were weighed. Leaf area of dried leaves was measured using ImageJ.

2.3.2. Statistical Analysis

For statistical analysis, the percent germination, plant height, leaf area, and biomass were modeled as a function of soil type (simulant, peat moss, etc.), species, and soil × species interaction with a generalized linear mixed model using PROC GLIMMIX in SAS (SAS Institute, Cary, NC), assuming each response variable is a normal or lognormal distribution with block as a random effect. I used an *a priori* linear contrast to test the effect of the varying ratios of peat moss to simulant and a Tukey multiple comparison post hoc analysis for pairwise tests among treatments, respectively. I log-transformed leaf area prior to analysis to improve normality.

2.3.3. Soil Analysis

Quantitative soil analysis was conducted at the Kansas State University Soil Testing Laboratory in Manhattan, Kansas. Soil analysis tests were conducted on all soil types used in this study (Project # 31005). Tests conducted included analysis of nutrients, cation exchange capacity, pH, soil texture, and organic matter. A summary of tests used can be found in Table 7. No statistical analysis was conducted on soil analysis results.

Table 7. Soil Analysis Tests.

Analysis	**Method**[a]
P	Melich III
Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, Cu^{+}, Fe^{3+}, Mn^{2+}, Zn+	Flame Atomic Absorption or ICP Spectrometry
NO_3, NH_4	Cadmium reduction, colorimetric assay
Al	ICP Spectrometry
Total N & C	LECO TruSpec CN combustion
CEC	Displacement method with ammonium acetate
pH	Direct measurement
Soil Texture	Hydrometer
Organic Matter	Loss on Ignition

[a]Methods conducted by Kansas State University Soil Testing Laboratory.

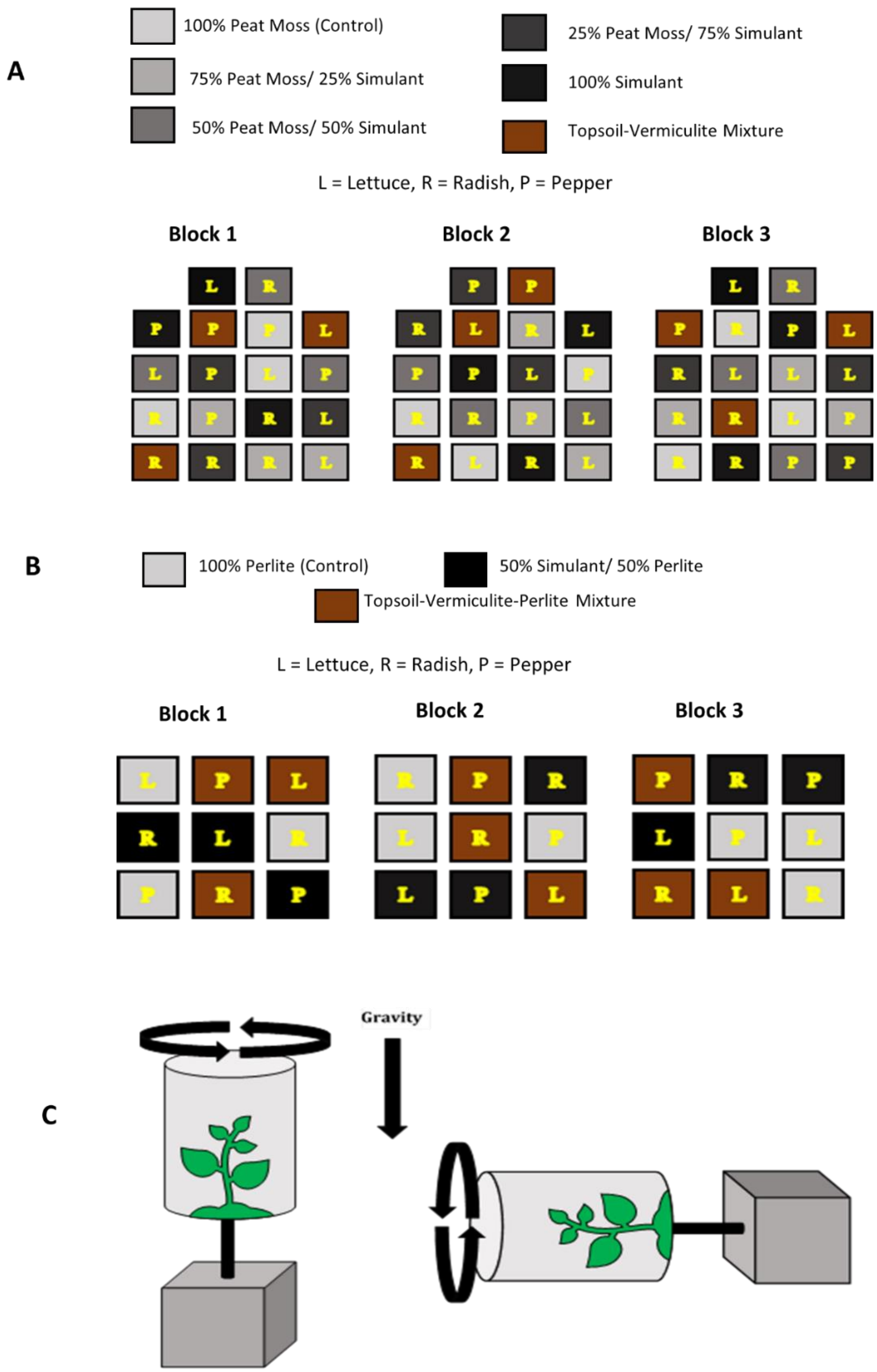


Figure 4 Experimental designs. Visual representation of the experimental design. Peat moss and simulant with a topsoil control (A) Perlite/simulant mixture and topsoil control (B). 2D Clinorotation of a plant. Normal gravity conditions rotate perpendicular to the surface, experiencing the force of gravity (left). Microgravity conditions rotate parallel (90o) to the surface, mimicking the state of free-fall, or microgravity (right). Radishes were tested under these conditions where the control is normal gravity (left) and treatment is microgravity (right) both with simulant/perlite mixture (C).

CHAPTER III

RESULTS

In every instance, plants did not germinate or grow in the 100% simulant and simulant-perlite environments. Due to this, data from treatment (zeroes) were left out of the statistical analysis. A summary of ANOVA statistical results for experiment A can be found in Table 8 below. For brevity, soil mixtures will be denoted as peat moss:simulant (100:0, 75:25, 50:50, 25:75, 0:100).

Table 8. ANOVA table of F-values from the generalized linear mixed model of germination, plant height, leaf area, and biomass.

Source	Num df	Den df	*F*	*p*
Germination				
Soil	4	28	5.04	0.0035
Linear Contrast	1	28	17.56	0.0003
Species	2	28	5.53	0.0094
Soil × Species	8	28	0.73	0.6611
Plant Height				
Soil	4	28	1.35	0.2754
Linear Contrast	1	28	4.20	0.0499
Species	2	28	24.67	< 0.0001
Soil × Species	8	28	4.29	0.0018
Leaf Area				
Soil	4	28	2.67	0.0594
Linear Contrast	1	28	7.02	0.0147
Species	2	28	19.65	< 0.0001
Soil × Species	8	28	2.30	0.0587
Biomass				
Soil	4	28	8.22	0.0002
Linear Contrast	1	28	28.91	<0.0001
Species	2	28	66.56	< 0.0001
Soil × Species	8	28	5.09	0.0006

3.1 Plant Growth Results – Experiment A

3.1.1. Germination

Overall, the percent germination decreased with increasing amounts of simulant. Germination decreased from 80% germination to 33%, with 100:0 having the most and 25:75 having the least number of seeds germinate. Figure 5 shows the germination rates of all species plotted against soil type. Overall, both 100:0 and 75:25 germinated significantly more than 25:75. There was a significant soil main effect on the germination rate with a significant linear trend. Additionally, there was a significant species effect where radishes had an average ~30% higher germination rate than the lettuce and peppers. However, there was no significant interaction effect indicating all species reacted similarly to the soil types.

