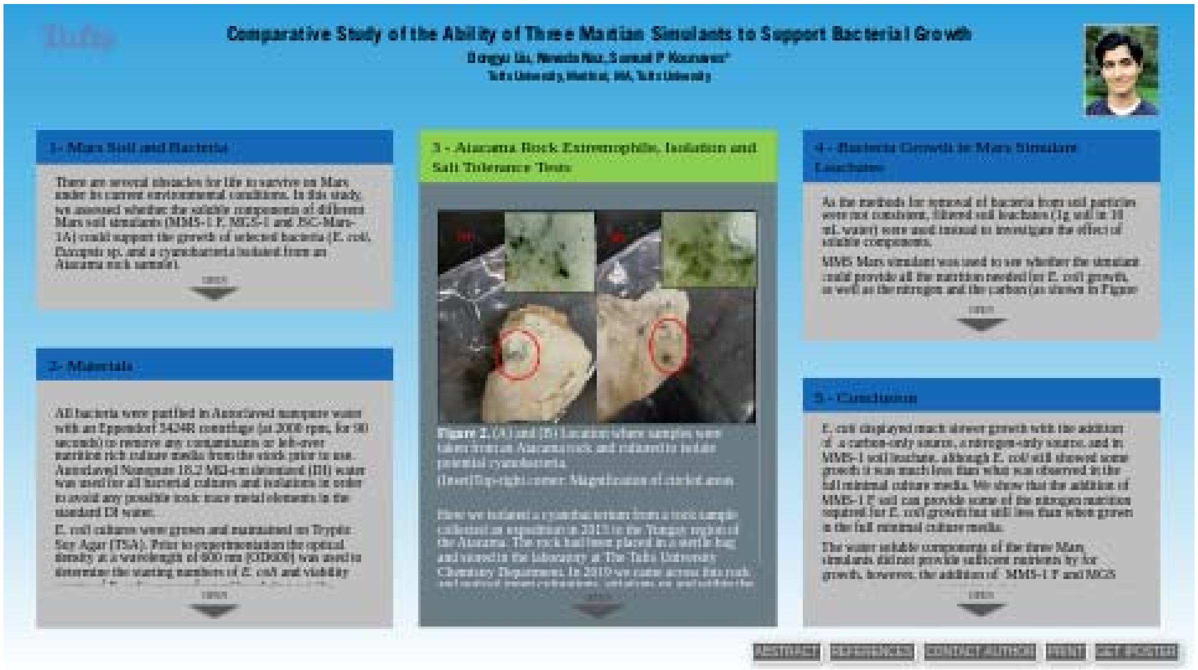
Comparative Study of the Ability of Three Martian Simulants to Support Bacterial Growth



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PRESENTED AT:

1- MARS SOIL AND BACTERIA

There are several obstacles for life to survive on Mars under its current environmental conditions. In this study, we assessed whether the soluble components of different Mars soil simulants (MMS-1 F, MGS-1 and JSC-Mars-1A) could support the growth of selected bacteria (*E. coli*, *Eucapsis* sp. and a cyanobacteria isolated from an Atacama rock sample).

E. coli can survive via aerobic respiration at Martian oxygen levels.[1] Cyanobacteria are aquatic and photosynthetic and were among the earliest forms of life to appear on the early Earth around 3.8 billion years ago. The similarity between early the Mars environment and that of early Earth indicates that cyanobacteria also could have survived on Mars.[2] In addition, our experiments have shown that the cyanobacteria isolated from an Atacama rock to be halophilic, which makes it more likely they would be able to survive in the brines of early Mars.

The three Mars analogues were chosen to simulate the mineral composition of Mars. However, it should be noted that these simulants are not free from terrestrial organic contaminations.[3] Previous analysis of JSC-1 Mars simulant found the presence of water-soluble organics.[4]

2- MATERIALS

All bacteria were purified in Autoclaved nanopure water with an Eppendorf 5424R centrifuge (at 2000 rpm, for 90 seconds) to remove any contaminants or left-over nutrition rich culture media from the stock prior to use. Autoclaved Nanopure 18.2 MΩ-cm deionized (DI) water was used for all bacterial cultures and isolations in order to avoid any possible toxic trace metal elements in the standard DI water.

