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Survival of Chroococcidiopsis cubana and Nostoc sp. NHVL1 under simulated Martian conditions

By Sabrina Aasted Rohde

“Between the red mountains”, by Max Rymsha

Title Page

Survival of *Chroococcidiopsis Cubana* and *Nostoc sp. NHVL1* under simulated Martian conditions

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Front page picture: "Between the red mountains", by Max Rymsha winner of HP Mars Home planet rendering challenge (Image credit: Courtesy of HP, Inc.) - https://www.space.com/41697-hp-mars-colony-challenge.html, 17.06.2022

ABSTRACT

With Space exploration, the possibility of life on extraterrestrial planets, such as Mars, has become of bigger interest. Due to the limiting factors found on Mars, such as radiation, low temperatures, perchlorate salts, and water deficit, life as we know it may not survive. Hence, investigations on the survival of microorganisms, found in some of the harshest areas on Earth, are increasing. This study aimed to investigate the potential survival of two cyanobacterial strains under simulated Martian conditions. This was done by exposing Chroococcidiopsis cubana and Nostoc sp. NHVL1, to perchlorate salt and UVC radiation. Cyanobacteria were chosen due to their growth under harsh conditions and their phototrophic lifestyle. Nostoc sp. NHVL1 and C.cubana exposed in up to 4% Ca-perchlorate, showed no significant growth. In Nostoc sp. NHVL1 exposed to 0.5%$Ca(ClO_4)_2$ a minor growth was spotted in the first 20 days of exposure, whereafter a drop appeared. Nostoc sp. NHVL1 exposed to 0.5 and 1% $CaCl_2$ showed significant growth. Survival was observed in Chroococcidiopsis cubana exposed to UVC in up to 30min, whereas irradiated Nostoc sp. NHVL1 cells showed moderate survival in up to 5min. This study also investigated the synergistic effect of 2%Ca-perchlorate on the survival of C.cubana under UV radiation. Data suggested that there is a bacteriostatic effect observed. Cyanobacterial growth under simulated Martian conditions could potentially be increased, by introducing growth under a modest covering and with perchlorate-reducing bacteria.

INTRODUCTION

The idea of terraforming extraterrestrial planets has become of bigger interest throughout time since there are no extraterrestrial planets suitable for colonization found so far. With this in mind Mars has been considered to be one of the most likely candidates for terraforming.

Mars formed 4.5 billion years ago and is the fourth planet in the solar system. It is 1.5 astronomical units away from the Sun and has a radius of 3’390 km, about half the size of Earth. It takes Mars 669.6 sols, or 687 Earth days, to rotate the sun and has a day length of 24.6 hours, with an axis tilt of around 25 degrees. Like Earth, Mars has four seasons but due to its elliptical orbit around the Sun, they vary in length. The longest season is spring, which lasts for 194 sols, and is to be found in the northern hemisphere. With a duration of 142 sols, autumn in the northern hemisphere is the shortest season. [1] When seen from space, the Martian surface seems to

have a red color, which is due to the oxidation of iron in the Martian rocks, regolith, and dust. The Martian landscape as we see it today has been formed by various dust storms, impact craters, crustal movements, and volcanos. Due to the lack of plate tectonics, the Martian crust as we see it today is as old as the planet itself, and therefore it can give a good description of what might have been. The Martian crust is more basaltic than granitic and therefore it reminds of the oceanic crust on Earth. In the northern hemisphere, a flatter non-volcanic lowland is to be found, whereas the southern hemisphere has highlands and volcanos. [1]

When it comes to colonizing Mars a lot of different factors become relevant and have to be taken into consideration. Therefore, it is important to both look at the challenges such as possibilities that arise on Mars.

The Martian atmosphere is very thin and consists of 95% CO_2, 2.6% N_2, 1.9% Ar, 0,16% O_2.The atmospheric pressure on Mars is only 0,6% of the pressure we experience on Earth.[2] Due to the loss of magnetic field throughout Martian history, the Martian atmosphere was no longer protected from strong and intense solar winds and due to sputtering, atmospheric molecules got lost to space. The electromagnetic environment changed and important ions such as CO_2^+ and O_2^+ got lost. [3] As a consequence of the thinning of the atmosphere, Mars could no longer hold a stable climate. Pressures got higher and temperatures got lower equaling in an environment no longer capable of holding liquid water, and hereby water either disappeared into space or got frozen. [2] Even temporarily, liquid water would be unlikely on the surface of Mars, due to evaporation in areas where it neither freezes nor boils. [4] Therefore, water on Mars found today, only exists as water vapor or as ice. The Martian climate is cold, and its temperatures can vary from -153 to 20 ℃. To support a warm climate one bar of CO_2 is needed, whereas Mars only has 6-7 mbar of CO_2. [5]

Challenges on Mars

As a consequence of a thin atmosphere, a high radiation climate is found on Mars. Both energetic particle radiation, such as galactic cosmic rays (GCRs) and solar energetic particles (SEP), and ultraviolet radiation irradiate the Martian surface. [6]

UV radiation

The ultraviolet radiation environment found on Mars today is very equal to the environment found on early Earth. The lack of an ozone layer, low temperatures, and atmospheric pressure results in a higher surface flux of ultraviolet radiation. [7] DNA is the primary target of UV radiation and is the greatest factor found responsible for the decline in organism's function. It has been found that most biological action spectra that describe UV damage, have their peak at wavelengths of 200nm to 280nm (UVC) and 280nm to 315nm (UVB). The death rate of microorganisms exposed to wavelengths as such is expected to be high. [8]The highest UV radiation measured on Mars is at Gale crater at midday and was detected to be 20 W/m^2. [9]

There are three different types of UV radiation, UVA, UVB, and UVC. UVB and UVC are the most important radiations to look at when it comes to biological hazardousness, due to their short and damaging wavelengths.[7] UVA (315-400nm) causes long-term photosensitized oxidation.[10] It primarily affects organisms by causing oxidative damage resulting from reactive oxygen species (ROS). This is different from UVB and UVC´s photochemical damaging effect. On Earth the atmosphere shields from most UVB and all UVC radiation, due to the ozone layer. UVC and UVB get absorbed by UV-absorbing oxygen and ozone gases responsible for screening UV radiation. [11] As a consequence of the thinning of the atmosphere, an increase in UVC and UVB radiation has been seen on Mars. UV radiation becomes damaging to biological life by breaking DNA strands caused by biomolecules, such as nucleotides, undergoing photodamage.[11]. Hereby day length plays an important role, due to the organism´s ability to repair its DNA damage. Days on Mars are longer than on earth and therefore organisms need an effective repair system. If their repair system is sufficient in keeping up with DNA damage, the damage caused might not be of concern. The action spectrum for loss of viability is quite similar to the inactivation spectrum of DNA in organisms and therefore with an increase in radiation, a decrease in viability is seen.[12] UVC can rapidly inactivate unshielded cells and is believed to have created oxidizing surface conditions in the topsoil, which can destroy organic molecules. UVC can only penetrate a few mm into the Martian regolith and therefore it might not be a problem if cells are covered, but due to the lose topsoil and wind mixing of oxidants, it could still be a biological hazard. [13]

It has been reported that some microorganisms such as cyanobacteria have different UV screening compounds such as Mycosporines, which are small water-soluble molecules that absorb UV in the spectra of 310nm to 365nm. Mycosporine-like amino acids (MAAs) are mycosporines substituted with amino acid residues. Their correlation with UVB irradiance has been reported in numerous cyanobacteria, by the induction of MAAs synthesis under UVB irradiated environments.[10] Specific cyanobacteria also produce scytonemin, which is a secondary metabolite synthesized by cyanobacteria capable of producing an extracellular matrix, such as some of the genus *Nostoc.* It has been shown to have a high UVA protection capacity.[14] To cope with extreme UV conditions, it has also been shown that the presence of phycobilisomes in cyanobacteria might have an impact on their survival. [8] Phycobilisomes are multi-subunit protein complexes that absorb light, due to their light-harvesting antennas. In contrast to chlorophyll, they absorb green and yellow light. They play a big role in photosynthesis, by conducting energy absorbed into the photosystem II. Visible light (>400nm) can bleach autofluorescence proteins after sufficient exposure time, and due to the Martian UV flux being stronger, it has been shown that it is four times more effective at bleaching these proteins. UVB is shown to prevent energy from being transferred from the light - harvesting complex to Chlorophyll, by causing disassembly of the accessory pigment complexes. Seemingly resulting from a breakdown of linker polypeptides.[8]

Perchlorate

The perchlorate concentrations found in Martian regolith, are another important factor when it comes to habitability on Mars. Perchlorate was first detected by wet chemistry laboratory (WCL) on the Phoenix landing site at concentrations up to 0.6wt%. [15]

Perchlorate is a soluble ion that is non-reactive due to its high energy of activation associated with its reduction. It has a central chlorine atom surrounded by a tetrahedral array of four oxygen atoms. Perchlorate has one anionic charge and a large molecular volume. Because of its low affinity for cations perchlorate often forms salts such as Calcium perchlorate ($Ca(ClO_4)_2$), Magnesium perchlorate ($Mg(ClO_4)_2$), Ammonium perchlorate (NH_4ClO_4), and Sodium perchlorate ($NaClO_4$). Perchlorate salts are highly soluble and are completely dissociated in aqueous solutions. On Earth, perchlorate is only found naturally in the Atacama Desert in Chile, with an average content of 0.03% in total mineral mass.[16] Even though perchlorate is not common

on Earth some microorganisms, such as cyanobacteria, can survive in some perchlorate concentrations.

The amount of perchlorate found in Martian regolith has raised questions about the production of perchlorate on Mars. It is believed that Perchlorate can be produced through photochemical interactions of chloride by ozone (O_3) in the upper atmosphere. Although, studies show that the atmospheric production is not sufficient enough in order to explain the amount found. It is believed that CO_2-rich chlorine - bearing ice exposed to galactic cosmic rays, produces ClO_3^- and ClO_2^-, bearing high order chlorine oxidants. In contradiction to that, the same study has also shown that Martian perchlorate is radiolytically degraded and destroyed by ionizing radiation over time. [15] Due to its short wavelengths and high energy, UVC plays an overall role in the oxidation of Cl^- to ClO_4^-. It has been shown that there is an overall correlation between the high amount of perchlorate found on Mars and increasing UVC. Therefore, it has been suggested that the concentrations of perchlorate furthermore will raise, while a decrease of chloride will happen.

It is suggested that liquid water in form of brines can exist on present-day Mars, despite the conditions found. Perchlorate salts can absorb water from the atmosphere and hereby form brines. Brines are liquid in temperatures down to 195K, and some even down to 150K, due to perchlorate concentrations.[17] Perchlorate salts such as $Ca(ClO_4)_2$, $Mg(ClO_4)_2$, and $NaClO_4$ are extremely relevant when it comes to brine formation via deliquescence and melting because they have a low eutectic temperature and are distributed globally. Especially in polar and equatorial regions.[4] Perchlorate and Chlorate are ubiquitous and are therefore suggested to be present in liquid brines, in the subsurface of Mars, protected by UV and oxychloride, making them possible subterranean habitats for chemotrophic microbes.

Life on Mars

Mars may not be habitable for life as we know it, but there might be some microorganisms capable of surviving the extreme conditions found on Mars.