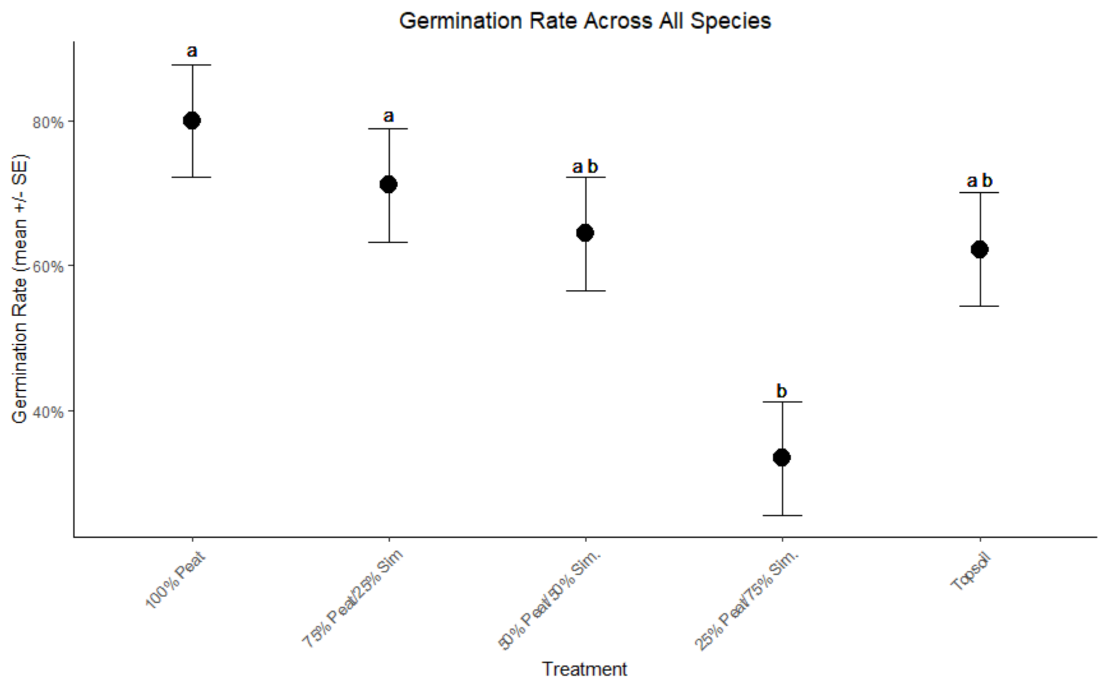


Figure 5. Effects of increasing concentrations of CI regolith simulant on germination across all three crop species. Averages (+/- standard error) of germination percentage were plotted against the treatment soil types. A compact letter display above data points in graphs is used to denote significant difference between points. Means with the same letter are not significantly different. The 25:75 treatment had significantly lower average germination percentage

3.1.2 Plant Height

Similar to germination, the average plant height (cm) decreased with increasing amounts of simulant. Plant height decreased from approximately an average of 3 cm in both the 100:0 and the 75:25 to an average of 2.1 cm in the 25:75. There was not a significant soil main effect in the average plant height. However, there was a significant linear trend in the soil effect. Additionally, there was both a significant species and interaction effect indicating that some species reacted differently to the soil. Overall, the average plant height for each species was significantly different than each other. Figure 6 shows that both the *C. annuum* and *L. sativa* did not have any significant differences between average plant height. However, *R. sativus* grown in 25:75 had significantly lower average plant height of 0.63 cm compared to those grown in the 100:0 with an average of 4.08 cm.

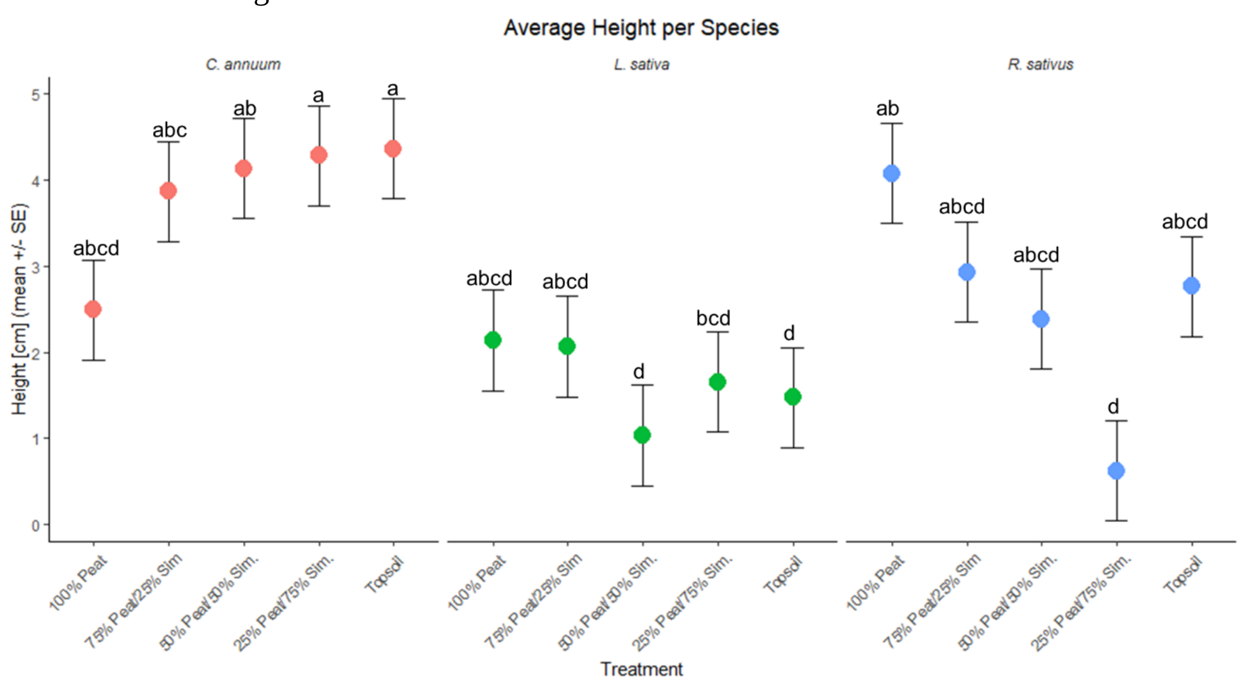


Figure 6. Plant height of radishes reacted differently than both lettuce and peppers. Plant height (cm) was measured from soil to leaf canopy. Averages (+/- standard error) of plant height were plotted against the treatment soil types, separated by species. A compact letter display above data points in graphs is used to denote significant difference between points. Means with the same letter are not significantly different. Both the lettuce and peppers were not significantly affected by soil type. In contrast, the radishes grew significantly less in the 25% Peat/ 75% Simulant treatment compared to the peat moss.

3.1.3. Leaf Area

Leaf area (cm^2) also decreased with increasing amounts of simulant. The means reported here are back transformed from the log transformed data. 100:0 had an average leaf area of 1.30 cm^2 and 25:75 had an average leaf area of 0.430 cm^2. Figure 7 shows the back transformed means of leaf area plotted against soil type. Analysis indicated that there was neither a significant main effect, though there was a significant linear trend. Additionally, there was a significant species effect, where peppers had an overall significantly lower average leaf area compared to the lettuce and radishes. However, there was no significant interaction effect between species and soil type.

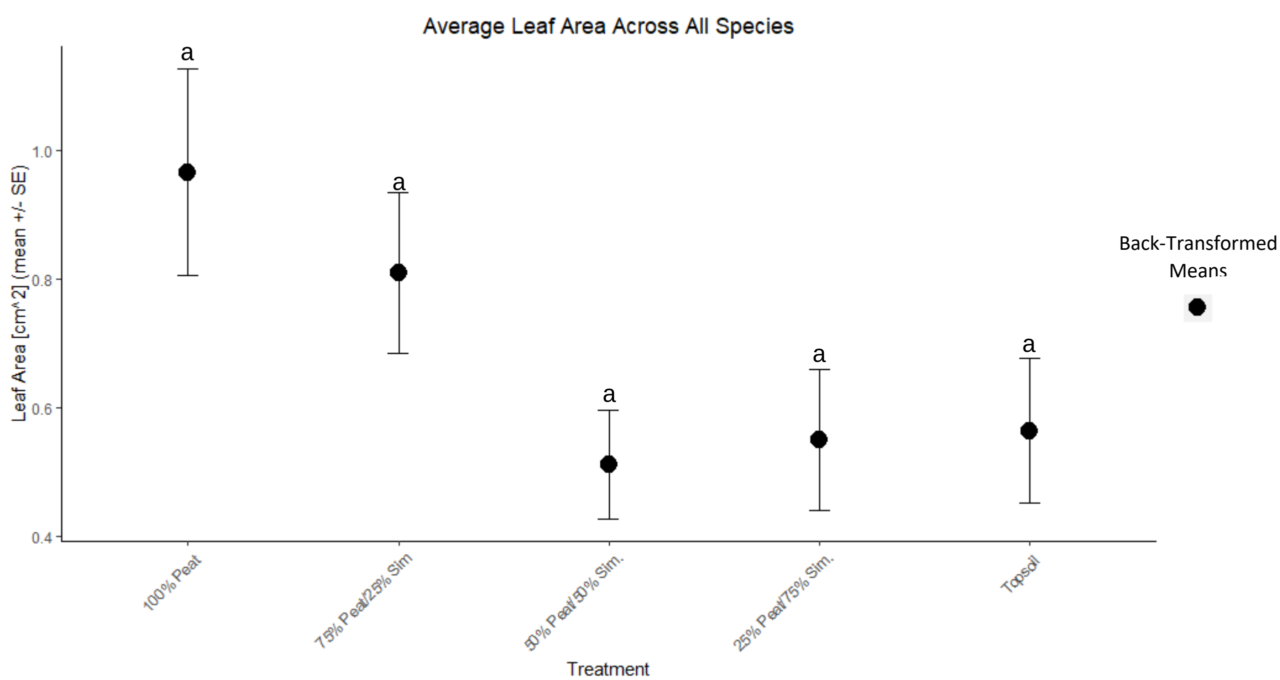


Figure 7. Back-transformed means of leaf area in cm^2. Data was analyzed on a log-normal scale as was back transformed to be plotted. Back-transformed averages (+/- standard error) of leaf area were plotted against the treatment soil types. A compact letter display above data points in graphs is used to denote significant difference between points. Means with the same letter are not significantly different.