E. coli cultures were grown and maintained on Tryptic Soy Agar (TSA). Prior to experimentation the optical density at a wavelength of 600 nm (OD600) was used to determine the starting numbers of *E. coli* and viability counts of *E. coli* were performed by plating out the sample on TSA plates. *E. coli* samples were kept in an Incufridge Pro 1.2 at 30°C during the experiment.

A minimal culture media was utilized to mimic the minimal requirements for *E. coli* growth.[5] To minimize the effects from complex organics and to better simulate the possible environment present on Mars, the only organic energy source in the minimal culture media was acetic acid. Acetic acid could be utilized as the (carbon source) and has also been found in carbonaceous meteorites with abiotic origin, which could have been brought to Mars via meteorites.[6] $((NH_4)_2SO_4)$ was selected as the nitrogen source in the Minimal culture media.

Eucapsis and rock cyanobacteria were grown and maintained in a DIY growth chamber (Figure 1). The lamp (Dual-lamp Grow Light Autien 36 LEDs 4 Levels 3 Modes Timing Plant Lights) was selected to adjust output intensity and wavelength (455 nm-650 nm). The inner wall of the culture box was covered with aluminum foil and the four lamp heads were aligned so that the light inside the box was evenly distributed.

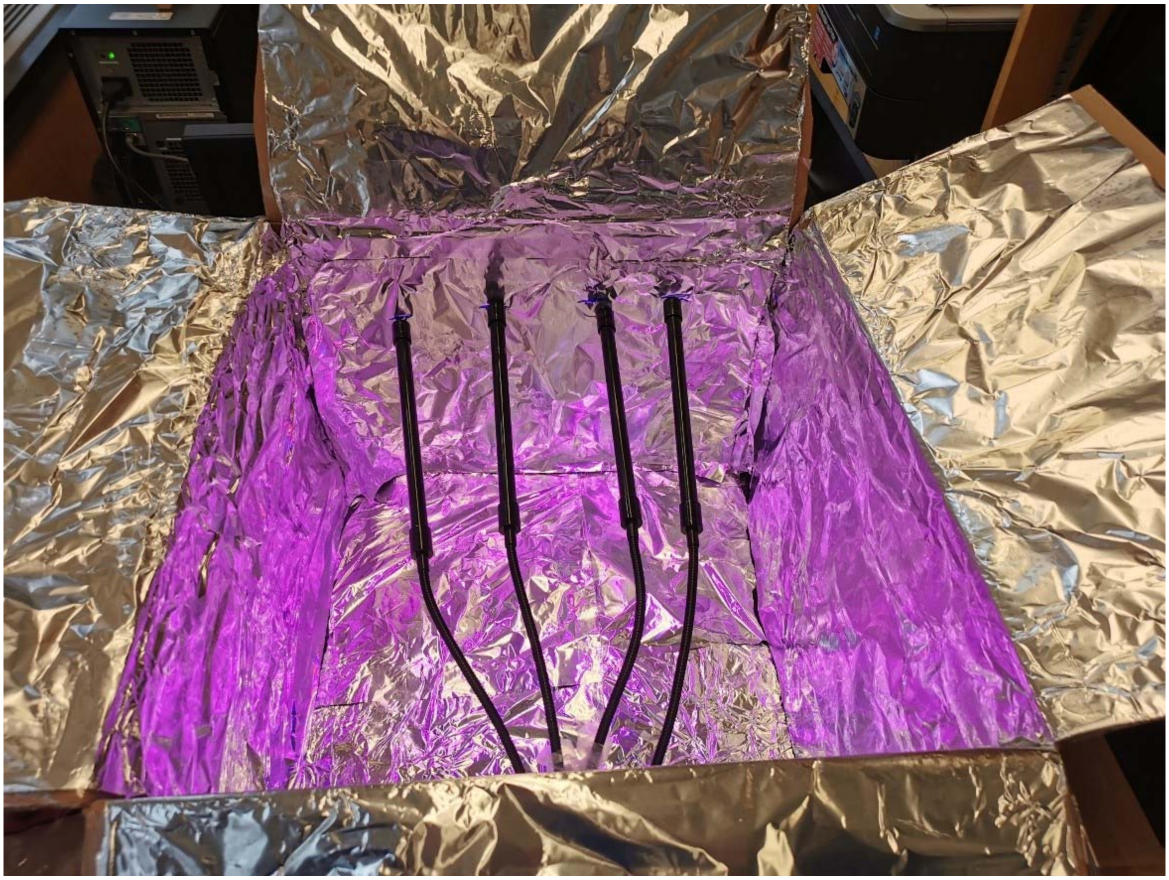

Figure 1. Culture box for cyanobacteria growth.

3 - ATACAMA ROCK EXTREMOPHILE, ISOLATION AND SALT TOLERANCE TESTS

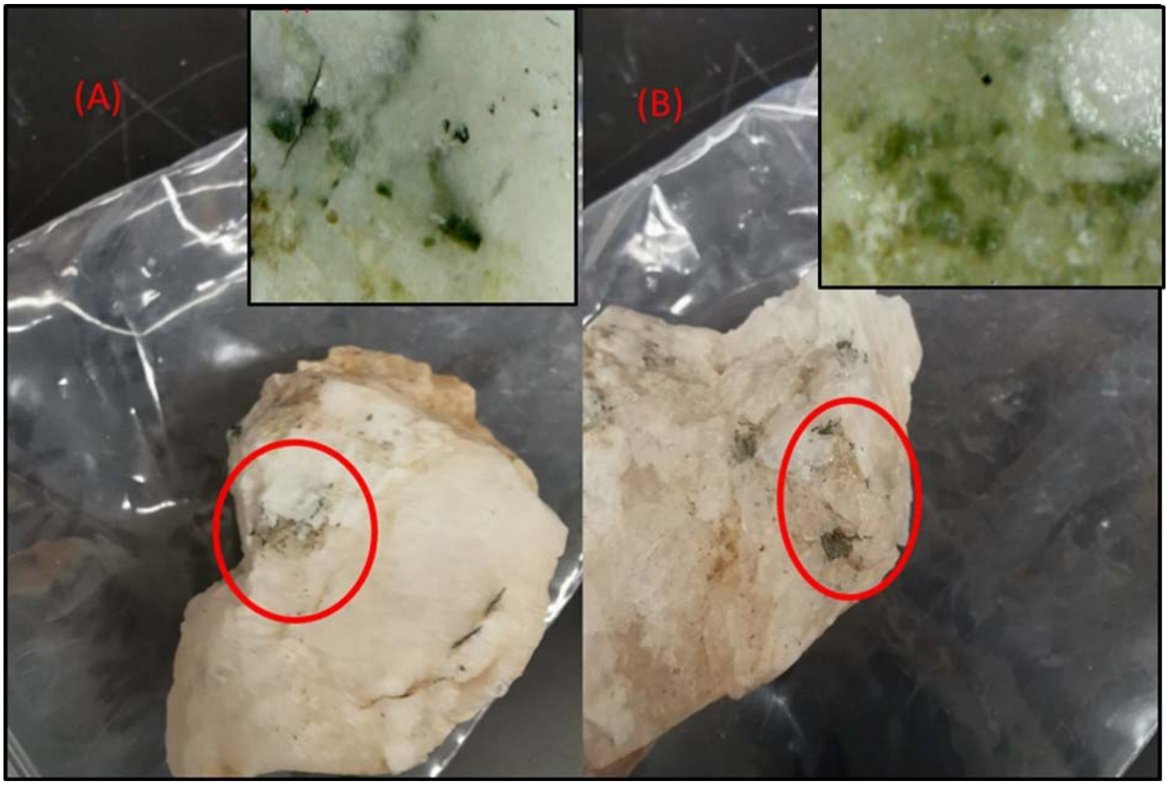

Figure 2. (A) and (B) Location where samples were taken from an Atacama rock and cultured to isolate potential cyanobacteria.

