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**Independent Study and Mentorship Original Work**

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**Astrobiology**

**Lab Design For a Manned Mission to Mars:**

**Effect of Nitrate Limitation on Extremophiles**

## Introduction

Life is seen as something that is fragile for a vast majority of organisms on Earth. However, certain creatures have shown the ability to live in extreme environments. As a result, these life forms have earned the name extremophiles. For many years, scientists researched extremophiles in order to understand how life developed and evolved on Earth but scientists recently found out that extremophiles could be key to understanding if life could exist on the red planet, Mars. NASA is currently planning to send humans to Mars in the 2030s, which will allow astrobiologists to conduct research at a scientific level we have never seen. With that said, astronauts should study extremophiles while on the surface of Mars.

## Martian Environment

It is no lie that Mars is far different from Earth, in terms of atmosphere and climate. In order to successfully study extremophiles on Mars, we need to send the most suitable organisms. To do that, we have to thoroughly understand Mars'

environment. Unlike Earth, the atmosphere of Mars is 90% carbon dioxide, 3% nitrogen, 1.6% argon, trace amounts of oxygen, and no amount of water vapor. Additionally, the atmospheric pressure is low compared to Earth. It is also important to note that Mars has an extremely thin atmosphere and has no magnetosphere. This means that the planet has little to no protection from the dangerous radiation. With all this in mind, we need to send organisms that require little to no oxygen and can withstand high amounts of radiation. In addition to the atmospheric constraints, Mars has exceedingly low temperatures due to its distance from the sun. The planet also experiences large dust storms that last for approximately 23 days. The dust storms cause a thin layer of dust to cover Mars. The Martian soil is also known to contain large amounts of salts. The red planet also lacks a sufficient amount of nitrate, perchlorate, and other elements that are essential for life to flourish.

## Selection of Extremophiles

The Martian climate is far too dangerous for most organisms to live, making the selection of extremophiles extremely important. As I was designing the experiment, I researched various extremophiles. I specifically looked at the location where they were found, the temperature range, nutritional needs, and unique characters of the organism. After compiling all the information, I selected four extremophiles that were most fit for Mars' climate. The extremophiles that I chose were *Deinococcus radiodurans*, *Tardigrades* ("water bears"), *Cryptoendoliths*, and *Cyanobacteria*.

*Deinococcus radiodurans* are known to thrive in a wide range of temperatures, survive in settings that are exposed to large amounts of radiation. In fact, astronauts, aboard the International Space Station, exposed a colony of *Deinococcus radiodurans* to outer space and found that this extremophile survived in the vacuum for six weeks. This organism is a clear candidate for astronauts to study on Mars because it can withstand the cold temperatures and the radiation. Tardigrades are another great candidate for this experiment because they can also withstand extreme temperatures, pressures, and radiation. Astronauts have also tested this in outer space and found that this organism can also survive in a vacuum. In addition to these extremophiles, I found cryptoendoliths to be of great interest for this particular experiment. This extremophile has been known to live inside of rocks in Antarctica. This continent is probably one of the few regions on Earth that resemble Mars, making cryptoendoliths a great applicant. The last organism that I selected to send to Mars is cyanobacteria. This extremophile can live without oxygen. In fact, there is evidence that shows this extremophile caused the increase of oxygen during the early times of Earth, through photosynthesis. On Mars, there is only trace amounts of oxygen, making cyanobacteria a prime candidate for this investigation. It is also important to note that this extremophile can be used to cultivate crops in a Martian greenhouse and serve as a potential food source for astronauts.

## Mission Length

This lab experiment is initially designed for a short term mission to Mars. Astronauts will be on Mars for 90 days and have a round trip of 650 days. The experiment will be conducted over a period of multiple manned missions for accuracy.