3.1.4 Biomass

Lastly, the average biomass (mg) decreased with increasing amounts of simulant with both 100:0 and 75:25 having an average total biomass of 41.15 mg and 40.10 mg respectively, to 11.80 mg in 25:75. There was a significant soil main effect on biomass with a significant linear trend. There was also a significant species and interaction effect on biomass and indicating that some species reacted differently to the soil types. Where the overall the average biomass of the radishes was significantly higher than the lettuce and peppers. Figure 8 shows that both the *C. annuum* and *L. sativa* did not have any significant differences between average biomass. However, *R. sativus* grown in 25:75 and 50:50 had significantly lower average biomass of 15.44 mg and 54.60 mg respectively, compared to those grown in the 100:0 with an average of 94.80 mg.

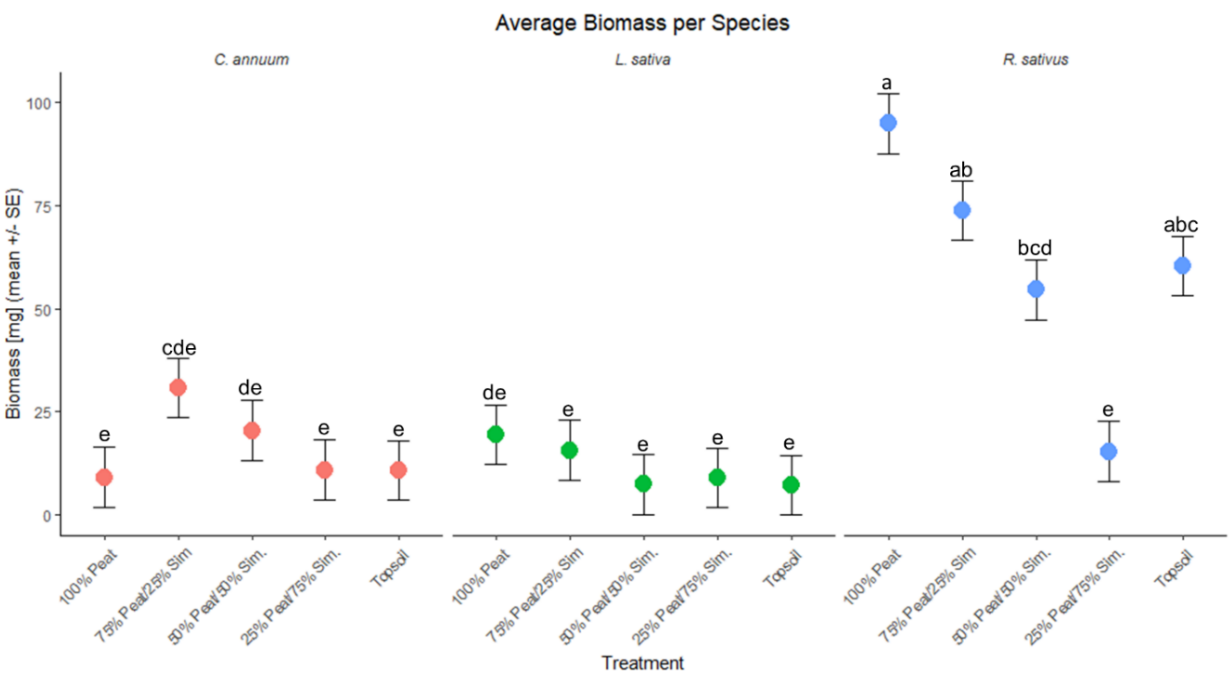


Figure 8. Biomass of radishes reacted differently than both lettuce and peppers. Crops were dried at 60°C for 64hrs and total biomass (mg) was collected. Averages (+/- standard error) of biomass were plotted against the treatment soil types, separated by species. A compact letter display above data points in graphs is used to denote significant difference between points. Means with the same letter are not significantly different. There was significantly less biomass in the 25:75 treatment of the radishes compared to the other treatments. Additionally, both 25:75 and 50:50 had significantly less than the 100:0.

3.2 Plant Growth Results – Experiment B and C

3.2.1 Simulant-Perlite

Seeds planted in a simulant-perlite mixture were unable to germinate and grow, with the exception of one *C. annuum* seed. No further analyses were conducted on these pots. Data for germination in pure perlite and topsoil can be found in Appendix B.

3.2.2 Microgravity

Similarly, seeds for this experiment were planted in a simulant-perlite mixture but were subjected to simulated microgravity. Again, no seeds germinated in this mixture and no further analyses were conducted on these pots.

3.3 Soil Analysis

Results from the soil analysis were placed into Table 9. Additionally, soil texture was plotted in figure 9. In general, the analyses showed a deficiency in essential nutrients such as K, P, and N (in the form of nitrate and ammonium) in the increasing simulant mixtures compared to both peat moss and topsoil. Also, regarding the peat moss:simulant mixtures, there was an increase in pH and a decrease in CEC and organic matter with increasing simulant. It was also determined that the simulant is classified as a silty loam soil containing 26.00% sand, 68.00% silt, and 6.00% clay material (Fig. 9).

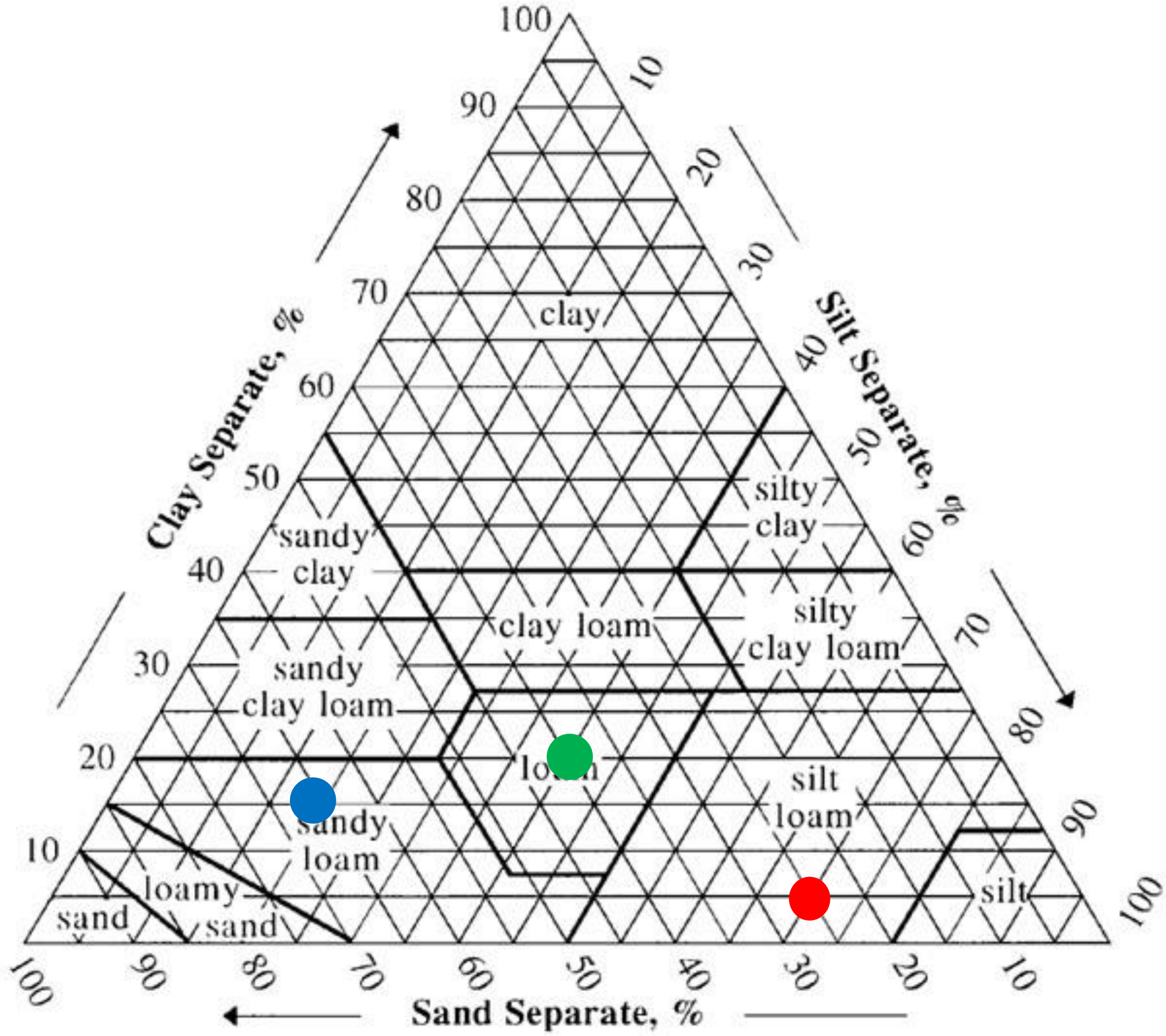


Figure 9. Soil Texture Pyramid of the soil analysis. Soil texture analysis (hydrometer method) indicated that the CI simulant is comprised of 26% sand minerals, 68% silt minerals, 6% clay minerals. Classifying the regolith as a silt loam soil (red). Topsoil from this experiment was found to be 68% sand, 18% silt, and 14% clay, classifying it as a sandy loam soil (blue). An ideal soil, or loam, is comprised of 40% sand, 40% silt, and 20% clay (green). Peat moss and subsequent mixtures were not analyzed as they were not dense enough and did not have enough minerals for the method utilized. Soil texture pyramid provided by the USDA's Natural Resources Conservation Service.