2.5 billion years ago cyanobacteria started to introduce oxygen into Earth's atmosphere when Earth had a climate similar to the Martian climate found today.[18] Cyanobacteria were some of the main organisms responsible for the oxygenation of Earth.[19] Stromatolites are microbial

reefs created by cyanobacteria and are the first evidence of life on Earth. Evidence of cyanobacteria on Mars has not yet been detected, but investigators have identified sedimentary structures which closely resemble stromatolites. It is believed that cyanobacteria may have begun constructing stromatolites on Mars, around 3.2 to 3.7 billion years ago.[20] It is said that photoautotrophy, such as performed by cyanobacteria, was an early invention on Earth. Due to the lack of ozone shielding prior to the oxygenation of Earth's atmosphere, wavelengths as low as 200nm would have reached the surface of Earth. [8] Due to the survival of cyanobacteria under these extreme conditions, they are believed to be some of the microorganisms capable of colonizing Mars.

It has been shown that required resources, such as H_2O, carbon, and solar energy, to sustain cyanobacteria are available on Mars. Therefore, they have been investigated as a potential biological component of life-support systems, for missions to Mars. Numerous benefits, such as oxygen production and the formation of organic nitrogen for potential use by other life forms, would follow with cyanobacterial colonization. Cyanobacteria could serve as a source of food and fertilizer for plant growth. [21] Furthermore, they could be used as a platform for the production of biofuels, to power ground vehicles on the Martian surface. Water is often produced as a by-product and can further support survival on Mars. [22]

Cyanobacteria such as *Chroococcidiopsis* are key photoautotrophic microorganisms and primary producers in stony hyper - arid deserts. They are distributed globally in dry, warm, and cold climates. They live in places where it becomes too dry to support higher plants and most eukaryotic and prokaryotic life, such as the driest areas of the Atacama Desert in Chile or Dry valleys in Antarctica.[23] *Chroococcidiopsis* often inhibits microscopic fissures of rocks, or forms biofilm at stone soil interface in deserts.[8] They survive by colonizing stones that retain sufficient moisture, and filter surface light from lethal to a wavelength suitable for photosynthesis.[24] *Chroococcidiopsis* is part of the "Culture Collection of Microorganisms from Extreme Environments (CCMEE)", due to their modalities of different rock colonization and their involvement in different biofilm formation and performance.[25] They are non-motile organisms and form unicellular or few celled packets[8] usually aggregates of two to ten cells. [20] They do not fix nitrogen under aerobic conditions and are very slow - growing, due to their slow doubling time.[8]

Chroococcidiopsis is extremely resistant to UV radiation, desiccation, high/low temperatures, and salt toxicity. This is due to their endolithic lifestyle of living under rocks or a few mm below the surface.[20] They can protect themselves by the formation and self - embedment into slime. Other than that, it is shown that by growing in biofilms they protect themselves better under stressful conditions.[20] Due to growth in few celled aggregates, the light path becomes elongated, and therefore it works as a protection against UV radiation. [8]They are able to withstand $13kj/m^2$ of UVC, which can be ascribed to the occurrence of multicellular aggregates, due to the inner cells being protected by the outer layer of cells. Therefore, inner cells cannot be reached by UV radiation.[18] Their sheath of organic - rich polysaccharides, that encapsulates *Chroococcidiopsis*, provides an extra protection layer against UVC.[8] *Chroococcidiopsis* has evolved different strategies to survive UV radiation, some of these are I. the avoidance of stress by gliding mechanisms, II. Different DNA repair mechanisms such as excision repair and photoreactivation, and III. their ability to stress defense by having UV absorbing compounds, antioxidant enzymes, and molecules.[26] Some *Chroococcidiopsis* are able to effectively repair and/or protect their genome under DNA-damaging conditions.[18] Their ability to survive these harsh conditions is also due to their phycobilisomes that can provide photoprotection since they are known to absorb UV radiation.[26] UV radiation gets attenuated due to their cellular organization and protection against oxidative stress, by carotenoid accumulation.[26] Along with that, they have evolved secondary metabolites such as mycosporines that are UV absorbing, and are a relevant factor when it comes to photoprotection. They act as antioxidants and reactive oxygen species (ROS) - scavengers. Mycosporines have been detected in *Chroococcidiopsis* exposed to UVC.[26] Due to their ability to cope with high levels of oxidative stress, which is an imbalance between the production and accumulation of ROS, they might be capable of coping with the presence of perchlorate, in their growing media.[27] This would be of interest due to the high concentrations of perchlorate in the Martian regolith, and due to the formation of salt brines on Mars. It has been shown that *Chroococcidiopsis* survives near the surface of salt deposits.[20] Therefore, if they are able to cope with high concentrations of salt, they might be able to survive and harvest water from salt brines on Mars.

In my experiments I am working with *Chroococcidiopsis cubana*, isolated from dry soil in Pinar del Rio, Cuba. *C.cubana* is phototropic, and it cannot be excluded that the strain produces cyanobacterial toxins that can be dangerous to humans and pets.[28]

Nostoc is another globally widespread cyanobacterium. In the genus *Nostoc,* many highly diverse species are found. They are found both as aquatic, semi-aquatic, and terrestrial species, and are some of the most common cyanobacteria in moist substrate environments.[29] They are reported in different parts of the world, such as the arctic and Antarctica to tropical regions.[19] They are commonly found on gravel, ground cloths, and aisles.[29] They differ in morphology, functional properties, habitat distribution, and biotic relations. Some of these species are free-living while others form symbiotic relationships with other organisms, such as plants or fungi.[19] *Nostoc* can form large gelatinous colonies of around 1 to 5mm thickness, that are variable in shape and structure[30] or grow into macroscopic mats. *Nostoc* colonies are known to grow in aggregated and entangled trichomes. They are often unbranched, flexuous, uniseriate, or curved and are always constricted at the cross walls.[29] Trichomes of *Nostoc* cells can be composed of vegetative cells, and heterocytes, which are cells found in some species, that are specialized in nitrogen fixation. Akinetes are survival cells formed by *Nostoc*. To form and maintain their colony shape, *Nostoc* colonies have an extracellular matrix composed of polysaccharides of high viscosity and molecular weight. They are also responsible to protect cells against environmental hazards and pathogens.[30] It is known that some *Nostoc* species can produce cyanotoxins and allelopathic compounds, that might affect plant growth and physiology.[29]

Nostoc was considered to be a good candidate for extraterrestrial agriculture, due to its high tolerance to extreme conditions.[19] Their physiologically suitable temperature is between 0 to 30 °C, but they are known to tolerate various temperatures. They survive freezing temperatures down to -60°C and -269°C in liquid helium.[30] They have well-developed adaptive strategies, which makes it possible for them to withstand repeated desiccation, salt stress, UV radiation, and pathogen infections.[19] It has been shown that *Nostoc commune* was able to withstand desiccation for 60 years. Both *Nostoc* and *Chroococcidiopsis* avoid desiccation damage by avoiding DNA fragmentations.[23] Due to their extracellular matrix, they can withstand UV radiation and in addition to that, some species have trehalose, sucrose, and glycan that are UV absorbing compounds, providing them protection. [31] Some *Nostoc* species have a modest salt tolerance. It has been shown that some retain their photosynthetic capacity upon exposure to 20 g/kg in alkaline freshwater, enriched with NaCl but were not able to survive 30 g/kg.[30] Sucrose and trehalose play a dual role when it comes to salt resistance, due to their

accumulation under desiccation and exposure to low salt concentrations. With that said, other cyanobacteria are more salt-tolerant, due to the synthesis of glycosylglycerol, glycine betaine, and glutamate betaine.[30]

The study aimed to examine *Chroococcidiopsis Cubana* and *Nostoc sp. NHVL1´s* ability to survive and grow under Martian conditions. This was tested by exposing them to different Mars-like conditions. A perchlorate threshold experiment was performed to show *C. Cubana* and *Nostoc sp*. *NHVL1´*s growth in different perchlorate concentrations. The interest of the experiment was to show growth and not only survival. It was hypothesized that *C. Cubana* would be able to grow in Ca-perchlorate concentrations up to 4%, whereas *Nostoc sp*. *NVHL1* would only show growth in concentrations up to 1% $Ca(ClO_4)_2$, due to its modest survival in salt. The hypothesis was tested by incubating *C. Cubana* and *Nostoc sp. NHVL1* in falcon tubes with BG11 media containing different concentrations of Ca-perchlorate. Additionally, *Nostoc sp. NHVL1´s* ability to grow in other salts, such as $CaCl_2$was investigated. This was tested by incubating *Nostoc sp. NHVL1* in falcon tubes with BG11 media containing 0.5 and 1% of $CaCl_2$.

Furthermore, an Ultraviolet-response experiment was performed to distinguish the survival of *Chroococcidiopsis Cubana* and *Nostoc sp. NHVL1* under UVC exposure. This was performed by exposing them to UVC radiations at different time intervals. Additionally, it was hypothesized that the radiation-tolerant cyanobacteria*, C.cubana* exposed to $Ca(ClO_4)_2$ prior and during the experiment, would have a higher survival rate under UVC exposure, due to their developed stress response to perchlorate salt. There was a higher expected survival rate for *C.cubana* compared to *Nostoc sp. NHVL1*. *Nostoc sp. NHVL1* will from now on be referred to as *NHVL1.*

In all experiments involving salt survival, growth was measured by measuring the quantity of light absorbed by the media they were incubated in. Growth of the cyanobacteria were measured every 10th day for 50 days. Their ultraviolet response was measured by gas chromatography to check for metabolism. This was done by measuring how much CO_2 had been taken from the incubated bottles every third day, over 12 days.

METHOD AND MATERIAL

Organisms and cultural conditions

Chroococcidiopsis cubana DSM No.: 107010, was isolated by František Hindák in 1966 from dry soil in Pinar del Rio, Cuba. *NHVL1* was isolated from the leaves of the moss *Hylocomium splendens*, by Danilo O. Alvarenga, Copenhagen University. The sample was collected in a boreal forest from Jokkmokk, Norrbotten, Sweden (66°1'13.6" N, 19°51'8.7" E). Both cyanobacterial strains were grown in 300mL Erlenmeyer flasks containing BG11 medium. These were sealed by parafilm and plugs made of water-repellent cotton wool with elastic fixation bandage, making gas exchange possible. They were incubated at 25°C, without shaking, under a photon flux density of 30 ± 1 µmol/ m^2/s, measured with a Quantum meter model MQ-200 (apogee instruments, 721W 1800N Logan, UT 843221). All Cyanobacteria strains were grown under a 16/8h light/dark circle provided by LD straps.

Effect of Ca-perchlorate on the growth of C.cubana and Nostoc sp. NHVL1

To measure *C.cubana´s* perchlorate threshold five different concentrations of $Ca(ClO_4)_2$ were used. 0.5 (21mM), 1 (42mM), 2 (84mM), 2.4 (100.6mM) and 4% (168mM) were each added to BG11 media and were used as growth medium. *NHVL1* was grown in BG11 medium with two different concentrations of $Ca(ClO_4)_2$; 0.5 and 1%. Each growth medium was prepared separately by adding the calculated wt.-percentage of $Ca(ClO_4)_2$ to a 100mL blue cap bottle containing BG11 medium, see appendix 1. After adding perchlorate salt, the BG 11 medium was autoclaved whereafter BG11-mix, vitamin B12, and $NaNO_3$ were filter sterilized and added after cooling. The experiment was performed in triplicates of 15mL falcon tubes, each containing 9mL of BG11 + $Ca(ClO_4)_2$ medium and 1mL of cyanobacteria culture. All plastic-/glassware and lids were sterilized by autoclaving prior to their use. Cells were concentrated by centrifuge for 10min at 5000rpm. This process was repeated twice, whereafter 20mL of clean BG11 medium was added. Cells were homogenized by vortexing, before adding them to 15mL falcon tubes, to ensure that each inoculum contained the approximately same amount of cells. Additionally, triplicates of positive control for each cyanobacterium strain were made. Each falcon tube was sealed with plugs made of water-repellent cotton wool with elastic fixation bandage and parafilm, making gas exchange possible. Whereafter they were incubated at 25°C with a 16/8hour light/dark circle.

