How *Anabaena* can improve plant growth on Mars by acting as a biofertilizer

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October 2022

Abstract:

The human race has the need to explore and colonize as seen throughout history. Humanity has conquered land, sea, and sky. It's time for humanity's greatest mission to the next frontier as we plan to colonize Mars and make it a second home. Companies such as SpaceX, Blue Origin, and governmental agencies such as NASA are planning to conduct missions to colonize Mars by 2030. However, the colonization process is very difficult, expensive, and time consuming. The Martian surface is barren, cold, and dry making it impossible to grow crops on the surface (Gonçalves, 2021). Early Martian colonies will be completely reliant off of Earth for their resources. However, transport of goods from Earth to Mars takes about 7 months, this is a very costly endeavor, and can only be done every twenty six months when Earth and Mars are closest. For Mars to become a sufficient self sustaining colony there needs to be a way for the colonizers to make their own food. Martian soil is similar to Earth's consisting of nitrogen, potassium, and phosphorus but the concentrations are too low to support any plant growth. Thus, Martian colonists must grow crops in controlled greenhouses. To help increase the nitrogen content in low nutrient Martian soil, cyanobacteria could be used to help. Cyanobacteria are ubiquitous photosynthetic microorganisms that can live in strenuous environments. Cyanobacteria, especially members of the genus *Anabaena*, are known to fix nitrogen, the process of converting atmospheric nitrogen into biologically available forms. If cyanobacteria are introduced into martian colonies for agricultural purposes, nutrient levels in soil could increase thus increasing crop yields on the planet. To test whether cyanobacteria can improve plant growth and increase nitrogen content in soil , four groups of six plants were grown in a hydroponic system; six with added nutrients and cyanobacteria, six with only nutrients (Miracle-gro), six with the combination of both nutrients and cyanobacteria, and six with no added nutrients or cyanobacteria.

Keywords - Mars, Biofertilizer, Cyanobacteria, *Anabaena*, Martian colonization, NASA, Nitrogen fixation, Soil, sustainability

Introduction:

Martian background:

"For all its material advantages, the sedentary life has left us...edgy, unfulfilled. Even after 400 generations in villages and cities, we haven't forgotten. The open road still softly calls, like a nearly forgotten song of childhood… Maybe it's a little early--maybe the time is not quite yet--but those other worlds, promising untold opportunities, beckon. Silently, they orbit the Sun, waiting" (Sagen, 1994). The human race developed from hunter gatherers with an itch to explore. It's time to expand outside Earth and into the next frontier of space exploration. Mars is our first step to becoming an interplanetary species meaning that humans can survive on other planets besides Earth (Fig. 1). Mars is the fourth planet from the sun estimated to be around 4.6 billion years old forming around the same time as the Earth. However, Mars has been hypothesized to once have had oceans and lakes as it began to cool 3 million years ago (ESA, 2019).


Figure 1: This image made available by NASA shows the planet Mars. This composite photo was created from over 100 images of Mars taken by Viking orbiters in the 1970s. (NASA,2014)


Figure 2. The 45 km wide Jezero Crater, landing site of the MSL Perseverance. (Cowart, 2021)