Table 9. Soil analysis results.

	N & C		Nutrients (mg/kg)															Texture		
Soil Type	Tot N %	Tot C %	Al	Ca	Cu	Mg	Mn	Na	P	NO_3	NH_4	K	Zn	Fe	CEC meq/100g	pH	Org. Matt. %	% Sand	% Silt	% Clay
Topsoil	0.48	6.84	0.30	3,377.5	1.6	656.5	11.8	77.9	380.0	29.6	5.1	1,788.3	12.9	72.0	23.65	8.0	12.3	68.00	18.00	14.00
Topsoil/ Perlite	0.51	7.64	<0.00	3,658.7	2.2	830.4	15.1	120.1	438.0	139.9	4.0	1,974.3	14.9	77.6	19.80	7.7	10.0	64.00	22.00	14.00
100:0	1.21	43.65	1.97	6,028.1	0.5	1,814.0	64.0	41.6	30.0	82.4	129.5	226.0	9.5	553.6	43.98	4.7	87.6			
75:25	0.71	27.23	0.02	5,370.5	12.5	4,333.3	56.6	51.9	15.0	87.5	17.9	184.0	7.7	316.0	35.29	5.4	60.8			
50:50	0.51	19.53	<0.00	3,826.1	13.8	6,338.6	57.5	66.4	6.8	32.1	46.7	177.7	7.1	229.8	33.86	6.2	35.3			
25:75	0.23	11.74	<0.00	3,234.5	15.1	6,532.8	30.4	65.5	4.9	8.0	24.9	146.6	6.6	111.0	23.38	7.0	14.6	24.00	68.00	8.00
0:100	0.06	4.87	<0.00	1,779.9	20.3	6,927.0	8.4	74.7	2.8	1.6	2.5	102.0	6.3	62.2	8.15	8.1	2.5	26.00	68.00	6.00
Perlite/Sim	0.10	4.32	<0.00	1,721.6	14.2	6,469.6	5.5	76.6	3.0	1.1	4.3	119.7	4.8	45.4	4.30	7.9	2.6	22.00	70.00	8.00
Perlite	0.25	1.12	<0.00	325.8	0.6	142.0	2.3	77.8	9.7	2.8	3.9	65.2	1.0	11.0	1.16	8.7	0.7			

CHAPTER IV

DISCUSSION AND FUTURE DIRECTIONS

4.1 Plant Growth Discussion

There was an effect on the plant growth of all three crops based on the soil type they were grown in. In all the variables measured, there was a decrease with increasing simulant. Particularly notable is the lack of any germination in the 100% simulant environments. These results may be, in part, due to drought stress on the plants. Low germination rates, decreased plant height, leaf area, and biomass are all indicators of drought stress (Farooq et al., 2009). As stated in the background, plants use water to take in the nutrients from the soil into the roots for growth and development. This is further evident by the composition and soil classification of the simulant, which will be discussed in section 4.2. Though water holding capacity was not measured, it was noticed that, qualitatively, water drained slower and mainly around the edges around the pot when there was more simulant. Furthermore, the sowing depth of 2.54cm across all species may have also been a factor in the lower germination rates among the higher concentrations of simulant.

Interestingly, whenever there was an interaction effect, only the *R. sativus* had any significant differences between means and both *L. sativa* and *C. annuum* were not significantly affected. For instance, in both the results of the plant height and biomass, there was a significant drop between the 100:0 and 25:75 treatments. This may in large part be due to a decrease in the organic matter in the treatments (Kumar et al., 2014) which helps aid soil fertility, aeration, and decreases compaction. Soil compaction can impair root development where radishes primarily grow. Similarly, in every case, plants grown in the topsoil reacted similarly to treatments with

50% simulant or more. Topsoil, without amendments, is dense and had similar weighed pot mass to that of the majority of simulant treatments. Indicating that the simulant may have similar plant-soil interactions as topsoil, besides nutrient content, given that the soils were in individual pots.

Additionally, the amount of organic matter affected the growth and development of the plants in both this study and that of Wamelink et al. (2014), Wamelink et al. (2019) and Duri *et al.* (2020) on the JSC-1A lunar, Mars 1A, and Mojave Mars regolith simulants. Particularly, they found that there was increased biomass and fresh weight at harvest in their regolith simulants when organic matter was added. Similarly, in this study, treatments with more peat moss had more total biomass than those with more simulant. However, Wamelink *et al*. (2014) were able to see germination in both 100% Martian and lunar regolith simulant, whereas there was zero germination in 100% CI simulant.

As stated above, there was zero growth in the perlite/simulant mixture except for one seedling of *C. annuum,* but it did not experience any growth besides two small leaves. Though the intent was to increase aeration to the seeds and roots in the simulant, there still seems to be a deleterious effect on germination in the simulant treatments. Similar to that of Eichler *et al*. 2021, the zero germination in all cases may be due in part by the relatively high pH (8) of the CI simulant and the fine particle size.

4.2 Soil Analysis Discussion

Soil analysis proved to be fruitful in understanding the simulant and how it may interact with the plants. Beginning with the nutrients, nutrient concentrations varied across treatments though showing a decrease as the simulant increased, particularly in the essential nutrients. The amount of nitrate and ammonium is of particular concern as they are considerably low, < 5 mg/kg in the simulant. This indicates little to no nitrification of the simulant, leaving little available soil nitrogen to the plants. Similarly, there is a very low concentration of plant available P in the simulant, < 3 mg/kg. This is likely due to the lack of organic and mineral phosphorus. Surprisingly, there was an adequate amount of plant available potassium, 102 mg/kg. Though, as stated in Britt *et al.* 2019, K_2O in the simulant may be more elevated compared to the Orgueil meteorite due to the use of terrestrial materials and may not represent non-simulated CI regolith. This is in contrast with what is known about the CM meteorites, as Mautner 2014 stated that K could be a limiting element in a space population using carbonaceous resources.

Other soil properties such as pH, CEC, and organic matter were also analyzed. Peat moss is quite acidic at 4.2, which is not entirely conducive for optimal plant growth. Interestingly, the pH increased as the amount of simulant increased with the simulant having the highest at 8.1, indicating a neutralizing effect from the simulant. Though CEC had the opposite response where it decreased in the increasing simulant treatments. This means that the nutrient retention of the simulant is likely to be low and likely cause leaching (Sonon *et al.* 2017). There was less organic matter in the simulant than was anticipated. This had likely contributed to the soil compaction and the decrease in fertility that was noticed.

For soil texture and classification, it was determined that the simulant is a silty loam soil with it being a majority silt (68%). Silt soils have unique challenges when growing plants. This includes crusting and compression. Crusting occurs when the silt particles dry after being watered, which was noticed during the growing process. This crust can be difficult for plants to grow through as it can be dense and tough to crack. Silty soils are also prone to compression as they have a weak structure between the pores and the particles (Warren and Taylor, 2017). This crusting and compression also clogs pores in the soil and subsequently does not allow water to the roots. Additionally, the simulant is comprised of mostly serpentine. Serpentine based soils can prove to be a very challenging environment for plants, especially vegetable crops. These types of soils are low in nutrient content, high in toxic metals, and tend to have high Mg compared to Ca (Gough et al., 1989; Shewry & Peterson, 1975; Turitzin, 1982).

Comparing the results of the soil analysis of the CI simulant to that of both the Murchison and Allende extracts, and the JSC-1A lunar and Mars 1A simulant may provide some useful information. Table 10 compares the values of the plant available nutrients, pH, and CEC measured in Mautner (1997) and Wamelink *et al.* (2014). There are some interesting similarities between each plant medium/extract. For instance, the concentration of NO_3 is low in all three carbonaceous samples (CI simulant or Murchison and Allende). The CI simulant also had a higher CEC value than the Murchison or Allende extract, indicating more slightly organic matter. Additionally, the concentration of all nutrients measured was higher in the CI simulant compared to the Lunar simulant. Lastly, the pH of the CI simulant fell directly between the JSC-1A lunar and Mars-1A simulant.

Table 10. Comparison of nutrients (mg/kg), pH, and CEC (meq/100g) between the CI, JSC-1A Lunar, Mars-1A simulants, and Murchison and Allende extracts[a].