(Inset)Top-right corner: Magnification of circled areas

Here we isolated a cyanobacterium from a rock sample collected an expedition in 2015 to the Yungay region of the Atacama. The rock had been placed in a sterile bag and stored in the laboratory at The Tufts University Chemistry Department. In 2019 we came across this rock and noticed green colorations, striations on and within the structure of the rock. Samples were chipped off from the two locations shown in Figure 1 and these were cultured on BG11+Agarose plates. The plates were kept at room temperature in a growth chamber. The plates were checked regularly, and no growth was visible for weeks. During the pandemic the university shutdown for 3 months. We discovered cyanobacteria growth after the department reopened (Figure 2).

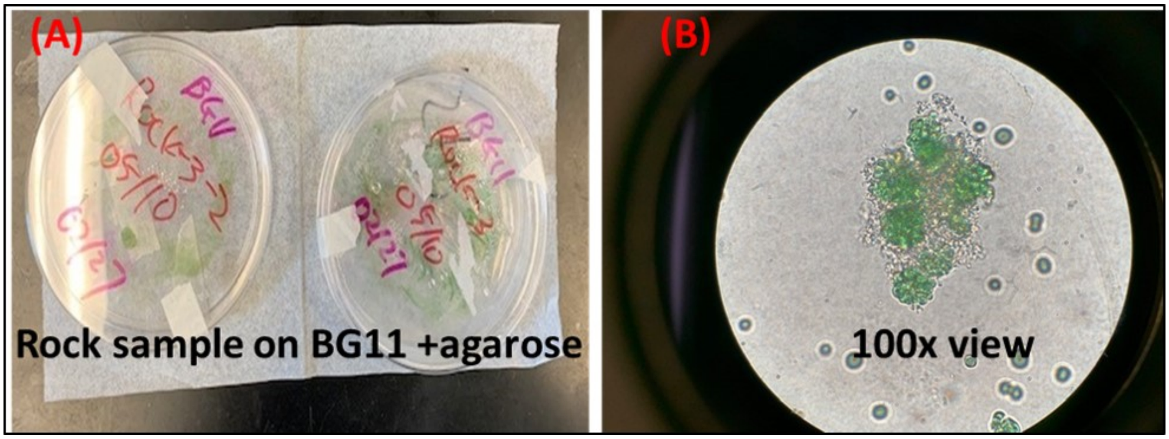

Figure 3. (A) Isolated rock cyanobacteria growing on BG11+agarose plate. (B) 100X magnification view of isolated cyanobacteria.

The salt tolerance of the isolated rock cyanobacteria was tested with NaCl in BG-11 culture media using *Eucapsis* as a control. As shown in Figure 4, no visible growth was detected for *Eucapsis* in two weeks while the rock cyanobacteria exhibited growth in up to 5% of NaCl.