Due to Mars's smaller size, its iron core cooled down quickly which stopped the generation of a magnetic field which left its atmosphere unprotected from solar winds and flares. Over time the liquid water got stripped away or frozen leaving Mars as the planet we know today - a corpse of what it once was. Mars is now a cold and desolate planet whose surface is a desert of red sand and polar ice caps of frozen carbon dioxide and water which can melt slightly in the summer months when the temperature reaches 20°C (ESA, 2008). Mars is its warmest during the summer at 20°C and its coldest at -140°C in the winter. The average temperature of Mars is about -62°C. Another issue is the surface pressure of the red planet. On Earth, sea level surface pressure is 1013 mbar whereas on Mars the pressure is only about 7-12 mbar. Low surface pressure is especially hazardous making respiration nearly impossible. Also, low surface pressures limit the amount of gasses in a planet's atmosphere, which can limit plant growth. The overwhelming evidence regarding Mars's past of having a warm atmosphere and oceans much like Earth has made it of significant interest to the scientific community. We have been sending rovers to Mars since the 1960s to the present day. The most recent rover to land on Mars is Perseverance and Ingenuity which landed on Mars February 18th 2021 (Fig. 3). Perseverance was carefully constructed to test and move around Mars - specifically in the Jezero crater. Perseverance Geology, Astrobiology Sample Caching. For geology Perseverance tests the rocks and soil on Mars to understand the history of the planet. The rover also tests the area it is in to identify if the area was able to sustain life in the past. Lastly the rover collects samples of rock and soil that will eventually be taken back to Earth for more intensive study. Perseverance is exploring the Jezero crater; an ancient river delta. (Fig. 2). Rivers similar to Jezero crater have clays that are known to host microbial life (NASA, 2020). The entire Martian surface has been mapped out and is being constantly studied and monitored by satellites orbiting the planet which will make future colonization easier.

Figure 3. Perseverance rover on Mars, Computer simulation created by NASA.

Why Mars?

Although Mars does not possess any of the livable conditions that Earth does, it could very well be a suitable planet to colonize. Mars does not have oxygen, in fact humans could only last about two minutes on Mars before their organs ruptured. Mars also has no liquid water, vital to sustaining any life. Scientists believe that there may be liquid water running underneath the Martian surface and that by adding hydrated salt to the frozen Martian ice caps, it could lower its freezing temperature enough to become

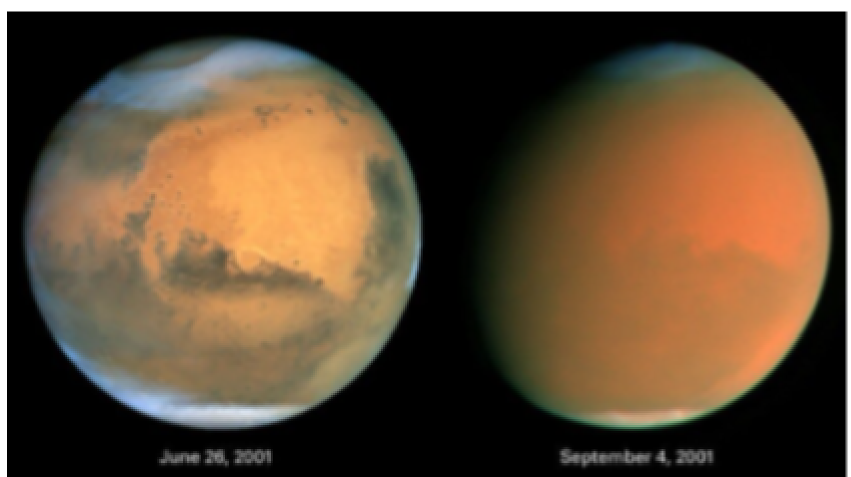

Figure 4. Comparison images of Mars taken by Hubble (left) and showing a global dust storm that engulfed it (right). Astronomers studying dust storms in the Aonia-Solis-Valles Marineris region over eight years have found a distinct periodicity in their occurrence. Credit: NASA (staff, 2019)

liquid (NASA, 2017). Mars is no stranger to violent dust storms. Every year, Mars will be frequented by continent sized storms with wind speeds up to 60mph that can last for weeks blocking out the sun. Every 3 years on Mars (5 1/2 Earth years) massive planet wide dust storms can occur (Smith, 2015; Fig. 4). Additionally, Mars has no magnetic field and a very weak atmosphere leaving the surface defenseless against the sun's ionizing radiation of 8 rads per year whereas Earth only gets 0.62 rads per year (Williams, 2022). The question still stands, why Mars? Mars is just within the right distance from the sun so that it isn't too cold. It has a similar day length to Earth at 24 hours and 37 minutes long. Mars has an atmosphere consisting of 95% CO_2, 3% N_2 and 1.6% Ar, there are also trace amounts of methane, water, and oxygen. Mars is at the edge of our habitable zone making it the perfect pit stop to explore the rest of the universe. With a weak atmosphere, little fuel will be needed to get off the planet. Also, it is a quicker trip compared to Earth to gas giants such as Jupiter and Saturn and their many moons. Mars offers an opportunity for humanity to expand outside of Earth and explore the universe.