Sample	**P**	**K**	**NO_3**	**NH4**	**Ca**	**Mg**	**Na**	**Fe**	**Al**	**pH**	**CEC**
CI Sim.	2.8	102.0	1.6	2.5	1779.9	6927.0	74.7	62.0	0.0	8.1	8.15
Murchison[b]	6.0	650.0	<10		4000	1700	570	126000	3000		5.8
Allende[b]	1600	30	2		130	130	60	43000	160		0.4
JSC-1A Lunar[c]	0.2	27		0.3				0	0.6	9.6	
Mars-1A[c]	0	138		3.9				0	0	7.3	

[a]Methods used to collect nutrient concentrations do differ from each other.
[b]Values derived from Mautner 1997
[c]Values derived from Wamelink 2014

4.3 Future Directions

The results from these plant growth experiments and the soil analysis pave paths for future research with this simulant. Recommendations for future research would primarily focus on alleviating the compaction of the simulant. Adding organic matter via plant waste from crops grown in a different planting medium then subsequently adding it to the simulant is an option. This mimics that of plants grown on spacecraft that leave leftover waste that could be used in recycling efforts for BLSS. Along these lines, experimenting with various soil amendments to adjust soil texture, such as adding clay and increasing pore size, may improve yield on crops. Similarly, the addition of nitrogen fixing bacteria to the simulant may aid in nitrification of the simulant, increasing the amount of plant available N.

Additionally, extracting the nutrients from the simulant may be able to bypass the soil texture and physical properties. Similar to that of the Mautner experiments, extracting the nutrients from the simulant could aid in formulating hydroponic solutions using the extract. Nutrient extract may also have the benefit of being selective of the nutrients, so that toxic elements can be bypassed. Similarly, plant tissues after being grown in the simulant should be analyzed to determine nutritional value and identify any toxic heavy metals. This was not included in this study as the plants were too small to be analyzed.

Lastly, the other developed asteroid simulants should be evaluated. Though grounded Murchison (CM) meteorite has been tested, evaluating the CM simulant will help both verify the quality of the simulant, as well as allow a direct comparison to the CI simulant. CR simulant or actual meteorite has not been tested in regard to plants, so this could aid in the understanding of these asteroid simulants. Using the other simulants would provide good comparison studies to the CI simulant.

CHAPTER V

CONCLUSION

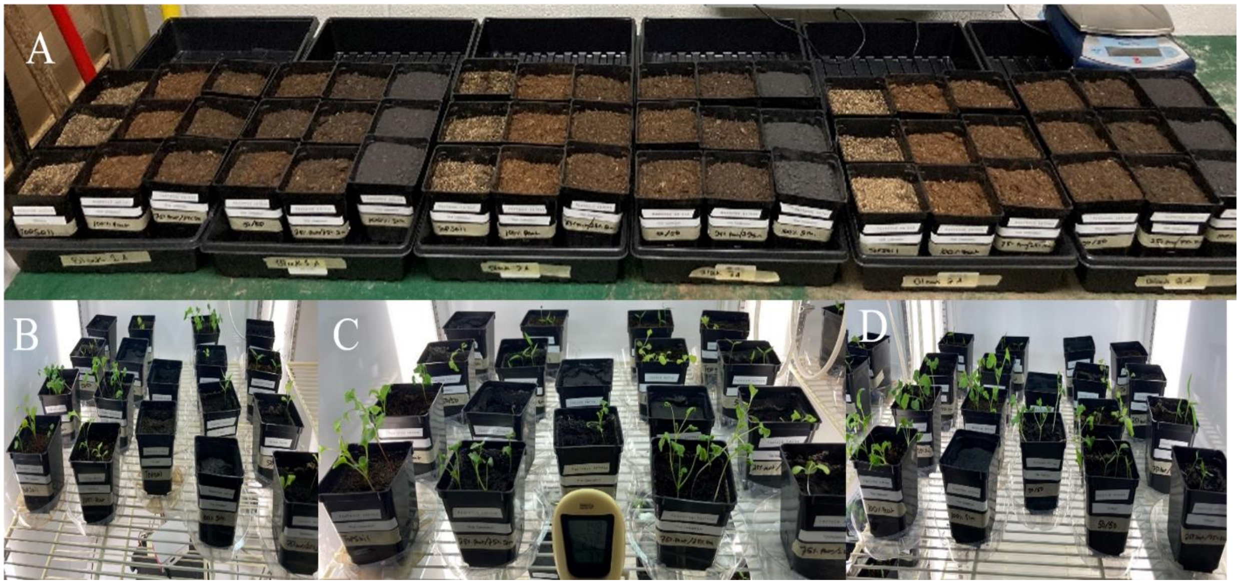
This research aimed to study the growth properties of plants grown in CI asteroid simulant, as well as its soil characteristics. The results of this study showed growth of crops in a simulated asteroid regolith from seed to mature plant, though there was decreasing growth with increasing simulant. However, it was clear that a total simulant environment was not conducive for plant growth. It is to be hypothesized that the simulant had difficulties providing enough air and water to the seeds and the roots due to its mineral composition and soil texture. Nutrient analyses showed that the simulant was deficient in nitrogen and phosphorus, though it does provide adequate amounts of trace elements and potassium.

While the simulant is not a 1:1 match to the Orgueil meteorite, it is a high-quality simulant that can be used for many applications such as this study. Along with this, soil analyses showed similar soil characteristics to that of CM meteorites and other simulants. Moreover, these data are an addition to the small dataset of plant studies in regolith simulants. Though recommendations of using plant waste, nitrogen fixing bacteria, different soil amendments, or extracting the nutrients should be a focus of future studies.

In all, this study provided interesting insights and data that could contribute to future missions to asteroids. Living off the land and utilizing the resources available is a key component in the development of a space-based population. Determining the interactions between plants and said resources is a steppingstone to accomplishing that. As humans continue forth into the solar system and beyond, understanding how plants grow and adapt for food production in a seemingly hospitable environment will be crucial for sustainability in space.

APPENDENCIES

Appendix A



Appendix Figure. Experimental Set-up. (A) Pots filled with planting media from 100% peat (left) to 100% CI simulant (right). (B-D) Complete randomized blocks of 3 blocks with 18 pots/block set up in the Percival AR-66L.

Appendix B

Appendix B Table. Germination percentage of Experiment B. Only 1 seed germinated in the Perlite/Simulant environment out of 45 total seeds.

Species	Soil Type	Germinated	Total Seeds	%
L. sativa 'Outredgeous'	Topsoil	7	15	47
	Perlite	15	15	100
	Perlite/Simulant	0	15	0
R. sativus 'Pink Celebration'	Topsoil	13	15	87
	Perlite	13	15	87
	Perlite/Simulant	0	15	0
C. annuum 'Chimayo'	Topsoil	10	15	67
	Perlite	14	15	93
	Perlite/Simulant	1	15	7

REFERENCES

Abell, P. A., Korsmeyer, D. J., Landis, R. R., Jones, T. D., Adamo, D. R., Morrison, D. D., Lemke, L. G., Gonzales, A. A., Gershman, R., Sweetser, T. H., Johnson, L. L., & Lu, E. (2009). Scientific Exploration of Near-Earth Objects via the Orion Crew Exploration Vehicle. *Meteoritics & Planetary Science*, *44*(12), 1825–1836. https://doi.org/https://doi.org/10.1111/j.1945-5100.2009.tb01991.x

Alexander, C. M. O., Bowden, R., Fogel, M. L., Howard, K. T., Herd, C. D. K., & Nittler, L. R. (2012). The Provenances of Asteroids, and their Contributions to the Volatile Inventories of the Terrestrial Planets. *Science*, *337*(6095), 721–723. https://doi.org/10.1126/science.1223474

Alexander, C. M. O., Grossman, J. N., Ebel, D. S., & Ciesla, F. J. (2008). The Formation Conditions of Chondrules and Chondrites. *Science*, *320*(5883), 1617–1619. https://doi.org/10.1126/science.1156561

Allen, C. C., Morris, R. V., Jager, K. M., Golden, D. C., Lindstrom, D. J., Lindstrom, M. M., & Lockwood, J. P. (1998). Martian Regolith Simulant JSC Mars-1. *Lunar and Planetary Science Conference*, 1690.

Anand, M., Crawford, I. A., Balat-Pichelin, M., Abanades, S., van Westrenen, W., Péraudeau, G., Jaumann, R., & Seboldt, W. (2012). A Brief Review of Chemical and Mineralogical Resources on the Moon and Likely Initial In Situ Resource Utilization (ISRU) applications. *Planetary and Space Science*, *74*(1), 42–48. https://doi.org/https://doi.org/10.1016/j.pss.2012.08.012

Anders, E. (1989). Pre-Biotic Organic Matter from Comets and Asteroids. *Nature*, *342*(6247), 255–257. https://doi.org/10.1038/342255a0

Ash, R. L., Dowler, W. L., & Varsi, G. (1978). Feasibility of Rocket Propellant Production on Mars. *Acta Astronautica*, *5*(9), 705–724. https://doi.org/https://doi.org/10.1016/0094-5765(78)90049-8

Barrat, J. A., Zanda, B., Moynier, F., Bollinger, C., Liorzou, C., & Bayon, G. (2012). Geochemistry of CI Chondrites: Major and Trace Elements, and Cu and Zn Isotopes. *Geochimica et Cosmochimica Acta*, *83*, 79–92. https://doi.org/https://doi.org/10.1016/j.gca.2011.12.011

Belyavskaya, N. A. (1996). Free and Membrane-Bound Calcium in Microgravity and Microgravity Effects at the Membrane Level. *Advances in Space Research : The Official Journal of the Committee on Space Research (COSPAR)*, *17*(6–7), 169–177. https://doi.org/10.1016/0273-1177(95)00631-n

Binzel, R. P. (2014). Human Spaceflight: Find Asteroids to Get to Mars. *Nature*, *514*(7524), 559–561. https://doi.org/10.1038/514559a

Brearley, A. J. (2006). The Action of Water. In D. S. Lauretta & H. Y. McSween (Eds.), *Meteorites and the Early Solar System II* (p. 584).