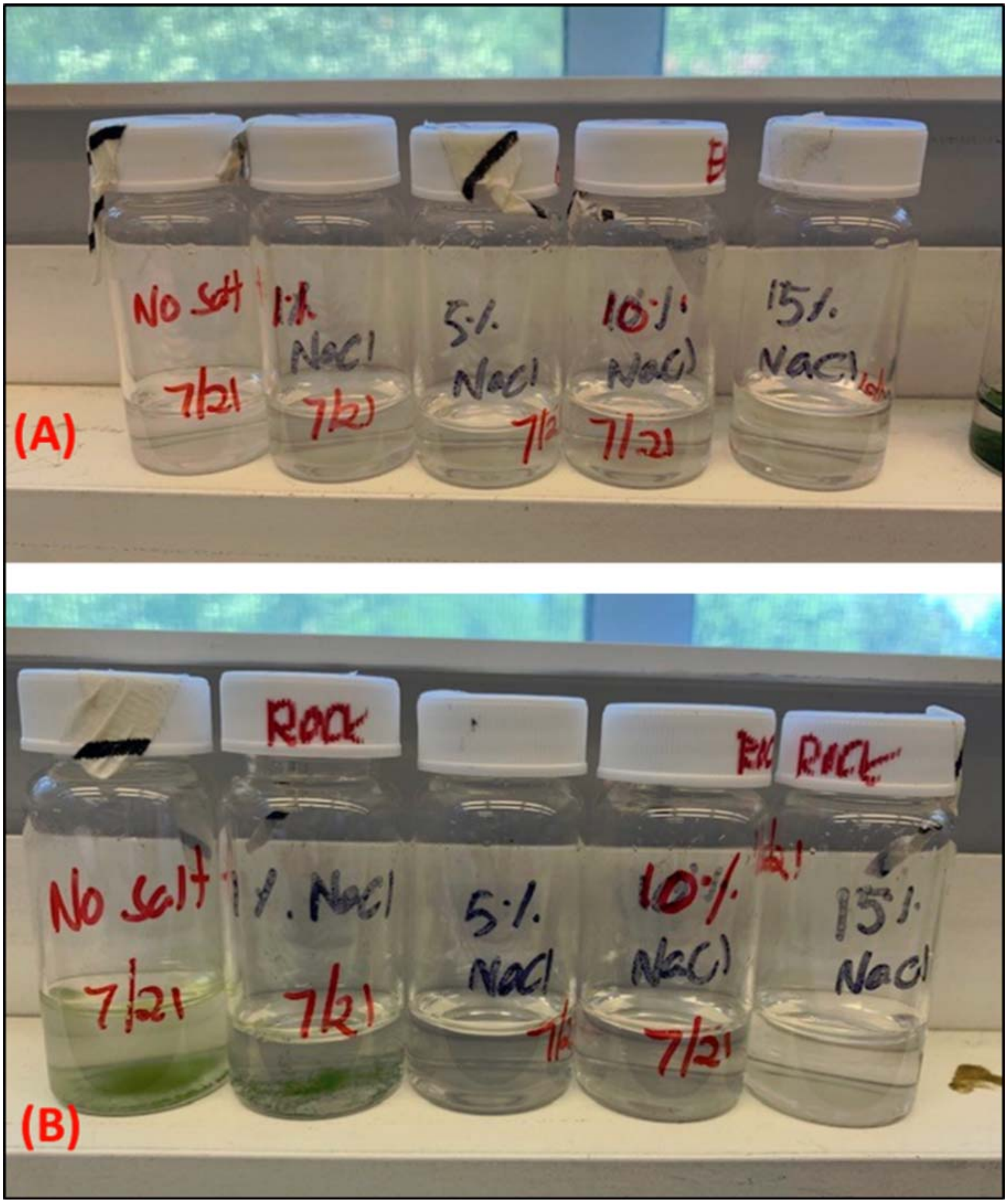

Figure 4. Rock cyanobacteria and *Eucapsis* salt tolerance test two weeks. Vials from left to right with increasing level of NaCl (no salt, 1%, 5% 10% and15% by weight). (A) *Eucapsis;* (B) Rock cyanobacteria

We suspect that the cyanobacteria we isolated belongs to the endolithic community of Cyanobacteria; *Chroococcidiopsis*, which is the only known inhabitant in the extremely inhospitable Atacama Desert.[7] We are currently in the process of using DNA sequencing to identify the strain of the isolated cyanobacteria.

4 - BACTERIA GROWTH IN MARS SIMULANT LEACHATES

As the methods for removal of bacteria from soil particles were not consistent, filtered soil leachates (1g soil in 10 mL water) were used instead to investigate the effect of soluble components.

MMS Mars simulant was used to see whether the simulant could provide all the nutrition needed for *E. coli* growth, as well as the nitrogen and the carbon (as shown in Figure 5).