Every 10th day growth measurements were performed by withdrawing 1mL of each triplicate from the falcon tubes. The substance was homogenized by vortexing, before the withdrawal. Aliquots were added to 2.2mL Eppendorf tubes containing beads. Before growth measurements were carried out, each tube got bead beaten by FastPrep-24 for 60second at a speed of 4m/s. Cyanobacterial growth was quantified by determining the optical density at 730nm. This was done for each triplicate, each one consisting of a 1mL aliquot. For each Ca-perchlorate concentration, a corresponding blank sample was used. Each triplicate was measured two times. Optical density measurements were performed by Thermo Spectronic Genesys 10 UV-visible Spectrophotometer. Different optical set-ups of spectrophotometers can lead to slightly differing absorbance values. Therefore, it was made sure that each measurement was performed by the same spectrophotometer.[32] Furthermore for each concentration blank measurements were performed. The experiment lasted for 50 days equalling six growth measurements.

***Nostoc sp. NHVL1´s* response to Calcium Chloride exposure**

An additional experiment was performed to examine *NHVL1´*s overall salt tolerance. Two different concentrations of $CaCl_2$ were added to the BG11 growth medium. Each concentration was calculated to contain the same molarity of Calcium, as in the previous $Ca(ClO_4)_2$ experiment. Calculations are shown in appendix 3. Each growth medium was prepared separately by adding the calculated mass of $CaCl_2$ to a 100mL blue cap bottle containing BG11 medium, whereafter it was autoclaved. The experiment was performed in triplicates of 15mL falcon tubes, each containing 9mL of BG11 + $CaCl_2$ medium and 1mL of *NHVL1* cells. All plastic-/glassware and lids were sterilized by autoclaving before their use. Cells were concentrated and homogenized before adding, to ensure that each inoculum contained the approximately same amount of cells. Additionally, triplicates of positive control of *NVHL1* were made. Each falcon tube was sealed with a lid made of water-repellent cotton wool with elastic fixation bandage and parafilm, making gas exchange possible. They were incubated at 25°C with a 16/8hour light/dark circle. Growth of *NHVL1* was measured identically as in the previously described experiment, by determining the optical density at 730nm of each triplicate, after they have been bead beaten for 60second at a speed of 4m/s. Growth measurements were performed every 10th day for 40 days.

Effect of UV radiation on the survival of C.cubana and Nostoc sp. NHVL1

An ultraviolet radiation experiment was performed, to test *C.cubana* and *NHVL1´*s survival under Martian stimulated UV flux. To test *Chroococcidiopsis cubana* survival under UV radiation five different UV treatments were performed; 0.5, 1, 5, 10, and 30min of 12.42 W/m^2 UV radiation. To test for positive synergistic effects of Ca-perchlorate on the survival of *C.cubana* under UV radiation, the same experiment was conducted for *C.cubana* exposed to 2% $Ca(ClO_4)_2$ six days prior to, and under UV exposure. *NHVL1´s* UV survival got tested by using three different treatments: 0.5, 1, and 5min of 12.42 W/m^2 UV radiation. Each time interval got performed in triplicates. To compare our results, triplicates of positive controls for each cyanobacterium were made.

Preparatory to the performance of the experiment all materials used were sterilized by autoclaving. To prepare the cultures, strains of *C.cubana* and *NHVL1* were concentrated, by adding 25mL of stock culture to a 50mL falcon tube, whereafter they were centrifuged for 10min at 5000rpm. After 10min the supernatant got removed and the process was repeated until all cells were clear of media. Afterwards, each 50mL falcon tube got filled with 30mL of its respective new media, to resuspend the cells; One with *C.cubana* in BG11 medium, one with *C.cubana* in BG11 medium + 2% $Ca(ClO_4)_2$, and one with *NHVL1* in BG11 medium. Tubes were sealed with cotton plugs made of water-repellent cotton wool with elastic fixation bandage and parafilm, making gas exchange possible. Each cyanobacterium was now incubated at 25°C for six days, at a 16/8-hour light/dark circle. After six days, OD measurements were performed on each culture to make sure that each strain starts with the same amount of cells. Three samples of each 1mL were collected in 1.5mL Eppendorf tubes containing glass beads. One tube of clean BG11 medium was also collected to function as our blank sample. Each tube got bead beaten for 60seconds at a speed of 4m/s. Afterwards, 700µl of our samples were transferred to cuvettes and were measured at 730nm. OD measurements were afterwards compared. It was preferable that each culture contained around 10^7cells pr. mL. Next 60 agar plates were made, 30 with BG11 medium and 30 with BG11medium + 2% $Ca(ClO_4)_2$. 0.2µm filters were used to transfer our cells from the falcon tubes to agar plates. Separation of cells from media was performed by vacuum filtration. 1.5mL of cells were added to the suction plate in the buncher funnel, on top of the buncher flask, which was connected to a vacuum machine. Once cells were separated from the media, filters were transferred to agar plates with a sterile pincer. Here it was made

sure that the cells were faced upwards and that the filter was touching the agar. To check for contamination a negative control was made by adding 1mL of BG11 media and BG11 + 2% $Ca(ClO_4)_2$ media to separate plates. *C.cubana* cells grown in 2% Ca-perchlorate media were added to agar plates containing BG11 media + 2% $Ca(ClO_4)_2$, whereas *NHVL1* and *C.cubana* grown in BG11 medium were added to agar plates made of clean BG11 medium.

The UV treatments were performed under a UV lamp with a wavelength of 257nm and a power of 15W. The lamp was set up inside of a flow chamber, on two tall 2L beaker glass. This gave the lamp a height of approximately 31cm from the under-laying plates, giving the plates a treatment of approximately 12.42 W/m^2. To see the calculation see appendix 4. Furthermore, the lamp was covered by aluminum foil to create a closed system. The plates were now added to the UV chamber, where they each got the subscribed treatment. After each treatment, the filters were cut in half with a sterile scissor, where half of the filter got collected for DNA damage and transcriptome. The other half of each filter was added to a 50mL falcon tube with 20mL of clean BG11 medium. Cells were now vortexed for 30sec to make sure that the cells were released from the filter. The liquid got transferred to 50mL gas bottles, which were sealed tight with rubber plugs. In total, we had 60 gas bottles. $Ca(ClO_4)_2$ - *C.cubana* cells were also incubated in clean BG11 media, to optimize their survival after their UV treatment. After sealing the bottles 10mL of approximately 4000ppm of CO_2, was added to each gas bottle. Bottles now had a concentration of approximately 5000-8000 ppm CO_2. This was done to ensure that there is enough CO_2 for the bacteria to consume. Afterwards, gas samples of 3mL with the helot of syringes were collected in gas sample bottles. This was done to measure the amount of CO_2 at day zero. Gas bottles were now incubated at 25°C, at a 16/8-hours light/dark circle. Gas sample bottles were stored at room temperature.

Gas sampling was performed every third day; days 0, 3, 6, 9, and 12. Each time 3mL of gas was taken out of the gas bottles and into gas sample bottles. This was performed for each triplicate. Whereafter they were stored at room temperature until further use.
After 12 days, 270 gas samples had been gathered. Gas analyses were performed by gas chromatography. Gas chromatography was performed over two days where 27 bottles were analyzed at a time. A standard curve was made by measuring the pressure of different known

concentrations. Afterwards all sample bottles were measured and the concentration of CO_2 in each bottle was calculated, with the help of Microsoft excel (appendix 5).

DATA ANALYSIS

Microsoft Excel was used to store and organize data, such as performing simple calculations and constructing simpler graphs. For data analysis, and data management R 4.1.1 was used. R 4.1.1 was also used to construct error bar graphs.

In the experiment performed, testing the survival of *Chroococcidiopsis cubana* and *NHVL1* in $Ca(ClO_4)_2$, negative measured OD values were put to zero, due to machine measuring errors. For both experiments, a first-order kinetic relationship was expected for the growth of both cyanobacteria, and therefore LN-transformation of the data was performed. Prior to LN-transformation non-positive values were removed. This was done prior to fitting linear regression models. Half – life and doubling time of *C. cubana* and *NHVL1* were calculated in Microsoft Excel using the growth rate found by linear regression.

Linear regression assumptions, such as assumptions of linearity, constant variance (homoscedasticity), and independence of errors, were analyzed using scatter plot fitted values versus residuals. In an ideal case, points would be scattered around the horizontal line, at a value of 0. Additionally, normality testing was performed by using a Q-Q plot, where theoretical quantiles were plotted against standardized residuals. If assumptions were not fulfilled, theoretical quantiles and standardized residuals, are from the same distribution. Hence, ideally, the data points fall approximately along the reference line. If the linear regression assumptions, in UV measurements, were not fulfilled, categorical variables for time were introduced.

Significance of parameters in models were tested, by calculating p – values using Wald test for parameters. P-values <0.05 were considered statistically significant.

RESULTS

Effect of Ca-perchlorate on the growth of C.cubana and Nostoc sp. NHVL1

Growths observed in *Chroococcidiopsis cubana* and *NHVL1* exposed to different Ca-perchlorate concentrations are shown in this section. Data is to be seen in appendix 6 tables 3, 4, and 5. All OD measurements are made at 730nm. To calculate the growth rate and estimated starting point, linear regression was used. Values were LN transformed, due to an expected first - order kinetic relationship.

Chroococcidiopsis cubana

In figure 1, we see the amount of growth observed in *Chroococcidiopsis cubana* control (0% $Ca(ClO_4)_2$) and *Chroococcidiopsis cubana* exposed to 0.5% $Ca(ClO_4)_2$ over 50 days. There is a significant growth of 0.025 (p-value ≈ 0, table 1) observed in *Chroococcidiopsis cubana* grown in normal BG11 medium. Whereas *C.cubana* exposed to 0.5% $Ca(ClO_4)_2$, shows a steady fall in OD measurements.