Britt, D. T., Cannon, K. M., Donaldson Hanna, K., Hogancamp, J., Poch, O., Beck, P., Martin, D., Escrig, J., Bonal, L., & Metzger, P. T. (2019). Simulated Asteroid Materials Based on

Carbonaceous Chondrite Mineralogies. *Meteoritics & Planetary Science*, *54*(9), 2067–2082. https://doi.org/https://doi.org/10.1111/maps.13345

Burbine, T. H., McCoy, T. J., Meibom, A., Gladman, B., & Keil, K. (2002). Meteoritic Parent Bodies: Their Number and Identification. In *Asteroids III* (pp. 653–667).

Buseck, P. R., & Hua, X. (1993). Matrices of Carbonaceous Chondrite Meteorites. *Annual Review of Earth and Planetary Sciences*, *21*(1), 255–305. https://doi.org/10.1146/annurev.ea.21.050193.001351

Cannon, K. M., Britt, D. T., Smith, T. M., Fritsche, R. F., & Batcheldor, D. (2019). Mars Global Simulant MGS-1: A Rocknest-Based Open Standard for Basaltic Martian Regolith Simulants. *Icarus*, *317*, 470–478. https://doi.org/https://doi.org/10.1016/j.icarus.2018.08.019

Certini, G., Scalenghe, R., & Amundson, R. (2009). A View of Extraterrestrial Soils. *European Journal of Soil Science*, *60*(6), 1078–1092. https://doi.org/https://doi.org/10.1111/j.1365-2389.2009.01173.x

Chapman, C. R. (2004). The Hazard of Near-Earth Asteroid Impacts on Earth. *Earth and Planetary Science Letters*, *222*(1), 1–15. https://doi.org/https://doi.org/10.1016/j.epsl.2004.03.004

Chyba, C., & Sagan, C. (1992). Endogenous Production, Exogenous Delivery and Impact-Shock Synthesis of Organic Molecules: An Inventory for the Origins of Life. *Nature*, *355*(6356), 125–132. https://doi.org/10.1038/355125a0

Clauwaert, P., Muys, M., Alloul, A., De Paepe, J., Luther, A., Sun, X., Ilgrande, C., Christiaens, M. E. R., Hu, X., Zhang, D., Lindeboom, R. E. F., Sas, B., Rabaey, K., Boon, N., Ronsse, F., Geelen, D., & Vlaeminck, S. E. (2017). Nitrogen Cycling in Bioregenerative Life Support Systems: Challenges for Waste Refinery and Food Production Processes. *Progress in Aerospace Sciences*, *91*, 87–98. https://doi.org/https://doi.org/10.1016/j.paerosci.2017.04.002

Connelly, J. N., Bizzarro, M., Krot, A. N., Nordlund, \r Ake, Wielandt, D., & Ivanova, M. A. (2012). The Absolute Chronology and Thermal Processing of Solids in the Solar Protoplanetary Disk. *Science*, *338*(6107), 651–655. https://doi.org/10.1126/science.1226919

Cooper, G., Kimmich, N., Belisle, W., Sarinana, J., Brabham, K., & Garrel, L. (2001). Carbonaceous Meteorites as a Source of Sugar-Related Organic Compounds for the Early Earth. *Nature*, *414*(6866), 879–883. https://doi.org/10.1038/414879a

Cronin, J. R., & Moore, C. B. (1971). Amino Acid Analyses of the Murchison, Murray, and Allende Carbonaceous Chondrites. *Science*, *172*(3990), 1327–1329). http://www.jstor.org.ezproxy.library.und.edu/stable/1732458

Cruikshank, D. P. (1997). Organic Matter in the Outer Solar System: From the Meteorites to the Kuiper Belt. *From Stardust to Planetesimals* (Vol. 122, p. 315-333).

Duri, L. G., El-Nakhel, C., Caporale, A. G., Ciriello, M., Graziani, G., Pannico, A., Palladino, M., Ritieni, A., De Pascale, S., Vingiani, S., Adamo, P., & Rouphael, Y. (2020). Mars Regolith Simulant Ameliorated by Compost as in situ Cultivation Substrate Improves

Lettuce Growth and Nutritional Aspects. In *Plants* (Vol. 9, Issue 5). https://doi.org/10.3390/plants9050628

Eichler, A., Hadland, N., Pickett, D., Masaitis, D., Handy, D., Perez, A., Batcheldor, D., Wheeler, B., & Palmer, A. (2021). Challenging the Agricultural Viability of Martian Regolith Simulants. *Icarus*, *354*, 114022. https://doi.org/https://doi.org/10.1016/j.icarus.2020.114022

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. M. A. (2009). Plant Drought Stress: Effects, Mechanisms and Management. *Agronomy for Sustainable Development*, *29*(1), 185–212. https://doi.org/10.1051/agro:2008021

Feierberg, M. A., Lebofsky, L. A., & Larson, H. P. (1981). Spectroscopic Evidence for Aqueous Alteration Products on the Surfaces of Low-Albedo Asteroids. *Geochimica et Cosmochimica Acta*, *45*(6), 971–981. https://doi.org/https://doi.org/10.1016/0016-7037(81)90121-6

Ferl, R., Wheeler, R., Levine, H. G., & Paul, A. L. (2002). Plants in Space. *Current Opinion in Plant Biology*, *5*(3), 258–263. https://doi.org/10.1016/s1369-5266(02)00254-6

Gaffey, M. J., Burbine, T. H., & Binzel, R. P. (1993). Asteroid Spectroscopy: Progress and Perspectives. *Meteoritics*, *28*(2), 161. https://doi.org/10.1111/j.1945-5100.1993.tb00755.x

Gaffey, M. J., Cloutis, E. A., Kelley, M. S., & Reed, K. L. (2002). Mineralogy of Asteroids. In *Asteroids III* (pp. 183–204).

Giese, C.-C., Ten Kate, I. L., Plümper, O., King, H. E., Lenting, C., Liu, Y., & Tielens, A. G. G. M. (2019). The Evolution of Polycyclic Aromatic Hydrocarbons Under Simulated Inner Asteroid Conditions. *Meteoritics & Planetary Science*, *54*(9), 1930–1950. https://doi.org/https://doi.org/10.1111/maps.13359

Gòdia, F., Albiol, J., Montesinos, J. L., Pérez, J., Creus, N., Cabello, F., Mengual, X., Montras, A., & Lasseur, C. (2002). MELISSA: A Loop of Interconnected Bioreactors to Develop Life Support in Space. *Journal of Biotechnology*, *99*(3), 319–330. https://doi.org/https://doi.org/10.1016/S0168-1656(02)00222-5

Gough, L. P., Meadows, G. R., Jackson, L. L., & Dudka, S. (1989). Biogeochemistry of a Highly Serpentinized, Chromite-Rich Ultramafic Area, Tehama County, California. In *Bulletin*. https://doi.org/10.3133/b1901

Gurevitch, J., Schiner, S.M., Fox, G.A., 2018. The Ecology of Plants, 2nd edn. Sinauer Associates, Inc., Sunderland, Massachusetts, pp 82-92.

Hamilton, V. E., Simon, A. A., Christensen, P. R., Reuter, D. C., Clark, B. E., Barucci, M. A., Bowles, N. E., Boynton, W. V, Brucato, J. R., Cloutis, E. A., Connolly, H. C., Donaldson Hanna, K. L., Emery, J. P., Enos, H. L., Fornasier, S., Haberle, C. W., Hanna, R. D., Howell, E. S., Kaplan, H. H., … the OSIRIS-REx Team. (2019). Evidence for Widespread Hydrated Minerals on Asteroid (101955) Bennu. *Nature Astronomy*, *3*(4), 332–340. https://doi.org/10.1038/s41550-019-0722-2

Hayatsu, R., Winans, R. E., Scott, R. G., McBeth, R. L., Moore, L. P., & Studier, M. H. (1980). Phenolic Ethers in the Organic Polymer of the Murchison Meteorite. *Science*, *207*(4436), 1202 LP – 1204. https://doi.org/10.1126/science.207.4436.1202

Hergenrother, C. W., Adam, C. D., Chesley, S. R., & Lauretta, D. S. (2020). Introduction to the Special Issue: Exploration of the Activity of Asteroid (101955) Bennu. *Journal of Geophysical Research: Planets*, *125*(9), e2020JE006549. https://doi.org/https://doi.org/10.1029/2020JE006549

Hummerick, M., Gates, J., Nguyen, B.-T., Massa, G., & Wheeler, R. (2012). The Effect of Plant Cultivar, Growth Media, Harvest Method and Post Harvest Treatment on the Microbiology of Edible Crops. In *42nd International Conference on Environmental Systems*. American Institute of Aeronautics and Astronautics. https://doi.org/doi:10.2514/6.2012-3506

Jewitt, D., Chizmadia, L., Grimm, R., & Prialnik, D. (2007). Water in the Small Bodies of the Solar System. *In Protostars and Planets V*, University of Arizona Press.