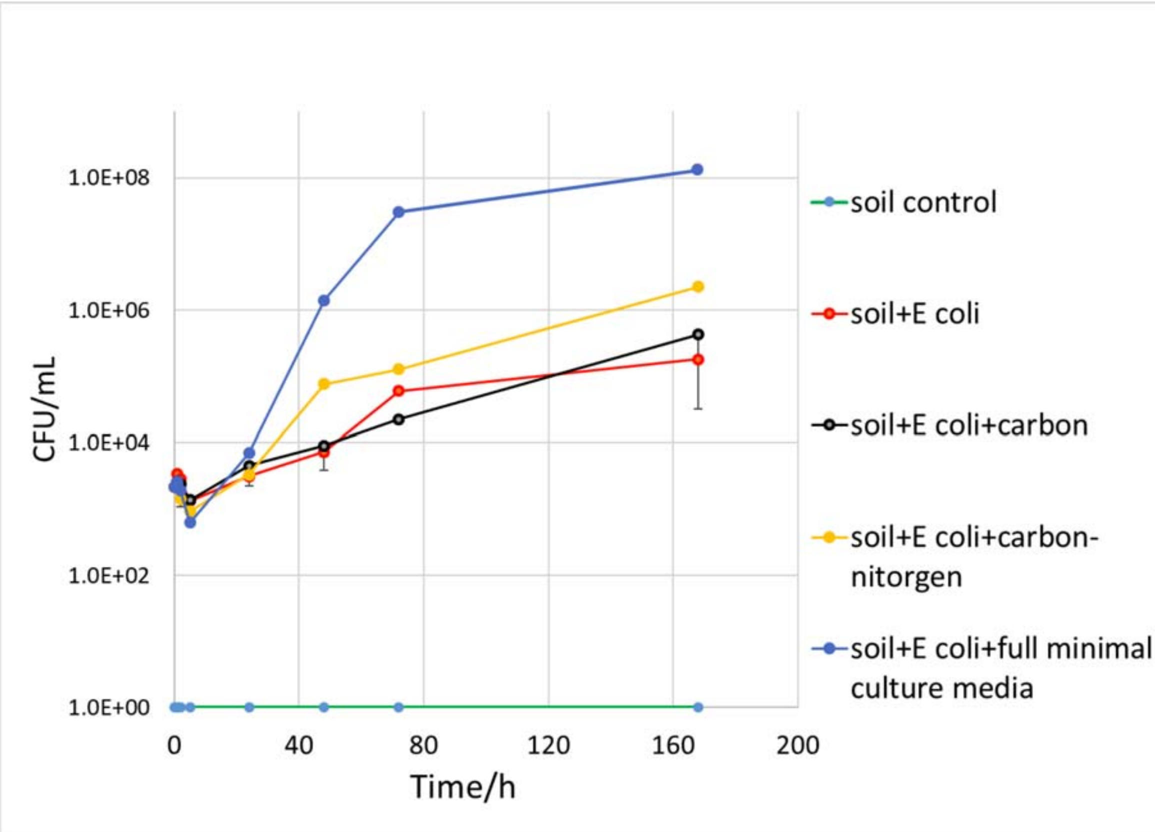

Figure 5. *E. coli* tests performed with Mars simulant soil and addition of different nutrients. Starting with 10 mL culture media or autoclaved water+1g MMS-1 soil (Initial $OD_{600}=1\times10^{-6}$).

Eucapsis growth in different Mars simulants leachates was tested using Allen's culture media as a control. All cyanobacteria samples were kept in the culture box at room temperature. Allen's minimal media (no trace elements added) was also used to determine if Mas soil simulants could provide the trace elements for bacterial growth. The *Eucapsis* counts were performed with a hemocytometer. As shown in Figure 6, using Allen's minimal media instead of water to leach Mars simulants led to a significant improvement in *Eucapsis* growth for both MMS-1 and MGS-1. However, JSC and JSC + Allen's Minimal did not show any significant difference regarding *Eucapsis* growth. Overall, MMS-1 was the most suitable for *Eucapsis* growth among the three Mars simulants.