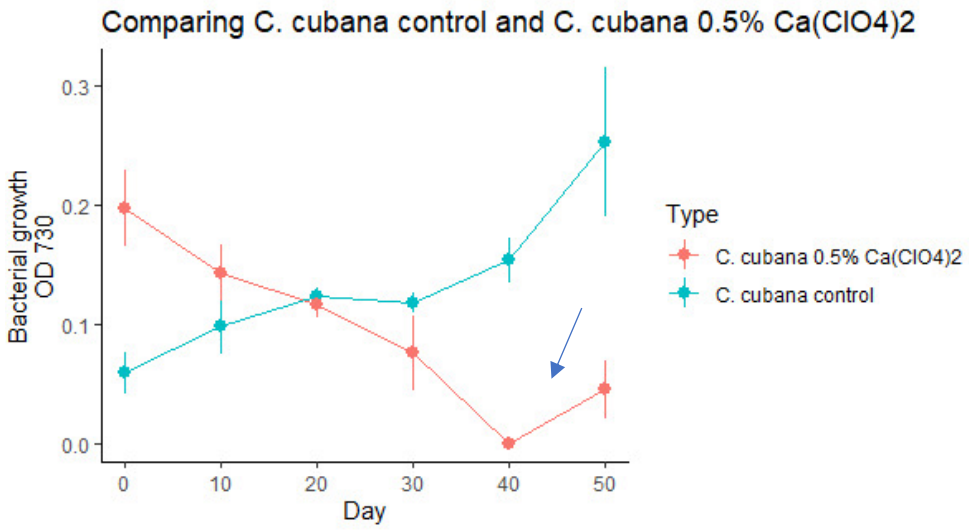

Figure 1: *This figure shows the amount of growth (mean and SD) observed in Chroococcidiopsis cubana control (blue), and Chroococcidiopsis cubana exposed to 0.5% $Ca(ClO_4)_2$ (red) over 50 days. The arrow seen in the graph, shows the bleaching time of C.cubana cells due to perchlorate. SD = standard deviation*

In table 1 shown below, we can see the estimated starting OD measurements and the estimated growth rates for *C.cubana* control and *C.cubana* 0.5% $Ca(ClO_4)_2$. We can see that there is a significant fall of -0.032 (p-value ≈ 0) in *C.cubana* 0.5% $Ca(ClO_4)_2$.

Chroococcidiopsis cubana control and 0.5% $Ca(ClO_4)_2$ compared		
R^2	0.7802	
$Ca(ClO_4)_2$ concentration (%):	Estimated OD_{730}:	P-value:
Control	-2.738	0.00163
0.5	-1.608	4.94e-07 (≈ 0)
	Estimated growth rate:	
Control	0.025	9.52e-07 (≈ 0)
0.5	-0.032	1.88e-10 (≈ 0)

Table 1: *This table compares the growth observed in Chroococcidiopsis cubana exposed to 0.5% Ca-perchlorate with C.cubana control. The estimated starting points (OD) are shown. It also shows the calculated growth rate for C.cubana control and C.cubana in 0.5% $Ca(ClO_4)_2$. P-values shown, tell us if there is a significant difference in the estimated staring point and growth rate in 0.5% Ca-perchlorate, compared to control.*

Chroococcidiopsis cubana was exposed to five different Ca-perchlorate concentrations, and in none of the concentration's growth was seen. Graphs are shown in appendix 7. The calculated p-values (all over 0.05) show that none of the parameters are significant and therefore we can reject that there is growth happening (see appendix 10 table 6).

Throughout the experiment, negative OD values were observed. For 1% Ca-perchlorate the first negative OD measurement was made after 20 days. This can be due to machine measuring errors since the value probably would be around zero. Due to negative measurements being inexplicable, these values were put to zero. After the first negative value, the subsequent values did not increase much during the next measurements. Similar observations were made for 2, 2.4, and 4% Ca-perchlorate. My results hereby indicate that *Chroococcidiopsis cubana* has a low threshold for Ca-perchlorate.

Doubling time of C. cubana and NHVL1

With the help of the calculated growth rate by linear regression of *NHVL1* and *Chroococcidiopsis cubana* in my control samples, an estimated doubling time was found. This is seen in table 2. We can see that *C.cubana* has an estimated doubling time of 28 days, whereas *NHVL1* has a doubling time of 22 days.

	C.cubana	***NHVL1***
Growth rate (OD/d)	0,025	0,032
Doubling time (Days)	28	22

Table 2*: In this table the calculated growth rates together with the doubling time for C.cubana and NHVL1 are shown.*

Effect of $CaCl_2$ on Nostoc sp. NHVL1 compared to Ca-perchlorate

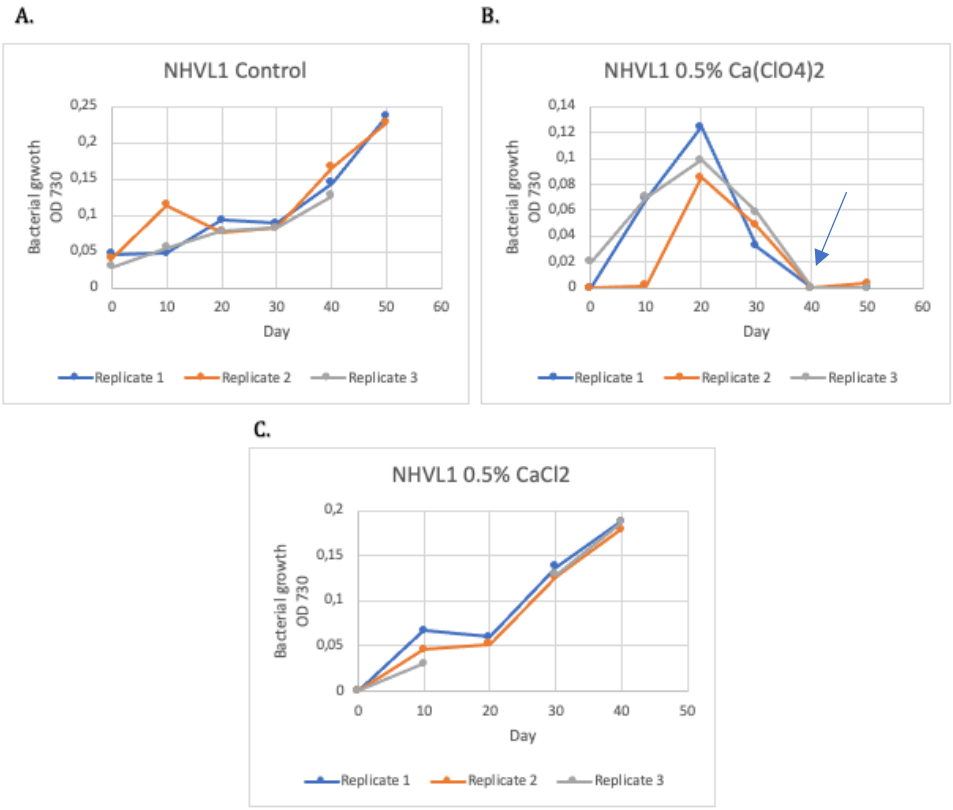

Figure 2: *This figure shows the amount of growth observed in NHVL1 control(**A**), 0.5% $Ca(ClO_4)_2$ (**B**), and in 0.5% $CaCl_2$ (**C**). The arrow in graph B shows the bleaching time of NHVL1 cells, due to perchlorate. The experiment was performed in triplicates, and each line represents one of the replicates. We can see that NVHL1 control shows normal growth in all of the replicates, where NHVL1 0.5% $Ca(ClO_4)_2$ shows growth the first 20 days whereafter a decline happens. In 0.5% $CaCl_2$ normal growth is to be observed.*

By looking at figure 2B, we observe a growth in the first 20 days in *NHVL1* exposed to 0.5% Ca-perchlorate, whereafter a drop is seen. Due to the small sampling size and due to a loss of data when LN transforming, the growth rate of *NHVL1* in 0.5% Ca-perchlorate was not calculated. No growth is observed for *NHVL1* grown in 1% $Ca(ClO_4)_2$ (see appendix 8 figure 2).

In figure 2C, the growth of NHVL1 in 0.5% $CaCl_2$ is shown. Here we can see that there is a significant growth (p-value ≈0) with a rate of 0.05 over 40 days of exposure. When comparing growth in 0.5% $Ca(ClO_4)_2$ to 0.5% $CaCl_2$ as done in figure 3 below, we can see that the growth observed in 0.5% $CaCl_2$ continues after 20 days, where 0.5% $Ca(ClO_4)_2$ drops. Better growth and survival are observed in $CaCl_2$, and therefore it seems like perchlorate has a toxic effect on the survival of *NHVL1*.

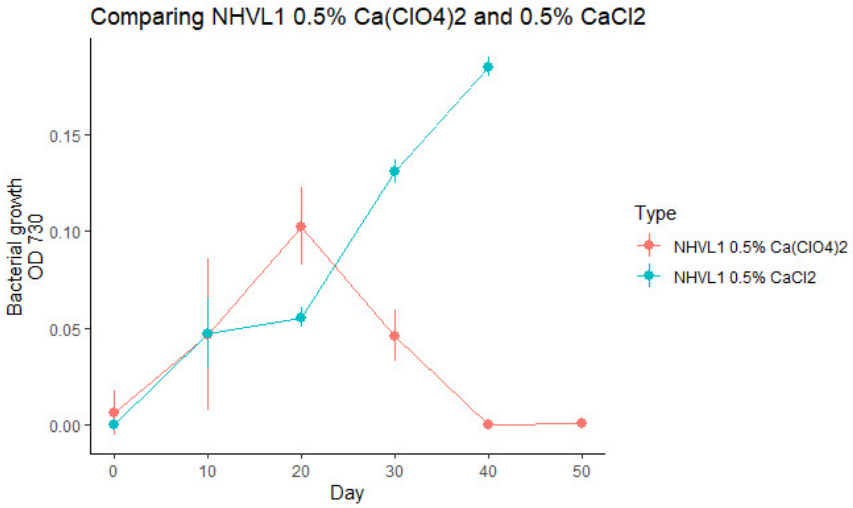

Figure 3: *In this figure the growth (mean and SD) of NHVL1 in 0.5% $Ca(ClO_4)_2$(red) and 0.5% $CaCl_2$(blue) throughout 50days is shown. It is to be observed that after 20 days a decline in growth is seen in 0.5% Ca-perchlorate compared to 0.5% $CaCl_2$ that continues to grow after 20 days. SD = standard deviation*

Graphs for *NHVL1* $CaCl_2$ control and *NHVL1* 1% $CaCl_2$ are shown in appendix 9. *NHVL1* control shows an estimated growth of 0.033 per day. When comparing the growth seen in 0.5% and 1% $CaCl_2$ to control, we see that *NHVL1* grown in 0.5% $CaCl_2$ shows a significant better growth then the control sample (p-value = 0.029). With a growth rate of 0,041 in 1% $CaCl_2$, we see a slightly faster growth compared to control. Although, the growth seen isn´t differing significantly (p-value = 0.32), see appendix11. The slightly higher growth seen in $CaCl_2$, may be due to a positive synergistic effect of the salt.

The bleaching effect of Ca-perchlorate

Over time the cells of *NHVL1* and *C.cubana* in Ca-perchlorate got bleached. *Chroococcidiopsis cubana* exposed to 4% Ca-perchlorate got bleached after 7 days. 2.4% Ca-perchlorate showed signs of bleaching in the top layer of cells after 10 days, whereafter they all were bleached on the 20th day. Most of *C.cubana* cells in all of the concentrations were almost fully bleached after 30 days. 0.5% still had signs of green cells after 50 days but most of them were bleached. There was no bleaching observed in the control sample. *NHVL1* exposed to 1% Ca-perchlorate was almost completely bleached after 20 days, and after 30 days all cells were bleached. *NHVL1* exposed to 0.5%$Ca(ClO_4)_2$ started to show signs of bleaching after 30 days but was

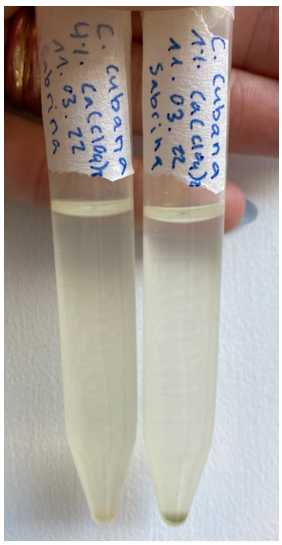

Figure 4: *Comparison of C.cubana in 4% Ca-perchlorate and 1% Ca-perchlorate after 7 days of exposure.*

fully bleached after 40 days. The control sample showed no signs of bleaching. To see the exact dates, see appendix 12.