But why Mars, why is this the next destination in human exploration? The population of the human race is hypothesized to reach 9.7 billion by the year 2050. The Earth is already largely overcrowded, disrupting wildlife and the natural order of the planet. Climate change is warming the Earth causing forest fires, ecological disasters, and mass extinction.

Colonization of Mars:

Elon Musk proposes that cargo ships will be sent to Mars in the early 2020’s with machinery capable of 3D printing. The company called Icon, which has 3D printed communities on Earth, has been running creating a real life model of the Martian habitat on Earth at the Lyndon B Johnson Space Center (Fig 5). The homes are made of a combination of concrete and synthetic Martian sand (Fig.6) To make these homes convert a mixture of Martian soil, water, and concrete (Icon, 2021) . The first colonists will have to land near the poles in order to use the melted ice to create the mixture. Initially, concrete will have to be transported by ship to Mars. However, concrete is a very heavy material to transport. A recent study suggested that the colonists could use the protein from their blood plasma bonded with sweat or urine mixed with Martian

Figure 5. ICON 3D Prints the First Simulated Mars Surface Habitat for NASA Designed by Renowned Architecture Firm BIG-Bjarke Ingels Group (ICON, 2021).

soil to create Biocomposites which have similar properties to concrete. Whereas Icon can supply strong building material for the habitats, the process can be very costly, therefore taking the resources from Mars such as the soil and plasma from the colonist blood can create a much cheaper, more self-sufficient form of building material once the colonists run out of the resources Earth has sent. (Roberts et al, 2021). Proposed Martian homes will be roughly 1,700 square feet. It takes the Icon printer 24 hours to create a 400-500 square foot, so Martian homes would be completed in roughly 70 hours. These homes may not be permanent as the long term effect of radiation is unknown, however, it has been proposed to make underground bases in dormant lava tunnels underneath the surface to avoid ionizing rays. The first steps of colonizers will be to secure power, food, and water. Because of Mars's distance from the sun, solar panels only receive 40% of the sun's energy compared to Earth. This means that the colonists would either have to cover a large surface area of 10 acres of solar panels just to power the initial base (Poston, & McClure, 2018). Solar panels are not the most efficient form of energy on Mars as it takes a lot of time, resources, and energy, to create, transport and install solar panels. On top of that, Martian dust storms will likely damage and make it impossible for solar panels to collect energy for weeks to months. The best option for initial colonists would be to adopt nuclear energy. Nuclear energy has been used before on missions such as the Curiosity Mars rover, Cassini mission to Saturn, and on the Voyager space crafts which are still in operation to this day. These craft use a radioisotope thermoelectric generator, which is powered by the decay of plutonium-238. While this is a well known form of energy, it only gives off enough energy to power radio signals on a rover or probe. The both also revive energy from solar panels however the farther away from the sun the less energy the probes receive, therefore the probes will need radioisotope thermoelectric generators. The best form of nuclear energy would be kilopower, a

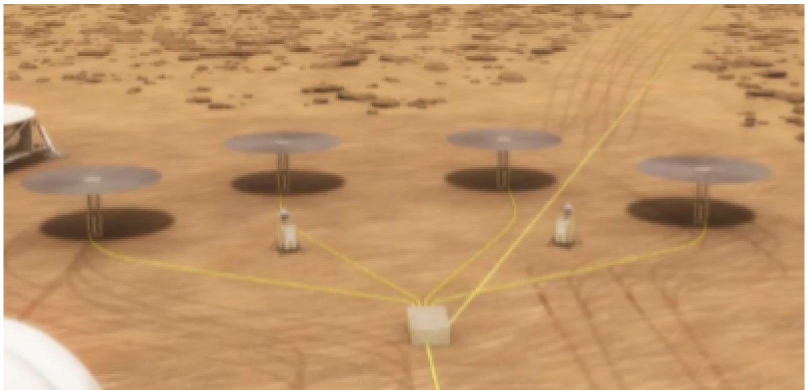

Figure 6. Artist's illustration of the umbrella-like heat radiators of four Kilopower nuclear reactors casting shadows on the Martian surface. (Image credit: NASA) (McClure & Poston, 2018)

