Basic Lunar Organism Observations and Measurements (Bloom); Investigation of Microorganisms and Plant Growth on the Lunar Surface in Lunar Regolith as a First Step in Protecting Health of Astronauts and Providing In -Situ Grown Food.

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Introduction: Determining how biology responds to the lunar environment will help in understanding how to maintain astronaut health by understanding potentially toxic/harmful lunar environment, sustainably explore space with humans by providing foundations for lunar farming, and inform planetary protection policy.

Organisms have never been grown in freshly collected lunar regolith in a habitat on the lunar surface; therefore, there are many open questions about how lunar regolith and the lunar environment will interact with biology (Table 1). The Chang'e 4 probe successfully demonstrated the germination and growth of cotton seeds in a sealed container on the Moon using cotton fiber as a growth support [1]; this accomplishment has provided foundational work for in-situ biological investigations. The next logical step is to investigate how organisms respond when exposed to lunar regolith inside a habitat on the Moon.

Table 1. Potential benefits and issues with using lunar regolith to support growth of organisms.

Benefits	Issues
Provide a solid-support matrix for plant growth	Potentially toxic elements
Buffering capacity	pH
Nutrient source	Nutrient availability
Nutrient storage	Air and fluid movement
Nutrient retention	Water retention
	Cation exchange capacity

The biological response to the lunar environment and the Moon's highly reactive regolith is not well understood. The potential toxicity of in -situ lunar regolith must not be underestimated and must be understood if we are to understand the potential for microbial survival and contamination to other planetary surfaces.

Metals like aluminum, iron, and chromium are present in lunar regolith. Aluminum is known to disturb plant growth and can lead to death [2]. Further, due to the lack of an atmosphere, lunar regolith is continuously exposed to some combination of ultraviolet radiation, solar wind, cosmic rays, and meteorite impacts, all of which can "activate" the soil and lead to the production of reactive surface species [3] that can be toxic. The agents of toxicity are thought to include reactive oxygen species (ROS) and highly-reduced nanophase metallic iron in the size rage of < 0.2 micrometer [4]. ROS are chemically reactive molecules that contain oxygen, superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH). Generally speaking, the aged lunar samples we have on Earth do not contain these reactive entities in representative abundances as many will decay, (re)combine, or otherwise passivate after their generation on time scales ranging from a sub-second to hours.

Broader contamination challenges lie ahead regarding a more sustained human presence on the Moon (and eventually on Mars). Such considerations should be kept in mind as we prepare for sustained human exploration [5,6].

Instrument: The Basic Lunar Organism Observations and Measurements (BLOOM) suite (Figure 1;Table 2) is a proposed Lunar science payload that will pioneer new knowledge about how living systems

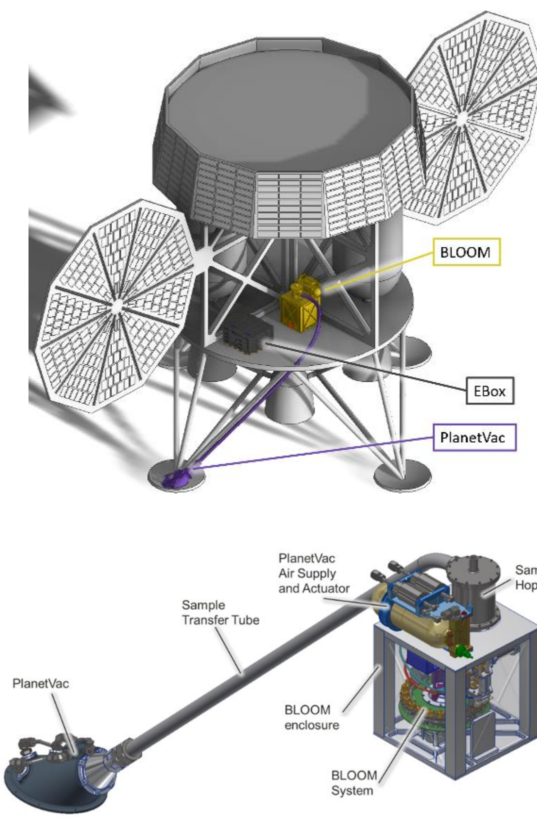

Figure 1. Conceptual design of the BLOOM System. Top: BLOOM, Electronics Box, and PlanetVac on a CLPS lander. Bottom: CAD design of the BLOOM system.

respond to the lunar environment by conducting a series of growth experiments in lunar regolith and by characterizing the regolith.

Table 2. List and description of the BLOOM instruments.

Instrument	Description
AIM: Analysis of lunar regolith Inclusion with Microorganisms	An instrument that consists of the cyanobacteria- and bacteria- spore growth chambers.
RISE: Regolith Inclusion Seed Experiment	Assembly consisting of the plant-growth chambers.
SWELL: Sensors in Well - an Environmental assessment for Lunar Life	The regolith-characterization sensors that measure pH, E_h, and ionic conductivity
Radiation Monitor	To measure total ionizing dose.

The BLOOM system will contain cyanobacteria, bacterial spores and plant seeds. Each organism will be evaluated to assess how it responds to the Moon's conditions of reduced gravity, space radiation, and lunar regolith, while using sensors to characterize the regolith and monitor radiation.

Experiments: BLOOM is a fully integrated suite of science instruments. The stand-alone suite of instruments will be capable of monitoring microorganism and plant growth in lunar regolith.

Regolith will be delivered to AIM, RISE and SWELL via PlanetVac, also selected as a part of the NASA Lunar Surface Instrument and Technology Payloads (LSITP) program to fly to Mare Crisium in 2023 onboard Firefly's Blue Ghost lander. PlanetVac will collect regolith from the lunar surface and transfer the material to the sorter and delivery system. Prior to launch organisms (bacterial spores, cyanobacterial and plant seeds) will be dried and adhered to the walls of the growth wells. Blanks, i.e., wells without organisms, will be included to ensure that there is no contamination or unexpected soil chemistry contribution to the measurement results. Once regolith is added to the growth wells, the wells will be sealed and then wetted, and gas will be added to produce a habitat-like atmosphere.

AIM will measure both optical density and fluorescence to determine the growth curves of the selected microorganisms. A camera will also be mounted for imaging wells to ensure appropriate regolith addition, hydration, and potential microbial mat growth. AlamarBlue will be measured using both absorbance and fluorescence (it is fluorescent as well in its reduced state and will not be affected by any scattering caused by regolith) at the end of the mission.

Plant seedlings will be imaged at periodic intervals (Figure 2). Given the short mission duration, the following growth and morphometric properties will be *measured when apparent in the images*: percent germination, germination time, number/area of leaves, number of branches, branch angles, root length, number of secondary roots, and total root length. The growth and morphometric properties will be compared between the flight experiments and ground control.

Figure 2. Images of RISE plant growth wells. The seed strip is visible in the middle of the well. On the left is an image of Raphanus sativus that has stated germination after 2 days and then again at 5 days with leaves clearly visible.

Conclusion: Ultimately, we seek to address the gap in knowledge in how microorganisms and cells will respond to critical elements of the Lunar environment and improve NASA's understanding of the potential risks for both forward and backward contamination and the ability to minimize it and set appropriate standards for future missions. BLOOM would benefit all NASA programs and disciplines whose underlying objectives involve planetary protection. Furthermore, this work will advance NASA's current understanding of bacterial spore survival and establish a process for in-situ regolith testing with biological organisms. These experiments would help guide future methodology and scientific approaches for studying bacterial spore survival in non-terrestrial environments, improving the models of survival for Mars, Europa, etc.

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