Changes in cell morphology

There was no change in cell morphology observed in *Chroococcidiopsis cubana* in either of the Ca-perchlorate concentrations. They still formed few celled aggregates, but it was to be observed that the cells appeared less green in all of the concentrations compared to the control. In big colonies exposed to 2.4% Ca-perchlorate bleaching of the outer layer of cells was observed, while the inner layer remained green. In *NHVL1* no change in cell morphology was observed but in 1% Ca-perchlorate, the cell wall appeared to be broken in some areas. Added to that, the cells of *NHVL1* exposed to perchlorate looked less green compared to the control. See figure 5.

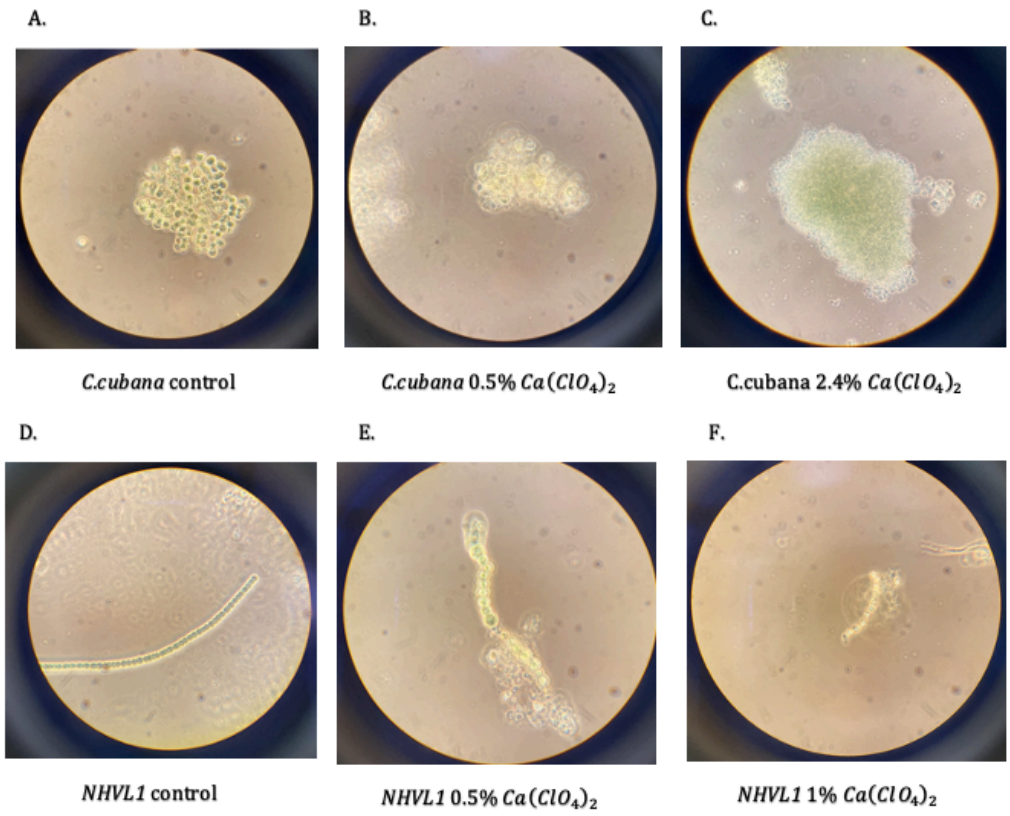

Figure 5: *In this figure we see cell morphology of C.cubana control (**A**), 0.5% Ca-perchlorate (**B**), and 2.4% Ca-perchlorate (**C**). We can see that there is no change in cell morphology, but that bleaching of cells can be observed. In 2.4% the outer layer of a bigger colony is bleached but the inner layer remains green. We also see NHVL1 control (**D**), 0.5% Ca-perchlorate (**E**), and 1% Ca-perchlorate (**F**). There is no change in morphology to be observed, but the cell wall in 1% is broken in some areas.*

Effect of UV radiation on the survival of C.cubana and Nostoc sp. NHVL1

For our experiment, UVC was chosen due to its short wavelengths and high energy, and therefore its demining effect on biological life. Based on my logarithm transformed rates ($\mu gCO_2/d$) (see appendix 13, tables 10, 11, and 12), a linear regression was performed, see figure 6. For each regression, the p-values were calculated

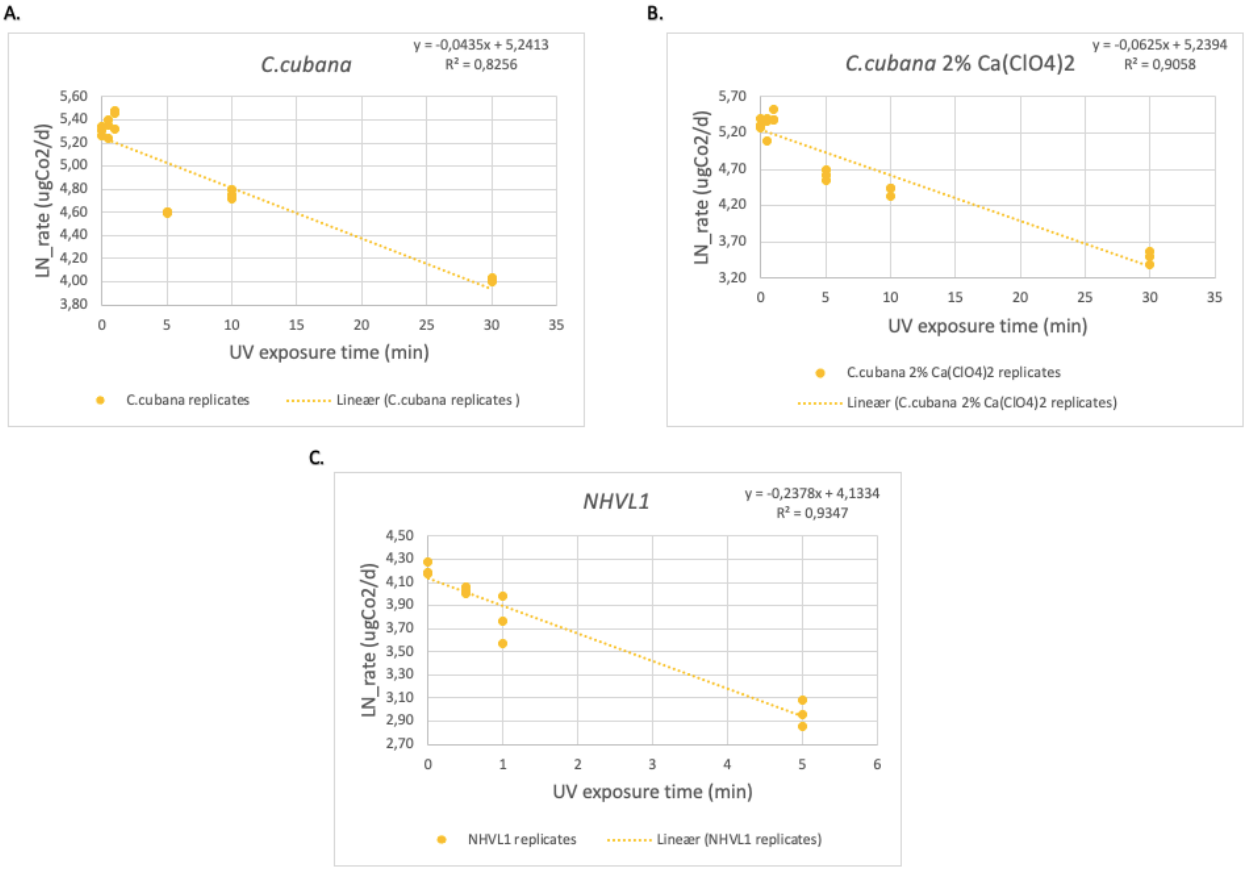

Figure 6 *: In this figure the linear regressions for Chroococcidiopsis cubana(**A**), Chroococcidiopsis cubana in 2% $Ca(ClO_4)_2$(**B**), and NHVL1(**C**) are shown. It shows the correlation between UV exposure time and the logarithm of the absolute value of rate ($\mu gCO_2/d$). Each dot seen on the graphs equals to the logarithm of each measured rate of the triplicates from each time exposure. It is observed that, in samples receiving longer UVC exposure, less μgCO_2 is taken per day. The correlation between UV exposure time and μgCO_2 pr. Day is explained mathematically by the equation seen in the right corner in each graph.*

Based on R^2 calculated for each graph seen above (A. 0.8256, B. 0.9058, and C. 0.9347), we can see that there is a good correlation between the relationship of the logarithm of the absolute value of rate and the UV exposure time.

The correlation between the amount of μgCO_2 taken per day for *C. Cubana* is shown by the mathematical equation $y = -0.0435x + 5.2413$. Due to the calculated p-value being close to zero, we can conclude that by time, the logarithm of the absolute value of rate is significantly

falling by 0.044 per minute. For *C.cubana* in 2% $Ca(ClO_4)_2$ the following equation was calculated $y = -0.0625x + 5.2394$. Here the calculated p-value, based on each coefficient, was close to zero, hence we can conclude that by time, the logarithm of the absolute value of rate is significantly falling by 0.063 per minute. The results suggest that Ca-perchlorate has a negative effect on the survival of *C.cubana,* compared to *C.cubana* grown in a normal BG11 medium prior to UV exposure. The equation for *NHVL1* is $y = -0.2378x + 4.1334$, due to the calculated p-value (≈ 0), we can conclude that the rate is significantly falling by 0.238. To see calculated p-values see appendix14.

To show the significance between UVC exposed cyanobacteria and non-exposed cyanobacteria (control), time as a categorical variable, was introduced. For each cyanobacterium the estimated rate of CO_2consumption was calculated. *C.cubana* is shown in table 3 below. To see the calculated estimates for *C.cubana* exposed to 2% Ca-perchlorate and *NHVL1*, go to appendix 15, tables 14 and 15.

Chroococcidiopsis cubana		
R^2	0.9923	
UVC time exposure (min):	CO_2 consumption($\mu gCO_2/d$):	P-value:
Control	5.31	~0
0.5	5.33	0.5830
1	5.42	0.0241
5	4.60	~0
10	4.76	~0
30	4.02	~0

***Table 3**.: This table shows, the estimated rate off CO_2 consumed by C.cubana under UVC exposure. Over longer periods of UVC irradiation, a change in CO_2 consumed by C.cubana is to be observed. The p-values shown, tell us if there is a significant difference observed in CO_2 consumed by cells over longer periods of UV exposure compared to control.*

Based on the calculated estimates for 0.5min of UV exposure compared to control, we can see that on average after 0.5 minutes the logarithm of the absolute value of rate is 0.02 higher than our starting point (control 0min). Due to this, we can say that there is a diminutive change seen. This is underlined by the calculated p-value (0.583), see table3. If we look at 10 and 30 minutes

of UVC exposure, we can see that based on the calculated p-values (both close to 0), a significant change in the estimated LN rates of CO_2 taken per day, compared to no UV exposure is observed.