## Biosafety Level

The safety of the astronauts is the top priority. We do not want to send life forms to Mars that will pose a danger to the astronauts. There is evidence of certain bacterias and viruses that become more resistant and virulent in outer space. It is crucial to make sure that these don't go to space with the astronauts. The extremophiles that were selected for this experiment do not pose any harmful threat to humans. As a result, the lab that the extremophiles will be placed in will be at a biosafety level of one.

## Materials

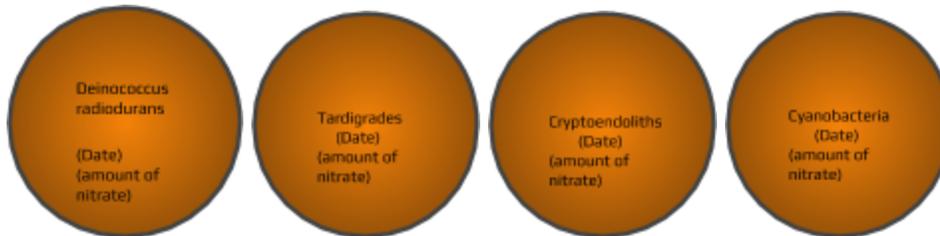
1. Petri Dish filled with agar (20)
2. Martian Soil
3. Colony of *Deinococcus Radiodurans* (5)
4. Colony of Tardigrades (5)

5. Colony of Cryptoendoliths (5)
6. Colony of Cyanobacteria (5)
7. Mars Simulation Chamber
8. Nitrite/Nitrate Colorimetric Assay Kit
9. Epifluorescence Optical Microscope
10. Spectrophotometer
11. Basic Protection Gear (gloves, eye goggles, etc.)
12. Soil Scooper (2g)
13. Sharpie
14. Parafilm
15. Nitrogen-Nitrate Standard Solution
16. Pipet Tip

## Procedure

1. Make sure the work space is clean. There should be no contaminants while performing this experiment.
2. Take 4 petri dishes and label each of the petri dish with the name of the extremophile that is going to be studied. You need to also label each petri dish with the date of which you started the experiment and also the amount of Nitrogen-Nitrate Solution that you will add.
3. Place a colony of each extremophile to its designated petri dish.

4. Sprinkle about 25 particles of martian soil onto each of the petri dish.
5. Cover all the petri dishes with a parafilm and lightly shake the petri dish to evenly distribute all the soil.



### *Set Up*

6. Repeat the steps above and add 4 drops Nitrogen-Nitrate standard solution into each petri dish, using a pipet tip.
7. Repeat the step above for the other extremophiles.
8. Repeat steps 1-5 but add 8 drops of the Nitrogen-Nitrate standard solution. Do this for all the extremophiles.
9. Once again, repeat steps 1-5 but add 12 drops of the Nitrogen-Nitrate standard solution. Do this for all the extremophiles.
10. Repeat steps 1-5 but add 16 drops of the Nitrogen-Nitrate standard solution. Do this for all the extremophiles.
11. Place all the petri dishes in the Mars Simulation Chamber and set the temperature, pressure, radiation, and atmospheric gases to what is found on Mars.
12. Check the petri dishes every 24 hours and place the samples in the Spectrophotometer to obtain the optical density growth. This will tell you how much microorganisms are present. Do this for 60 days.

13. Additionally, use the nitrate colorimetric assay kit to identify if any of the extremophiles are

conducting nitrogen fixation.

14. Use epifluorescence optical microscope to further investigate the extremophiles, if necessary.

## Laboratory Instrument Weights

A manned mission to Mars will not only require science but also a great amount of precise engineering. The math establishes that we cannot send an inordinate amount of weight all at once to Mars. If we do, there is no doubt that the mission will fail. This particular experiment does require a mars simulation chamber, which has a large weight. One way to solve this problem is to land next to the Curiosity Rover. Curiosity has the sample analysis at Mars (SAM) instrument, which investigates the martian soil composition. Within the instrument, it stimulates a martian environment. If engineers could figure out a way to extract all measuring instruments, we would have a viable mars simulation chamber. This would save a lot of weight and money for NASA.

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