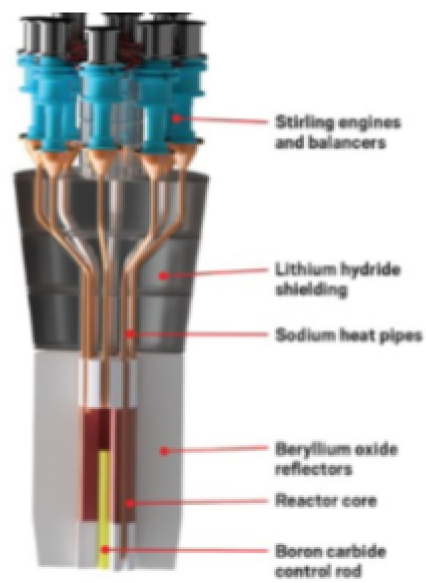

Figure 7. With no moving parts, this compact reactor was designed with simplicity in mind(Space Technology, 2022).

more efficient form of the radioisotope thermoelectric generator (Fig. 7). Kilopower five they can produce 10kW, a small household on Earth uses no more than 5kW of power; this would be enough to supply colonists the energy to power their base, filter water, circulate and create oxygen as well as make liquid oxygen fuel for trips back to Earth. (Fig. 8) Kilopower works by "The sealed tube in the heat pipe circulates a fluid around the reactor, picking up the heat and carrying it to the Stirling engine. There, the heat energy pressurizes gas to drive a piston coupled to a motor that generates electricity. Using the two devices in tandem creates a simple, reliable electric power supply" (Poston, 2018). Over time, safer and efficient power sources may arise such as geothermal energy. The colonization process is a very difficult, expensive, and time-consuming process. In order to create the most efficient Martian colony, colonists need to work off the land and use all available resources in an innovative way.

Making Mars a Self Sustaining Colony

The next step in Martian colonization would be to find an efficient food source that is not reliant off of Earth. Hydroponics are a quick and easy way to create food; they use 10% less water than traditional farming. By using Martian soil, water -created from recycled urine- and growth lights food can be made in these systems. Unfortunately, Martian soil is nutrient poor lacking the necessary nutrients that plants need such as nitrogen. However, if nitrogen fixing *Anabaena,* a genus of cyanobacteria, is used as a biofertilizer on Mars, then crops could grow significantly faster and with higher crop yields. Early Martian settlements will be dependent on Earth for their resources since the Martian surface is desolate, cold, and dry, making it very difficult to cultivate crops there (NASA, 2020). There must be a means for the colonists to produce their own food for Mars to become a self-sufficient planet. A type of cyanobacteria called Diazotrophs are known to be the best for creating biofertilizers. The cyanobacterial genus *Anabaena* is a part of the Diazotrophs family and is used in many biofertilizer experiments. With the rising population one's ability to access to contamination-free food, Cyanobacteria like *Anabaena* have been known to be able to break down pesticides and naturally deliver fertilizers and certain vitamins such as B12 to the plants and can fix up to 20–25 kg/ha of nitrogen (Chittora et al, 2020). Anabaena are ubiquitous and can fix nitrogen, a process in which atmospheric N_2 is

Figure 8. This blue-green microbe called a cyanobacterium was likely the first photosynthetic organism—and it was a game changer in the story of life on Earth. Over millions of years, oxygen continued to accumulate in the atmosphere, thanks to cyanobacteria and other photosynthetic organisms. It was this oxygen that would allow complex life to thrive in the millennia to come. (Smithsonian , 2019)

chemically converted into more bioavailable forms of nitrogen. This will enable the replenishment of nitrogen in the soil on Mars and serve as a biofertilizer for plant growth such as soybeans and lettuce protein bearing and nutritious crops. Using *Anabaena* will allow for the plants on Mars to be able to grow and be consumed by humans without the assistance and reliance of Earth.