Based on our results in appendix 15, we can see that *C.cubana* exposed to 2%Ca-perchlorate prior to UV exposure, shows no significant difference in the amount of CO_2 taken per day under 0.5min and 1min of UV exposure, compared to control. This is supported by our calculated p-values (0.5min = 0.628, 1min =0.221). After 5 minutes of UV irradiance, we start to see a significant fall in the amount of CO_2 taken per day. With a consumption of 3.48 $\mu gCO_2/d$, the biggest drop is observed after 30 minutes of UV exposure, with an estimated rate of 1.84 less CO_2 taken per day compared to our control starting point.

Our results suggest that NHVL1 has a low surviving rate when it comes to UVC exposure. It is observed that after 1 min of UVC exposure a significant drop in the amount of CO_2 taken per day, is to be seen compared to the control (p-value = 0.00239),

Calculated Half – life of NHVL1, C.cubana, and C.cubana 2% $Ca(ClO_4)_2$

Based on the calculated growth rates for each bacterium I now calculated their half – life under UVC exposure, see table 4.

Bacteria	**Half - life**
C. cubana	15.93min
C. cubana 2% $Ca(ClO_4)_2$	11.09min
NHVL1	2.91min

***Table 4**: In this table the calculated half – life of each bacteria is shown. All values are calculated by the growth rate given by linear regression.*

It is shown that the half-time calculated for *C.cubana* is 16min. This tells us that, after 16 minutes only one – half of the starting cells are left, and therefore half the consumption of CO_2 is seen. *C.cubana* in 2% $Ca(ClO_4)_2$ showed a half – life of 11 minutes and for *NHVL1* the calculated half – life is 3 minutes.

UV radiation´s differing effect on Cyanobacteria

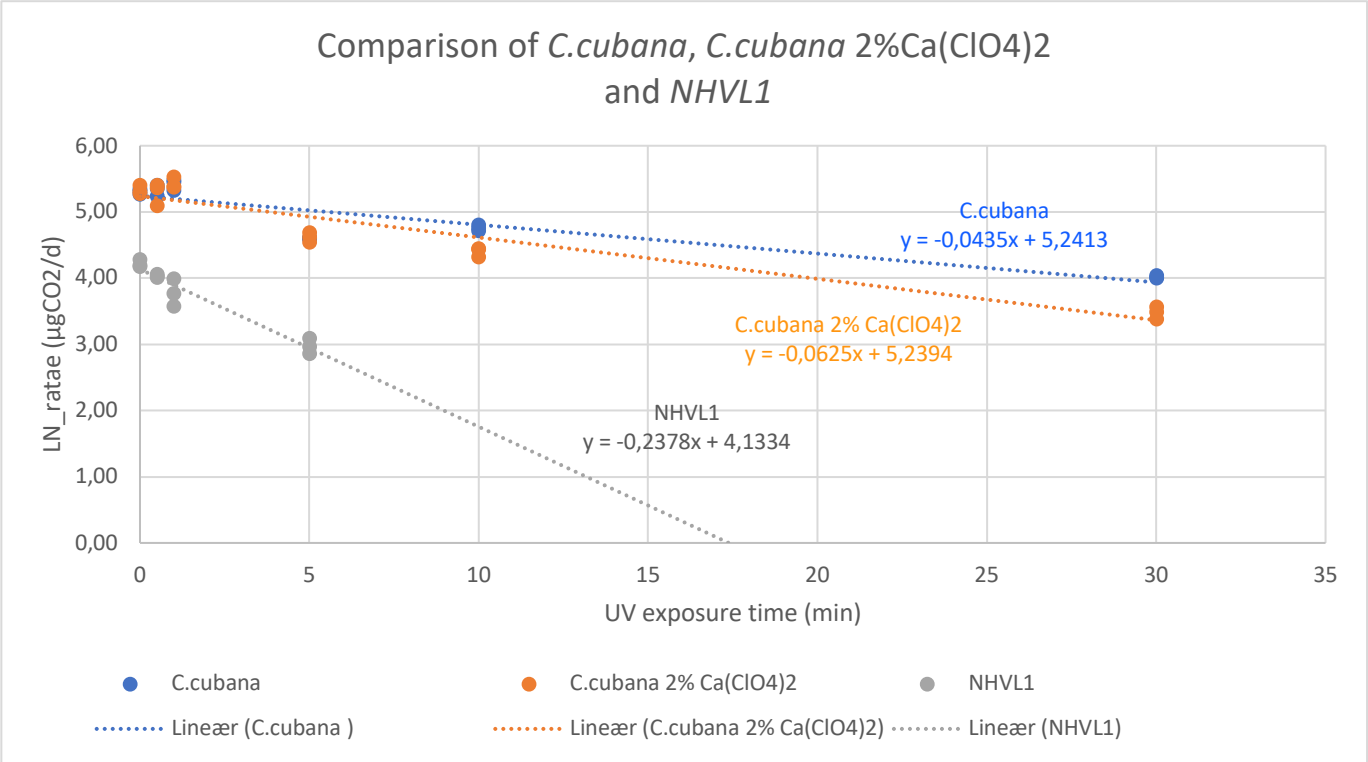

Figure 7*: This figure shows the linear regressions for Chroococcidiopsis cubana (blue), Chroococcidiopsis cubana in 2% $Ca(ClO_4)_2$(orange), and NHVL1 (grey). It shows the correlation between UV exposure time and the logarithm of the absolute value of rate ($\mu gCO_2/d$). We can see that the rate for NHVL1 drops the fastest by far. We can also observe that, when comparing C.cubana and C.cubana2% $Ca(ClO_4)_2$ a slightly difference is to be seen in the drop over time. For each cyanobacterium linear regression is shown mathematically.*

In figure 7, the CO_2 consumption over time of *NHVL1, C.cubana*, and *C.cubana* in 2% $Ca(ClO_4)_2$ are compared. When comparing the starting amount of CO_2 consumed by *Chroococcidiopsis cubana* exposed to 2% $Ca(ClO_4)_2$ prior to and during the experiment, to *Chroococcidiopsis cubana* without perchlorate exposure, no significant difference is observed (P-value 0.982). But when we compare them over time, we can see that there will occur a significant difference in CO_2 consumption (p-value 0.0058), which means that over time, there will be a greater fall in CO_2 taken per day in *C.cubana* 2% $Ca(ClO_4)_2$. The greater fall observed means that less CO_2 is taken and therefore fewer cyanobacteria are photosynthesizing. *NHVL1* compared to *C.cubana*, also shows a significant difference in CO_2 taken per day (p-value ≈ 0). This difference is seen in the amount of CO_2 consumed at the starting point of the experiment and also over time. See appendix 16.

My results hereby suggest that *C. cubana* has a higher threshold for UVC exposure compared to *NHVL1* and *C. cubana* exposed to 2% $Ca(ClO_4)_2$.

DISCUSSION

This work aimed to investigate the survival of *Chroococcidiopsis cubana* and *NHVL1* under simulated Martian conditions. This was done by investigating their threshold to Calcium perchlorate and examining their survival under ultraviolet radiation. The survival in salt was tested, by exposure to increasing perchlorate concentrations. *Chroococcidiopsis cubana* was exposed to five different concentrations, whereas *NHVL1* was exposed to two different concentrations. This was due to the expected moderate survival of *NHVL1* in salts.[33] The survival of UV irradiated cyanobacteria was also demonstrated in this study. This was done by exposing them to different time intervals of UVC radiation (256nm, $12.42 W/m^2$). *NHVL1* was exposed to UVC in up to 5min and *Chroococcidiopsis cubana* was exposed in up to 30min. This was decided due to the expected positive survival of *C.cubana* under UV radiation. Due to a lack of knowledge of *NHVL1´s* survival under UV radiation, pilot testing was performed (data not shown). Results suggested that no survival was seen after 10min of exposure, hence 5min was the highest exposure time used. It was also hypothesized that exposure to Ca-perchlorate prior to the experiment might give a positive synergistic effect under UV exposure, due to already developed stress responses and therefore cause better survival.

Effect of Ca-perchlorate on the growth of C.cubana and Nostoc sp. NHVL1

Perchlorate was first detected in 2008 [21] and was observed in concentrations of about 2.4mM perchlorate ions (0.4wt%-0.6wt%) at the Phoenix landing site. Whereas the curiosity rover found concentrations of up to 1% at the Gale crater. [34] Previous research has demonstrated the ability of two *Chroococcidiopsis* strains, *Chroococcidiopsis* sp. CCMEE 029 and CCMEE 029 P-MRS, to grow in Mars relevant concentrations of 2.4mM ClO_4^- ions provided in a 40% Mg- and 60% Ca-perchlorate mixture. Furthermore, perchlorate survival of these two *Chroococcidiopsis* strains, in concentrations up to 100mM of perchlorate ions, has also been demonstrated and therefore I chose to test their threshold in higher concentrations. The concentrations varied from 42 to 336mM ClO_4^- ions (appendix 2). The data suggest that neither *Chroococcidiopsis cubana* nor *NHVL1* shows a strong threshold to Ca-perchlorate. A minor growth was demonstrated in the first 20 days in *NHVL1* exposed to 0.5% Ca-perchlorate (42mM $ClO_4^- ions$), whereafter a drop was observed. No other growths were shown. Steady fall was observed in *C.cubana* exposed to 0.5% Ca-perchlorate, however, growth was completely inhibited in other concentrations. Concentrations such as 1% and 2% showed negative OD values after 20 days, whereas

in 2.4% and 4% negative values already appeared after 10 days. Numbers did not recover subsequently. Other research suggests that reduced growth is more evident in Mg - and Ca-perchlorate than in Na-perchlorate. While reduced growth was seen in 100mM Na-perchlorate, no growth at all was observed in 100mM Mg – and Ca-perchlorate (200mM perchlorate ions).[34] Previous studies testing for survival in perchlorate show that survival can vary a lot among different strains of cyanobacteria, and also differ a lot among the same strain. They show that out of 17 tested cyanobacteria only 5 cyanobacteria were capable of maintaining growth in 1% Mg-perchlorate containing 6mM of ClO_4^- ions. The other 13 showed moderate tolerance to perchlorate. In this study, *Chroococcidiopsis cubana´s* survival was estimated to a concentration of 0.25 – 0.5% of Mg-perchlorate, whereas *Chroococcidiopsis thermalis* survived in up to 1% Mg-perchlorate. These results underline that the concentrations used in my experiment are too high to see growth in *Chroococcidiopsis cubana*, due to survival only being seen in low concentrations. They also suggest that *C.cubana* has a lower threshold for perchlorate – salts compared to other *Chroococcidiopsis* strains. *Anabaena lax*, a cyanobacterium from the *Nostoc* family showed growth in concentrations up to 0.25% Mg-perchlorate, which is in agreement with the lack of growth seen in my data of *NHVL1.*[21] *NHVL1* shows a significant growth in concentrations of 1% $CaCl_2$. My results hereby suggest that the bad survival observed in Ca-perchlorate is not due to overall moderate survival in salts, but due to the toxic effect of perchlorate ions.