Jones, T. D., Lebofsky, L. A., Lewis, J. S., & Marley, M. S. (1990). TheComposition and Origin of the C, P, and D asteroids: Water as a Tracer of Thermal Evolution in the Outer Belt. *Icarus, 88*(1), 172–192. https://doi.org/https://doi.org/10.1016/0019-1035(90)90184-B

Jones, T., Davis, D., Durda, D., Farquhar, R., Gefert, L., Hack, K., Hartmann, W., Jedicke, R., Lewis, J., Love, S., Sykes, M., & Vilas, F. (2002). The Next Giant Leap: Human Exploration and Utilization of Near-Earth Objects. *ASP Conference Series*, *272*, 141–154.

Kitazato, K., Milliken, R. E., Iwata, T., Abe, M., Ohtake, M., Matsuura, S., Arai, T., Nakauchi, Y., Nakamura, T., Matsuoka, M., Senshu, H., Hirata, N., Hiroi, T., Pilorget, C., Brunetto, R., Poulet, F., Riu, L., Bibring, J.-P., Takir, D., … Tsuda, Y. (2019). The Surface Composition of Asteroid 162173 Ryugu from Hayabusa2 Near-Infrared Spectroscopy. *Science*, *364*(6437), 272 LP – 275. https://doi.org/10.1126/science.aav7432

Kumar, S., Maji, S., Kumar, S., & Singh, H. D. (2014). Efficacy of Organic Manures on Growth and Yield of Radish (Raphanus sativus L.) cv. Japanese White. *International Journal of Plant Sciences (Muzaffarnagar)*, *9*(1), 57–60.

Levine, H. G., & Krikorian, A. D. (2008). Changes in Plant Medium Composition After a Spaceflight Experiment: Potassium Levels are of Ppecial Interest. *Advances in Space Research*, *42*(6), 1060–1065. https://doi.org/https://doi.org/10.1016/j.asr.2008.03.019

Marcano, V., Matheus, P., Cedeño, C., Falcon, N., & Palacios-Prü, E. (2005). Effects of Non-Carbonaceous Meteoritic Extracts on the Germination, Growth and Chlorophyll Content of Edible Plants. *Planetary and Space Science*, *53*(12), 1263–1279. https://doi.org/https://doi.org/10.1016/j.pss.2005.05.003

Martínez-Jiménez, M., Moyano-Cambero, C. E., Trigo-Rodríguez, J. M., Alonso-Azcárate, J., & Llorca, J. (2017). Asteroid Mining: Mineral Resources in Undifferentiated Bodies from the Chemical Composition of Carbonaceous Chondrites. In J. M. Trigo-Rodríguez, M. Gritsevich, & H. Palme (Eds.) Assessment and Mitigation of Asteroid Impact Hazards, *Astrophysics and Space Science Proceedings, 46,* 73–101. Springer International Publishing.

Massa, G. D., Wheeler, R. M., Morrow, R. C., & Levine, H. G. (2016). Growth Chambers on the International Space Station for Large Plants. *Acta Horticulturae*, *1134*, 215–222.

https://doi.org/10.17660/ActaHortic.2016.1134.29

Massa, G, Newsham, G., Hummerick, M., Caro, J., Stutte, G., & Wheeler, R. (2013). Preliminary Species and Media Selection for the Veggie Space Hardware. *Gravitational and Space Research*, *1*, 96-106.

Massa, Gioia, Dufour, N., Carver, J., Hummerick, M., Wheeler, R., Morrow, R., & Smith, T. M. (2017). VEG-01: Veggie Hardware Validation Testing on the International Space Station. *Open Agriculture*, *2*, 33-41. https://doi.org/10.1515/opag-2017-0003

Mautner, M. N. (1997). Biological potential of extraterrestrial materials—1. Nutrients in Carbonaceous Meteorites, and Effects on Biological Growth. *Planetary and Space Science*, *45*(6), 653–664. https://doi.org/https://doi.org/10.1016/S0032-0633(97)00017-2

Mautner, M. N. (2014). In Situ Biological Resources: Soluble Nutrients and Electrolytes in Carbonaceous Asteroids/Meteorites. Implications for Astroecology and Human Space Populations. *Planetary and Space Science*, *104*, 234–243. https://doi.org/https://doi.org/10.1016/j.pss.2014.10.001

Mautner, M. N., Conner, A. J., Killham, K., & Deamer, D. W. (1997). Biological Potential of Extraterrestrial Materials. *Icarus*, *129*(1), 245–253. https://doi.org/https://doi.org/10.1006/icar.1997.5786

McAdam, M. M., Sunshine, J. M., Howard, K. T., & McCoy, T. M. (2015). Aqueous Alteration on Asteroids: Linking the Mineralogy and Spectroscopy of CM and CI Chondrites. *Icarus*, *245*, 320–332. https://doi.org/https://doi.org/10.1016/j.icarus.2014.09.041

McKay, D. S., Carter, J. L., Boles, W. W., Allen, C. C., & Allton, J. H. (1993). JSC-1: A New Lunar Regolith Simulant. *Lunar and Planetary Science Conference*, 963.

Merényi, E., Howell, E. S., Rivkin, A. S., & Lebofsky, L. A. (1997). Prediction of Water in Asteroids from Spectral Data Shortward of 3 μm. *Icarus*, *129*(2), 421–439. https://doi.org/https://doi.org/10.1006/icar.1997.5796

Merkys, A. J., Laurinavičius, R. S., & Švegždiene, D. V. (1984). Plant Growth, Development and Embryogenesis During Salyut-7 Flight. *Advances in Space Research*, *4*(10), 55–63. https://doi.org/https://doi.org/10.1016/0273-1177(84)90224-2

Metzger, P. T., & Britt, D. T. (2020). Model for Asteroid Regolith to Guide Simulant Development. *Icarus*, *350*, 113904. https://doi.org/https://doi.org/10.1016/j.icarus.2020.113904

Metzger, P. T., Britt, D. T., Covey, S., Schultz, C., Cannon, K. M., Grossman, K. D., Mantovani, J. G., & Mueller, R. P. (2019). Measuring the Fidelity of Asteroid Regolith and Cobble Simulants. *Icarus, 321*, 632–646. https://doi.org/https://doi.org/10.1016/j.icarus.2018.12.019

Milliken, R. E., & Mustard, J. F. (2007). Estimating the Water Content of Hydrated Minerals Using Reflectance Spectroscopy. I. Effects of Darkening Agents and Low-Albedo Materials. *Icarus*, *189*, 550–573. https://doi.org/10.1016/j.icarus.2007.02.017

Morgan, J. B. & Connolly, E. L. (2013) Plant-Soil Interactions: Nutrient Uptake. *Nature*

Education Knowledge 4(8):2

Morohashi, K., Okamoto, M., Yamazaki, C., Fujii, N., Miyazawa, Y., Kamada, M., Kasahara, H., Osada, I., Shimazu, T., Fusejima, Y., Higashibata, A., Yamazaki, T., Ishioka, N., Kobayashi, A., & Takahashi, H. (2017). Gravitropism Interferes with Hydrotropism via Counteracting Auxin Dynamics in Cucumber Roots: Clinorotation and Spaceflight Experiments. *New Phytologist*, *215*(4), 1476–1489. https://doi.org/https://doi.org/10.1111/nph.14689

Morrow, R. C., Remiker, R. W., Mischnick, M. J., Tuominen, L. K., Lee, M. C., & Crabb, T. M. (2005, July). A Low Equivalent System Mass Plant Growth Unit for Space Exploration. *International Conference On Environmental Systems*. https://doi.org/https://doi.org/10.4271/2005-01-2843

Morrow, R., Richter, R., Tellez, G., Monje, O., Wheeler, R., Massa, G., Dufour, N., & Onate, B. (2016). A New Plant Habitat Facility for the ISS. *International Conference On Environmental Systems*. 320.

Nathan, M. V., (2009) Soils, Plant Nutrition and Nutrient Management. University of Missouri Extension Publication MG4.