Materials and methods:

Four transparent containers were filled with six identical net cups, each measuring 2" in diameter. Butter lettuce was grown from seeds in potting soil to allow them to germinate.Once the plants started to show prominent leaf growth around 5-7 inches wide, they were transferred into a container filled with a mixture of 75% soil and 25% sand to mimic Martian soil. Before the soil was added to the net cups, its nitrogen content was assessed using Rapid Test nitrogen test kit (Luster Leaf). One part soil and five parts water were placed into a container and left to rest for 45 minutes to 24 hours for the soil to separate from the water. Once all the soil had settled on the bottom of the containers, water was placed to the top of the small side of the test kit, and one of the purple pill capsules was emptied into the water. The lid was placed onto the test kit and was shaken vigorously for one minute then left for another minute before measuring. The net cups were halfway filled with the soil mixture. Twenty four seeds were individually planted at ½" depth in the soil mix. All net cups were placed in a transparent, airtight container (container A). Container A received regular waterings and was placed near sunlight. Once the butterhead roots were visible, the cups were transferred from the airtight container to four transparent containers (container B). Every container was filled with 3 gallons of water- alga grow mixture. In one container, one tablespoon of miracle grows with a culture of cyanobacteria added into the water (container 1b). In another container (container 2b) only a tablespoon of miracle grow was added. In a different container(container 3b), just cyanobacteria and the medium. The last container (container 4b) should just have a water-algae grow mixture to act as the control. The nitrogen levels were measured every 3-4 days repeating the process above for each experimental group. Along with the plant's height until the plants have matured (30 days). A grow light was used for four hours a day on the plants.

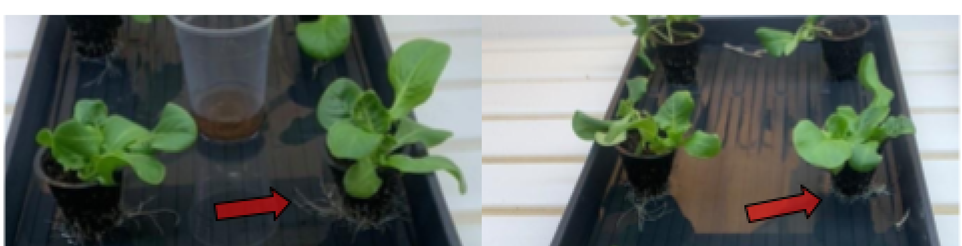

Figure 9. Miracle grow (right) DWC Butterhead lettuce roots (pointed out by red arrow) Vs Cyanobacteria (left) DWC Butterhead lettuce roots. Photos taken by me.

<u>Results and discussion</u>

The plant group with the largest leaf length (cm) and most efficient supply of nitrogen in its soil was the group that was given cyanobacteria and miracle growth (condition Z). The conditions with just cyanobacteria (X) and the condition with just Miracle grow (Y) had relatively similar plant growth and nitrogen levels. However, the conditions of the plants had a visual difference whereas condition X looked healthier; their leaves were not as wilted and their stems were thicker and their roots were much longer (Fig. 9). The control group (W) receiving just water had the smallest plants and very little nitrogen in its soil (Fig 10, Chart 2). When testing the nitrogen for the first time after three days of being in the DWC hydroponic system 2.5 tablespoons of water was added to ½ tablespoon soil from each experimental group and control. However, when these samples were tested for nitrogen content each was below the detection limit. The nitrogen levels were tested again where five tablespoons of water was added to 1 tablespoon of soil which resulted in accurate results. The plant's leaves were all 3cm when they were transported into the DWC hydroponic systems (chart,1).