In my research, I chose to work with Ca-perchlorate, due to the lack of provided results in other research. Calcium perchlorate is a chaotropic agent and therefore it can cause macromolecule destabilization. Growth inhibition is shown to be stronger in Ca-perchlorate compared to other perchlorate salts.[34] This might explain the lack of growth in my experiment compared to other experiments conducted with Na-perchlorate.

Throughout the 50 days of perchlorate exposure in my experiment, bleaching was observed. This might be due to the oxidizing actions of perchlorate. It has been shown that *Chroococcidiopsis* sp. CCMEE 029 bleaches in 680mM NaCl [34], and therefore this might be an ongoing habit for cyanobacteria when exposed to salts. The fall in OD measurements can hereby be due to the bleaching of chlorophyll in my cells. In appendix 7and 8, we can see that there is a correlation between the day of bleaching and the fall in OD measurements. Therefore, bleaching may cause bad results. In cyanobacteria grown in high perchlorate concentrations, a shift in carotenoid content in relation to levels of chlorophyll A has been observed. This can represent a protective

response, due to carotenoid inhibiting free radical reactions which mitigates oxidative stress. In general, cyanobacterial cells exposed to perchlorate showed a decrease in chlorophyll A and total carotenoid content. [21] Due to a decrease in chlorophyll content, a less green color would also be observed. Therefore, the bleaching observed in my experiment could be due to a decrease in chlorophyll. To support this argument, chlorophyll measurements should have been performed. The observed fall in OD measurements can also be caused by the cells dying or simply not growing due to inhibiting damage. The survival and growth of cyanobacteria in stressful environments, depend on the efficiency of their antioxidant system. Studies confirm that perchlorate exposure can lead to oxidative stress due to an increased level of lipid peroxidation.[21] The production and accumulation of ROS cause oxidative stress, which can cause lipid peroxidation. An increased level of MDA has been observed in *C.cubana* exposed to 0.5% Mg-perchlorate. Hence, the poor survival seen in my data can be due to, cyanobacteria not coping well enough with oxidative stress, to survive high concentrations of perchlorate exposure. An increased level of MDA was also to be observed in *Nostoc* strains, exposed to 0.25% Mg-perchlorate.[21] To investigate if the bad survival observed in my data, is due to cell death induced by oxidative stress, the levels of intracellular reactive oxygen species under perchlorate exposure could be monitored.

Morphological changes were not observed in *Chroococcidiopsis cubana* during the 50 days of exposure. Research shows that other bacteria such as *Hymenobacter marinus* changed their cell morphology to long cell chains whereas other bacteria such as *Planococcus halocryophilus* formed large cell clusters when exposed to perchlorate. Halophilic archaea were also shown to occur swollen when exposed to perchlorate ions.[34] *NHVL1* showed no morphological changes but in 1% of Ca-perchlorate, a breakdown of the cell wall in some parts seems to be observed. This can be due to osmotic lysis. Osmotic lysis is caused by an osmotic imbalance, caused by a severe amount of water leaving the cell which can lead to a collapse of the cell wall, equaling in cell death. It can also be due to lipid peroxidation damaging the cell membrane and causing changes in cell structure.

Survival of Chroococcidiopsis cubana and Nostoc sp. NHVL1 under UV exposure

At midday, the maximum UV irradiance (200-380nm) found at the Gale crater, is 20 W/m^2.[22] In my experiment I used an irradiance of 12.42 W/m^2, to determine their survival under UV exposure. My results suggest that there is a significant difference seen between *NHVL1* and *C.cubana* in their tolerance to UVC radiation. The half – life of *NHVL1* and *C.cubana* was calculated and showed that *NHVl1* had an estimated half – life of 3min under an irradiance of 12.42 W/m^2, whereas *C.cubana* had a half – life of 16min. There was an overall higher consumption rate observed in *C.cubana* compared to *NHVL1*. However, it is not clear what amount of CO_2 has been taken by the cyanobacteria and what amount by the media itself. This is due to gas diffusion to the growth medium. The amount of gas lost to the medium is unknown and due to that the calculated rate of CO_2 taken per day can be misleading. Therefore, we cannot conclude if the amount taken in 30min for *C.cubana* and 5min in *NHVL1*, is due to photosynthesis or gas diffusion.

Other research suggests that cyanobacteria strains of the *Nostoc* family, such as *Nostoc* sp., *Nostoc carmium,* and *Anabaena* sp., show no significant UV tolerance. It has been shown that under an irradiance of 10 W/m^2, for 30min almost 100% of the cells were killed. Although, exposure to 2.5 and 5 W/m^2 has no significant effect on the growth of these strains.[35] Furthermore, *Nostoc* sp. showed a strong decrease in photosynthetic activity, under Mars-like UV exposure [36], underlining the results found in my research. Contrariwise, *Chroococcidiopsis* strains have exhibited greater longevity compared to other cyanobacteria. Research shows that they are able to grow under 13 kj/m^2 of UVC.[8] As underlined by my research, *C.cubana* shows positive survival skills under UV radiation of up to 30min of exposure. Other cyanobacteria show a loss of 99% viability and no survivors after 30 min of simulated Martian UV flux.[8] It has been shown that *Chroococcidiopsis* cells exposed to Mars-like conditions for a longer period, show a reduction in photosynthetic pigments and autofluorescence.[36] This can be due to, as previously mentioned, the action spectrum for loss of viability being quite similar to the inactivation spectrum of DNA, therefore an increase in radiation causes a decrease in viability. Furthermore, visible light can bleach autofluorescence's proteins, which is four times more effective under Martian conditions. Hence, less CO_2 will be consumed.

As previously investigated, cyanobacteria show a higher tolerance to simulated Mars-like UV flux compared to non-photosynthetic bacteria.[8] This can be due to photosynthetic pigments,

such as the phycobilisome protein complex that absorbs light, and therefore less radiation reaches the cells. Research implies, that *Bacillus subtillis* shows no survivors after 15min of simulated Martian UV exposure and that they lost 99% of their viability after 15s of exposure.[8]

Research has shown a linear decrease in protein content with increasing UV exposure, indicating that cellular proteins are the main target of UV. In *Anabaena* sp. the CO_2 uptake after UV exposure was severely affected, due to a reduction in the supply of ATP and NADPH2, due to a deleterious effect on the photosynthetic apparatus.[35] UVC is more damaging than UVB radiation, due to causing thylakoid lamellar disorder and cell membranes disintegration. Disruption of the thylakoid membrane containing chlorophyll molecules, may cause partly or wholly destruction of the components required for photosynthesis and thus affect the rate of CO_2 fixation.[36] This may be an explanation for the small amount of CO_2 consumed by *NHVL1*.

According to my data, *Chroococcidiopsis cubana* shows greater tolerance to UV radiation compared to *NHVL1*, this can be due to the difference found in cell morphology. *NHVL1* grows in a chain of cells where each cell is exposed to UV radiation, this can equal in more cell damage and therefore a shorter half – life. *C.cubana* on the other hand, occurs as few celled aggregates, elongating the light path of UV radiation. This results in inner cells not being exposed to UV radiation, due to the protection of the upper layer of cells, causing higher survival.[8] As already indicated *Chroococcidiopsis* cells are also encapsulated by a polysaccharide sheath, which as well provides extra protection.

Bactericidal effect of UVC and perchlorate on Chroococcidiopsis cubana

On Mars both perchlorate and UV radiation are present, and therefore it was of my interest to examine the survival of *Chroococcidiopsis cubana* exposed to 2%Ca-perchlorate, prior to and during UV exposure. Furthermore, the CO_2 consumption was compared to *C.cubana* exposed to UV radiation but without prior exposure to Ca-perchlorate. My data suggest that there is a difference between the amount of CO_2 consumed by *C.cubana* exposed to 2% Ca-perchlorate and the ones not exposed to Ca-perchlorate. The results suggested that there was a slightly greater fall in the rate of CO_2 consumption of 0.063 for exposed *C.cubana*, whereas the rate for CO_2 consumption falls by 0.044 in non – exposed *C.cubana*. Furthermore, the calculated half – life of *C.cubana* exposed to 2% Ca-perchlorate, under UV exposure, was 11min. Hint, my results

suggest that perchlorate in combination with UVC has a toxic effect on the consumption of CO_2 and survival.

Other research suggests that Mg -, Na -, and Ca – perchlorate become bactericidal when irradiated under short – wave UVC radiation, as encountered on the Martian surface. It has been shown that it significantly reduces the viability of vegetative *Bacillus subtillis* cells when irradiated with 11.2 W/m^2 of UVC, compared to cells only exposed to UVC without perchlorate. *Bacillus subtillis* shows no sensitivity to perchlorate, when not exposed to UV radiation. Other than that, it was shown that *Bacillus subtillis* lost viability after 30 seconds of UVC exposure when exposed to perchlorate, whereas loss of viability only occurred after 60 seconds of exposure to UVC, without perchlorate.[37] Therefore it is believed that the combination of those two factors, has a negative synergistic effect on microorganisms. UVC has been shown to further activate perchlorate oxidizing powers and therefore become more toxic.[21]

Due to *Chroococcidiopsis cubana´*s bad tolerance to perchlorate as shown in my previous data, and the good survival under UVC exposure compared to other cyanobacteria, my results could suggest that this negative synergistic effect is also seen in my research. But due to the small sampling size and the moderate difference between their consumption, I am unable to generalize my results on a broader strain. My cells suggest that there is a correlation between the amount of exposure time and the amount of CO_2 consumed, and that the toxic effect of perchlorate is to be seen under longer periods of UV exposure. Therefore, the data oppose the hypothesis that stress response due to perchlorate helps with survival under UV exposure.

Possible solutions

Perchlorate has a large positive reduction potential and can be used as an energy source by numerous microbes.[37] These microorganisms can respire perchlorate to innocuous chloride, by using it as an electron acceptor for microbial metabolism. To the best of our knowledge, the enzymes used in perchlorate reduction, such as perchlorate reductase and chlorite dismutase, are not found in cyanobacteria and therefore they cannot eliminate perchlorate themselves.[21] The toxic effect of high concentrations of perchlorate on some cyanobacteria, such as *Chroococcidiopsis cubana* and *NHVL1* used in my experiments, could hereby be diminished by growing them together with Dissimilatory Perchlorate – Reducing Bacteria (DPRB). DPRBs are found in four subclasses ($\alpha, \beta, \gamma, and\ \varepsilon$) of Proteobacteria.[38] The majority are found in the

β −subclass and belong to *Dechloromonas* and *Azospira* species.[39] Bacteria can utilize perchlorate as a terminal electron acceptor, and effectively reduce ClO_4^- to ClO_2^-, with the help of the enzyme perchlorate reductase. Afterwards, ClO_2^- is completely reduced to Cl^- and O_2, with the help of chlorite dismutase.[40] The oxygen produced under perchlorate reduction can either be reduced to water and respired by the same bacteria[40], or it can be used, with the help of the produced O_2 by cyanobacteria, to support an ecosystem on Mars.
To prevent the bactericidal effect of UVC radiation, cells could be grown under a modest covering of rocks or in biofilms. Cyanobacterial cells grown in biofilms have been shown to have better survival under UV exposure.[25] The upper layer of cells functioned as a protective layer to the under-laying cells. The upper layer did bleach when exposed to $10 kj/m^2$ of UVC radiation, but no damage was seen in the under-laying cells.[41] It has also been shown that dried monolayer of *Chroococcidiopsis* sp. CCMEE 029 survived exposure up to 30 kj/m^2 of simulated Martian UV flux (>200nm) and was shown to be ten times more resistant than *Bacillus subtilis*. This has not been tested on hydrated cells and therefore the survival of hydrated cells is unknown.[26]

UVC rapidly inactivates unshielded cells but can only penetrate a few mm into the Martian regolith. Therefore, covering the cyanobacterial cells could solve this problem. By growing cyanobacteria in rock analog systems, the bactericidal effect of perchlorate in the combination of UVC might also be reduced. It has been shown that *Chroococcidiopsis* – like – organisms covered by 1mm of rock, can cope with intense UV radiation, as found on Mars.[8] It has also been shown that *Chroococcidiopsis* overlayed by 3mm of Antarctic sandstone, survived simulated Martian conditions for up to 1.5 years. On top of that, it has also been shown that they survived 548 days in actual space by being augmented into an epilithic microbial community.[41] This data, therefore, suggests, that UVC radiation alone will not be the limiting factor of the survival of cells on Mars if covered, although they have many other requirements for growth, such as nutrients, water, and temperature.[8] Thus, rocks provide certain UV refugia for life on Mars, the atmosphere remains hazardous due to the lack of UV protection, and therefore if *Chroococcidiopsis* cells or other cyanobacterial cells were to be released from their endolithic environment, a rapid loss of viability would still be seen.