Nechitailo, G., & Gordeev, A. (2001). Effect of Artificial Electric Fields on Plants Grown Under Microgravity Conditions. *Advances in Space Research : The Official Journal of the Committee on Space Research (COSPAR)*, *28*(4), 629–631. https://doi.org/10.1016/s0273-1177(01)00370-2

Nolan, M. C., Magri, C., Howell, E. S., Benner, L. A. M., Giorgini, J. D., Hergenrother, C. W., Hudson, R. S., Lauretta, D. S., Margot, J.-L., Ostro, S. J., & Scheeres, D. J. (2013). Shape Model and Surface Properties of the OSIRIS-REx Target Asteroid (101955) Bennu from Radar and Lightcurve Observations. *Icarus*, *226*(1), 629–640. https://doi.org/https://doi.org/10.1016/j.icarus.2013.05.028

O'Brien, D. P., & Sykes, M. V. (2011). The Origin and Evolution of the Asteroid Belt—Implications for Vesta and Ceres. *Space Science Reviews*, *163*(1), 41–61. https://doi.org/10.1007/s11214-011-9808-6

O'Leary, B. (1977). Mining the Apollo and Amor asteroids. *Science*, *197*(4301), 363–366. https://doi.org/10.1126/science.197.4301.363-a

O'Neill, G. K. (1974). The Colonization of Space. *Physics Today*, *27*(9), 32–40. https://doi.org/10.1063/1.3128863

Patel, A. B., Shaikh, S., Jain, K. R., Desai, C., & Madamwar, D. (2020). Polycyclic Aromatic Hydrocarbons: Sources, Toxicity, and Remediation Approaches. *Frontiers in Microbiology*, *11*, 562813. https://doi.org/10.3389/fmicb.2020.562813

Paul, A.L., Wheeler, R. M., Levine, H. G., & Ferl, R. J. (2013). Fundamental Plant Biology Enabled by The Space Shuttle. *American Journal of Botany*, *100*(1), 226–234. https://doi.org/https://doi.org/10.3732/ajb.1200338

Perchonok, M., & Bourland, C. (2002). NASA Food Systems: Past, Present, and Future. *Nutrition*, *18*(10), 913–920. https://doi.org/https://doi.org/10.1016/S0899-9007(02)00910-3

Peters, G. H., Abbey, W., Bearman, G. H., Mungas, G. S., Smith, J. A., Anderson, R. C., Douglas, S., & Beegle, L. W. (2008). Mojave Mars Simulant—Characterization of a New Geologic Mars Analog. *Icarus*, *197*(2), 470–479. https://doi.org/https://doi.org/10.1016/j.icarus.2008.05.004

Ross, S. (2001). Near-Earth Asteroid Mining. *Space Industry Report.*

Sacksteder, K., & Sanders, G. (2012). In-Situ Resource Utilization for Lunar and Mars Exploration. In *45th AIAA Aerospace Sciences Meeting and Exhibit*. https://doi.org/10.2514/6.2007-345

Schrader, C., Rickman, D., McLemore, C., Fikes, J., Stoeser, D., Wentworth, S., & McKay, D. (2009). Lunar Regolith Characterization for Simulant Design and Evaluation Using Figure of Merit Algorithms. In *47th AIAA Aerospace Sciences Meeting including The New Horizons Forum and Aerospace Exposition*. American Institute of Aeronautics and Astronautics. https://doi.org/doi:10.2514/6.2009-755

Scott, E. R. D., & Krot, A. N. (2007). Chondrites and Their Components. *Treatise on Geochemistry*, *1*, 1-72. https://doi.org/https://doi.org/10.1016/B0-08-043751-6/01145-2

Shewry, P. R., & Peterson, P. J. (1975). Calcium and Magnesium in Plants and Soil from a Serpentine Area on Unst, Shetland. *Journal of Applied Ecology*, *12*(1), 381–391. https://doi.org/10.2307/2401740

Singleton, V. L., & Kratzer, F. H. (1969). Toxicity and Related Physiological Activity of Phenolic Substances of Plant Origin. *Journal of Agricultural and Food Chemistry*, *17*(3), 497–512. https://doi.org/10.1021/jf60163a004

Smith, M., Craig, D., Herrmann, N., Mahoney, E., Krezel, J., McIntyre, N., & Goodliff, K. (2020). The Artemis Program: An Overview of NASA's Activities to Return Humans to the Moon. *2020 IEEE Aerospace Conference*, 1–10. https://doi.org/10.1109/AERO47225.2020.9172323

Soil Survey Staff. 2014. Keys to Soil Taxonomy, 12th ed. USDA-Natural Resources Conservation Service, Washington, DC.

Sonon, L.S., Kissel, D.E., & Saha, U., (2014) Cation Exchange Capacity and Base Saturation. University of Georgia Extension Publication C 1040.

Stutte, G. W., Monje, O., Hatfield, R. D., Paul, A.-L., Ferl, R. J., & Simone, C. G. (2006). Microgravity Effects on Leaf Morphology, Cell Structure, Carbon Metabolism and mRNA Expression of Dwarf Wheat. *Planta*, *224*(5), 1038–1049. https://doi.org/10.1007/s00425-006-0290-4

Tholen, D. J. (1989). Asteroid Taxonomic Classifications. University of Arizona Press. http://inis.iaea.org/search/search.aspx?orig_q=RN:22000986

Tsuchiyama, A., Uesugi, M., Matsushima, T., Michikami, T., Kadono, T., Nakamura, T., Uesugi, K., Nakano, T., Sandford, S. A., Noguchi, R., Matsumoto, T., Matsuno, J., Nagano, T., Imai, Y., Takeuchi, A., Suzuki, Y., Ogami, T., Katagiri, J., Ebihara, M., … Kawaguchi, J. (2011). Three-Dimensional Structure of Hayabusa Samples: Origin and Evolution of Itokawa Regolith. *Science*, *333*(6046), 1125 LP – 1128.

https://doi.org/10.1126/science.1207807

Turitzin, S. N. (1982). Nutrient Limitations to Plant Growth in a California Serpentine Grassland. *The American Midland Naturalist*, *107*(1), 95–99. https://doi.org/10.2307/2425191

Van Schmus, W. R., & Wood, J. A. (1967). A Chemical-Petrologic Classification for the Chondritic Meteorites. *Geochimica et Cosmochimica Acta*, *31*(5), 747–765. https://doi.org/https://doi.org/10.1016/S0016-7037(67)80030-9

Vandenbrink, J. P., & Kiss, J. Z. (2016). Space, the Final Frontier: A Critical Review of Recent Experiments Performed in Microgravity. *Plant Science : An International Journal of Experimental Plant Biology*, *243*, 115–119. https://doi.org/10.1016/j.plantsci.2015.11.004

Wada, K., Grott, M., Michel, P., Walsh, K. J., Barucci, A. M., Biele, J., Blum, J., Ernst, C. M., Grundmann, J. T., Gundlach, B., Hagermann, A., Hamm, M., Jutzi, M., Kim, M.-J., Kührt, E., Le Corre, L., Libourel, G., Lichtenheldt, R., Maturilli, A., … International Regolith Science Group (ISRG) in Hayabusa2 project (2018). Asteroid Ryugu Before the Hayabusa2 Encounter. *Progress in Earth and Planetary Science*, *5*(1), 82. https://doi.org/10.1186/s40645-018-0237-y

Wamelink, G W W, Frissel, J. Y., Krijnen, W. H. J., & Verwoert, M. R. (2019). Crop Growth and Viability of Seeds on Mars and Moon Soil Simulants. *Open Agriculture*, *4*(1), 509–516. https://doi.org/doi:10.1515/opag-2019-0051

Wamelink, G W Wieger, Frissel, J. Y., Krijnen, W. H. J., Verwoert, M. R., & Goedhart, P. W. (2014). Can Plants Grow on Mars and the Moon: A Growth Experiment on Mars and Moon Soil Simulants. *PLOS ONE*, *9*(8), e103138. https://doi.org/10.1371/journal.pone.0103138

Warren, J. & Taylor, R. (2017) Managing Soil Compaction. Oklahoma Cooperative Extension Publication PSS-2244

Wasson, J. T., & Kallemeyn, G. W. (1988). Compositions of Chondrites. *Philosophical Transactions of the Royal Society of London. Series A, Mathematical and Physical Sciences*, *325*(1587), 535–544. http://www.jstor.org/stable/37983

Weisberg, M. K., McCoy, T. J., & Krot, A. N. (2006). Systematics and Evaluation of Meteorite Classification. In Meteorites and the Early Solar System II. University of Arizona Press (p. 19-52).

Wheeler, R. (2010). Plants for Human Life Support in Space: From Myers to Mars. *Gravitational and Space Biology*, *23*.

Wheeler, R. M., Mackowiak, C. L., Stutte, G. W., Sager, J. C., Yorio, N. C., Ruffe, L. M., Fortson, R. E., Dreschel, T. W., Knott, W. M., & Corey, K. A. (1996). NASA's Biomass Production Chamber: A Testbed for Bioregenerative Life Support Studies. *Advances in Space Research : The Official Journal of the Committee on Space Research (COSPAR)*, *18*(4–5), 215–224. https://doi.org/10.1016/0273-1177(95)00880-n

Williams, D. (2002). Isolation and Integrated Testing: an Introduction to the Lunar-Mars Life Support Test Project. *Isolation - NASA Experiments in Closed-Environment Living*, *104*, 1-5.

Williamson, J., M. Kluepfel, and B. Lippert., (2012) Changing the pH of Your Soil. Clemson Cooperative Extension Publication HGIC 1650.

Zabel, P., Bamsey, M., Schubert, D., & Tajmar, M. (2016). Review and Analysis of Over 40 Years of Space Plant Growth Systems. *Life Sciences in Space Research*, *10*, 1–16. https://doi.org/https://doi.org/10.1016/j.lssr.2016.06.004

Zellner, B., & Gradie, J. (1976). Minor Planets and related objects. XX. Polarimetric evidence for the albedos and compositions of 94 asteroids. *The Astronomical Journal*, *81(4)*, 262–280. https://doi.org/10.1086/111882