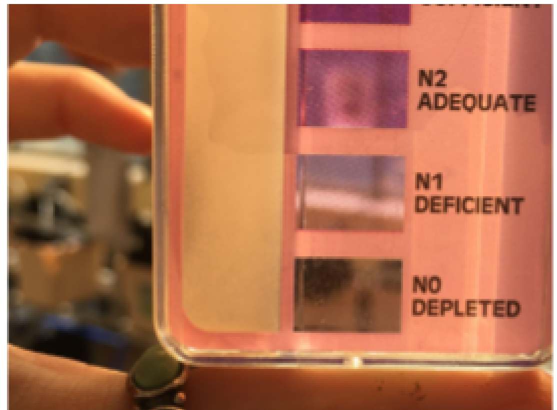

Figure 10. Photo taken by me on october 10th of the nitrogen levels in condition x.

The nitrogen levels of the four conditions were also assessed deficient (N1) shown as 1 on Figure Adequate (N2) shown as 2 on Figure X, Adequate (N3) shown as three on the graph, the Nitrogen kit's highest level was N4 representing surplus which isn't represented by the graph because none of the conditions exhibited that amount of nitrogen in the soil, however it is believed that if the plants were given more time and larger doses of their fertilizer they could have reached a surplus in nitrogen (Chart 2).

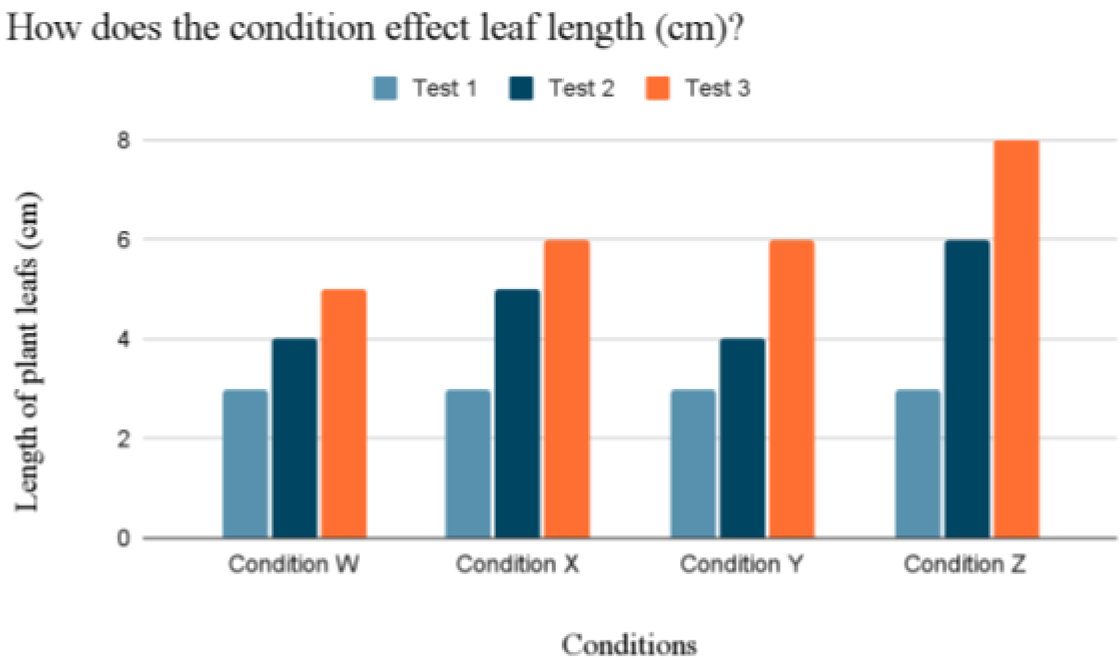

Chart 1. Condition W had the least growth over a 2 1/2 weeks growth period only growing 2 cm. Conditions X and Y leaves both grew 3 cm. Lastly condition Z grew the most, growing 6cm in total and having the healthiest looking leaves, stem, and roots.