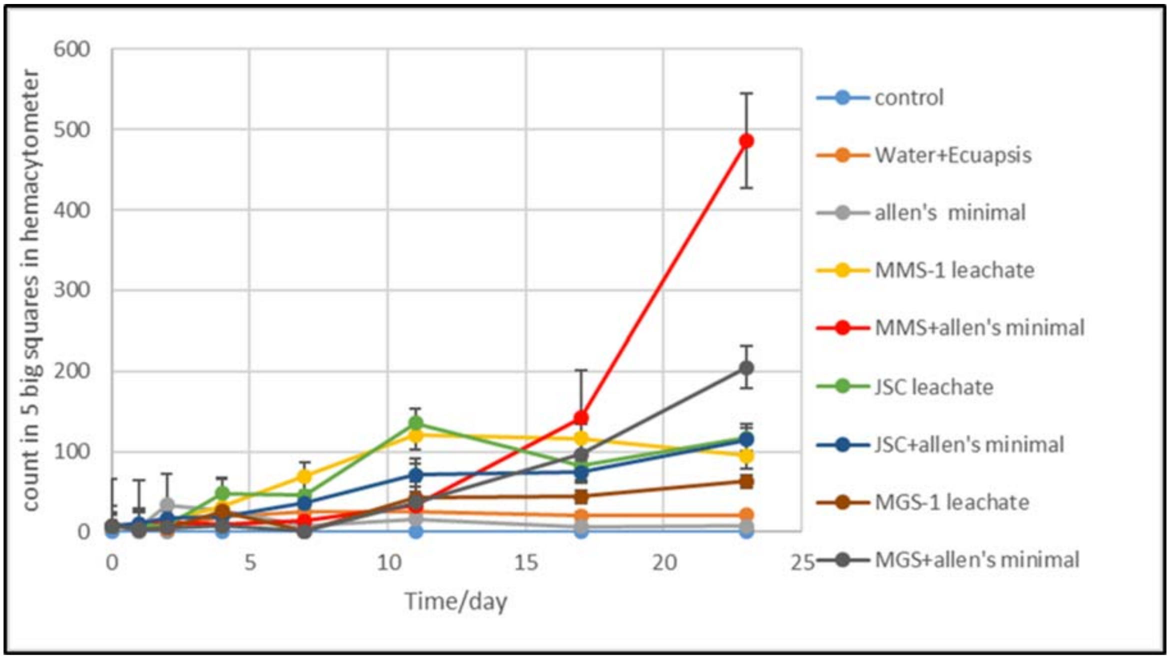

Figure 6. *Eucapsis* growth in different soil leachates and soil leachate + Allen's Minimal groups.

5 - CONCLUSION

E. coli displayed much slower growth with the addition of a carbon-only source, a nitrogen-only source, and in MMS-1 soil leachate, although *E. coli* still showed some growth it was much less than what was observed in the full minimal culture media. We show that the addition of MMS-1 F soil can provide some of the nitrogen nutrition required for *E. coli* growth but still less than when grown in the full minimal culture media.

The water soluble components of the three Mars simulants did not provide sufficent nutrients by for growth, however, the addition of MMS-1 F and MGS supported growth, with MMS-1 F showing the best results for *Eucapsis*.

ABSTRACT

One key question about Mars is whether life has been or still is present on or beneath its surface. For life to flourish, it requires a habitable environment with the appropriate physical and chemical regolith parameters. To better understand the parameters that constitute a habitable environment, leachates of three martian simulants (JSC Mars-1, MMS-1 fine, and MGS-1) were analyzed for their soluble ionic composition, pH, and conductivity in order to determine the presence of any beneficial or toxic elements and their effects on the two bacteria *E. coli* and *Eucapsis*.

E.coli was cultured in minimal media where acetic acid was the only organic source, and tested its requirements for a carbon source (acetic acid), nitrogen source ($(NH_4)_2SO_4$) as well as trace elements (Ca, Mn, Zn, Cu, Fe, Co, Mo). In minimal culture media with all nutrients available for healthy growth, *E. coli* showed substantial growth. In the case of a carbon-only source or nitrogen-only source in MMS-1 soil leachate, *E. coli* showed limited growth compared to that observed in the full minimal culture media. Assessing *Eucapsis* growth, among the three leachates tested MMS-1 displayed the best growth. Additionally, we observed that MMS-1+Allen's minimal and full Allen's medium groups displayed similar growth curves, indicating that MMS-1 can provide all the trace elements needed for *Eucapsis* growth.

Among all the leachates, MMS-1 showed the most promising results. MMS-1 + Allen's medium provided the highest *Eucapsis* growth. MGS-1 + Allen's minimal media also showed significantly higher growth than MGS-1 alone. However, JSC and JSC + Allen's minimal did not show any significant difference regarding *Eucapsis* growth. The results indicated that *Eucapsis* grew best in both MMS-1 F leachate only and MMS-1 F leachate with Allen's minimal medium.

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