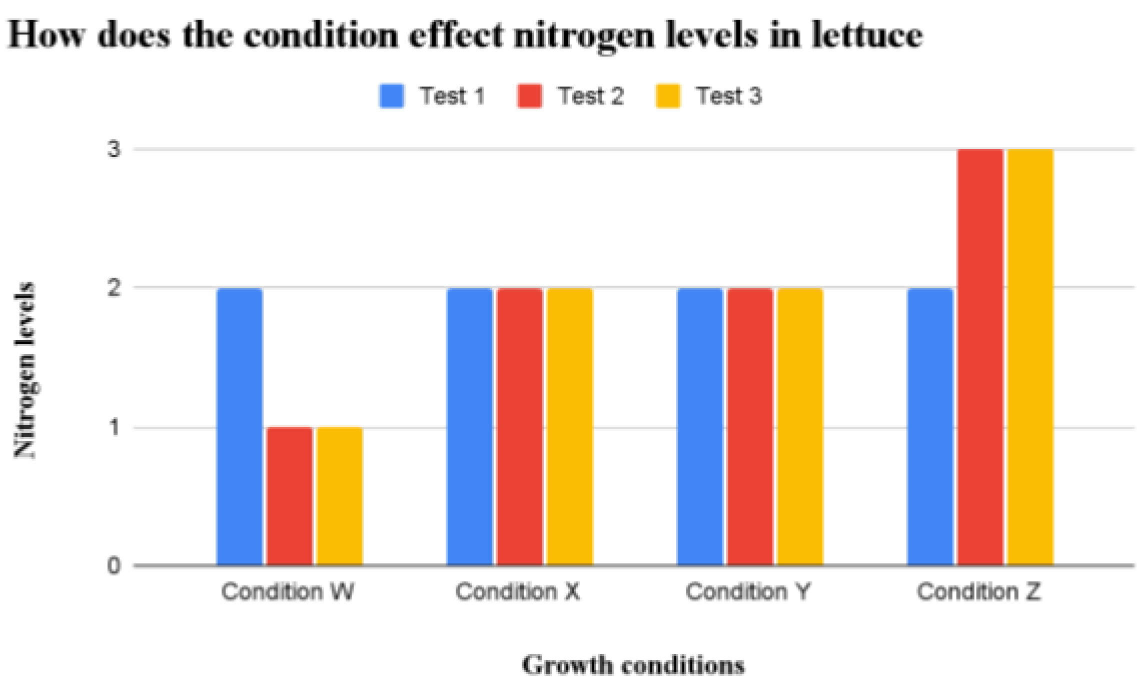

Chart 2. This test the nitrogen levels present in four conditions where butterhead lettuce was grown. Test 1 was conducted on September 28th 2022. Test two was conducted on october 4th 2022 and the last test, test 3 was conducted on October 10th 2022.

Conclusion:

How can cyanobacteria improve plant growth on Mars by acting as a biofertilizer? My hypothesis was supported by the data as condition Z grew the healthiest and largest plants over the 2 ½ week experimental period. The outcomes of this project provide insight into the role of cyanobacteria as a biofertilizer by acting as a source of nitrogen for plant life. The data suggests that cyanobacteria could be a sustainable alternative to using fertilizer for the growth of lettuce plants. In the future other experiments can be done on different plants that are higher in protein such as soybean and wheat plants. These plants take longer to grow than butterhead lettuce however they are more nutritious and high in protein. Different forms of cyanobacteria from the Diazotrophs family could be used to improve the nitrogen levels in the plants. In the future the PH,phosphorus, potassium and sulfur levels could have been tested. Testing more conditions such as different forms of nitrogen fixating bacteria to determine the best species such as Azotobacter, Legumes and Rhizobia. Money can be saved on expensive shipments of soil and fertilizer that must be brought from Earth, biofertilizer and nitrogen-fixing cyanobacteria can be a big aid in settling Mars with less of the cost. Current research being conducted by the Perseverance rover in the Jezero crater will greatly benefit future colonists when the time comes to finally step foot on the red planet. While the task of colonizing Mars is daunting, it is crucial for us to step beyond Earth and expand throughout the stars

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