CONCLUSION

Terraforming Mars has become of big interest along with the colonization of Mars. Due to toxic conditions found on Mars, life as we know it may not survive. Thus, it is believed that some microorganisms, such as cyanobacteria found in the harshest environments on Earth, might be able to colonize and grow on Mars. Perchlorate and UV radiation are some of the obstacles causing the toxic environment. In this study, the growth of *Chroococcidiopsis cubana* and *Nostoc sp*. *NHVL1* under simulated Martian conditions was investigated. *Chroococcidiopsis cubana* exposed to UVC radiation showed good survival skills, whereases *NHVL1* showed a lack of UV tolerance over longer periods of UV exposure. It is also shown that neither of the cyanobacteria show a big threshold to Ca- perchlorate and that the combination of Ca-perchlorate and UVC radiation might have a bactericidal effect on *Chroococcidiopsis cubana*. Significant growth was observed in *NHVL1* in $CaCl_2$, and therefore the lack of survival found in Ca-perchlorate is believed to be due to the toxic effects of perchlorate ions. Previous studies show that different strains of *Chroococcidiopsis* can tolerate simulated Martian conditions and that they show better survival under UV exposure compared to other microorganisms such as *Bacillus subtilis*. Growth in perchlorate concentrations such as found on Mars has been demonstrated, but higher concentrations of Ca-perchlorate show a toxic effect on cyanobacteria. With future studies it could be of interest to examine why higher concentrations of perchlorate become toxic to cyanobacteria, and how this can be avoided to secure better growth.

Together with their growth and their ability to form oxygen under harsh conditions, cyanobacteria still remain some of the microorganisms mostly capable of colonizing Mars. Due to their ability to function as food, produce biofuel, and oxygen, and indirectly support growth of other organisms they are able to form a cyanobacterial culture life-support system. With the help of this, an ecosystem could be built on Mars. Hence, making colonization possible. Altogether, terraforming Mars may be an option in the future, with the help of cyanobacteria. Without colonization transforming Mars into an Earth like environment seems impossible. Microorganisms such as cyanobacteria, could therefore function as a bridge between terraforming Mars and human colonization. If humanity truly wishes to colonize mars.

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APPENDIX

Appendix 1

Calculations of Ca-perchlorate concentrations in BG11 media:

There has been shown growth of a *Chroococcidiopsis* strain in 100mM of perchlorate in a study performed by Billi D, Gallego Fernandez B, Fagliarone C, Chiaverini S, Rothschild LJ[34]. Due to their results I chose to base my calculations of the added amount of Ca-perchlorate, on their highest concentration in which growth was found. To figure out how much 100mM of $Ca(ClO_4)_2$ is in wt%, I used the following equations.

First, I calculate how much the amount of substance (n) of 100mM is, by the following equation:

$$n = CV$$

$$n = 100mM * 10^{-2}L$$

$$n = 10^{-1}M * 10^{-2}L$$

$$\underline{\boldsymbol{n = 10^{-3}ML}}$$

To figure out how much 100mM is in wt%, I used the molar mass of $Ca(ClO_4)_2$ which is 238.5792 g/mol. With my calculated amount of substance and molar mass of $Ca(ClO_4)_2$ I can now calculate the mass equalling 100mM. See the following equation:

$$m = n * Mr$$

$$m = 10^{-3}mol * 238.6\frac{g}{mol}$$

$$m = 0.2386\ g$$

From the mass found I can now calculate my wt%:

$$wt\% = 0.2386\ g * 100$$

$$\underline{\boldsymbol{wt\%. \approx 2.4\ wt\%}}$$

ML MEDIA	0,50% $Ca(ClO_4)_2$	1% $Ca(ClO_4)_2$	2% $Ca(ClO_4)_2$	2,40% $Ca(ClO_4)_2$	4% $Ca(ClO_4)_2$
100	0,55g	1,1g	–	–	–
50	–	–	1,1g	1,32g	2,2g

***Table 1**: Shows the mass of Ca-perchlorate added to each 100mL and 50mL of BG11 media. The mass shown is based on the amount of wt% + 10%, due to the fact that 10% of the media added is without Ca-perchlorate. The different wt% are based on the calculation of 100mM of $Ca(ClO_4)_2$ equalling ≈ 2.4wt%.*

Appendix 2
To transform the added percentage of Ca-perchlorate to millimolar, the following calculations were made for all the concentrations. Here I have used 0.5% Ca-perchlorate to demonstrate.

First, I transformed the data 0.5% to g/L:

$$0.5\% \rightarrow 0.5\ g/mL \rightarrow 5g/L$$

Afterwards with the help of $Ca(ClO_4)_2$molar mass (238.5792 g/mol) I calculated the corresponding molar:

$$\frac{5\ g/L}{238.5792\ g/mol} = 0.02095\ mol/L$$

To get mM I multiplied the found molarity with 1000:

$$0.02095M * 1000 = \mathbf{21}\boldsymbol{mM}$$

See calculated mM masses in table 2:

$\boldsymbol{Ca(ClO_4)_2}$ **(wt%)**	**0,50**	**1**	**2**	**2,40**	**4**
Concentration of $\boldsymbol{Ca(ClO_4)_2}$ (mM)	21	42	84	100.6	168
$\boldsymbol{ClO_4^-}$ **concentration (mM)**	42	84	168	201.2	336

Table 2: *Shows the calculated concentration (mM) based on the wt% of $Ca(ClO_4)_2$ added to BG11 medium. It also shows the concentration of ClO_4^- ions.*

Appendix 3
To make sure that the same amount of salt was added in the experiment performed on NHVL1 exposed to 0.5% and 1% $CaCl_2$, as in the Ca-perchlorate experiment the following calculations were made:
First, I transformed the data 0.5% to g/L:

$$0.5\% \rightarrow 0.5\ g/mL \rightarrow 5g/L$$

With the help of the molar mass of $Ca(ClO_4)_2$ I then calculated the Molar concentration of $Ca(ClO_4)_2$ in 0.5%. This is shown below:

$$\frac{5g/L}{238.579\ g/mol} = 0.021mol/L$$

Based on my found molar mass of Ca-perchlorate I can now calculated the mass of CaCl2 that needs to be added to correspond to 0.5% Calcium. To do this I used the molar mass of CaCl2, which is 110.984 g/mol:

$$110.984g/mol * 0.021mol/L = \mathbf{2.326}\ \boldsymbol{g/L}$$

This was equally performed for 1% CaCl2, which gave me the result of **4.652g/L**.

Appendix 4

The irradiance our cyanobacteria experienced was calculated by the following equation.

$$I = \frac{P}{4\pi * r^2}$$

The power of the lamp was 15W, and the distance from the lamp to the irradiated agar plates was 31cm = 0.31m. The irradiance was hereby calculated as shown below:

$$I = \frac{15W}{4\pi * (0{,}31m)^2}$$

$$\underline{\boldsymbol{I = 12.42\ W/m^2}}$$

The UV irradiance the bacteria encounter throughout the experiment was hereby $\boldsymbol{12.42 W/m^2}$.

Appendix 5

First, with the help of the calculated ppm of CO_2 the amount of mols of CO_2 taken by the cyanobacteria were found. This was done by using the ideal gas equation.

Ideal gas equation:

$$pV = nRT$$

Isolated for mols of gas present (n -> μgCO_2):

$$n = \frac{pV}{RT}$$

The constants used in my calculations are seen below.

$$p\ (atm) = \left(\frac{ppm}{1000000}\right) due\ to\ 1atm = 1.000.000ppm$$

$$V = 59ml$$

$$R = 0,08206$$

$$T\ (K) = 298K$$

Calculation will be shown by using 0.5min of UV exposure in C.cubana:

$$\left(\frac{5034.29}{1000000}\right) * \frac{0.59}{0.08206 * 298} * 44 * 1000000 = \boldsymbol{534.44\ \mu gCO_2}$$

With the help of the calculated mols of CO_2, the CO_2 consumption could be calculated.

Appendix 6

C.cubana

$Ca(ClO_4)_2$ Concentration (%)	Day 0	10	20	30	40	50
0	0,046	0,071	0,1305	0,126	0,147	0,227
0	0,0515	0,1085	0,1225	0,1155	0,1745	0,323
0	0,079	0,1165	0,1155	0,1095	0,139	0,207
0,50	0,162	NA	0,115	0,0645	0	0,0575
0,50	0,2025	0,159	0,128	0,111	0	0,061
0,50	0,225	0,1255	0,1055	0,0505	0	0,017
1	0,0145	0,023	0	0	0,0345	0,0415
1	0,0565	0,0865	0	0	0,069	0,0215
1	0,1505	0,2025	0	0,0655	0,058	0,035
2	0,1405	NA	0,0225	0	0,009	0,0175
2	0,2305	0,107	0,007	0	0,036	0,0195
2	0,0925	0,191	0	0	0,0155	0,0175
2,40	0,2555	0	0,0425	0,0225	0	0,048
2,40	0,245	0	0	0	0	0,0735
2,40	0,3925	0	0,0015	0	NA	0,09
4	0,0765	0	0,0345	0	0,0345	0
4	0,0275	0	0,059	0	0,037	0
4	0,0165	0,001	0,04	0	0,027	0
	OD_{730} Measure-ments					

Table 3: *In this table OD measurements for C.cubana in $Ca(ClO_4)_2$ are shown. Negative values are put to zero. Samples lost during measurements are marked with NA. NA = not available*

NHVL1

$Ca(ClO_4)_2$ Concentration (%)	Day 0	10	20	30	40	50
0	0,0465	0,048	0,0935	0,0885	0,144	0,236
0	0,041	0,1135	0,0765	0,082	0,166	0,2265
0	0,029	0,055	0,0775	0,082	0,1265	NA
0,50	0	0,0685	0,124	0,032	0	0
0,50	0	0,0015	0,085	0,048	0	0,0035
0,50	0,02	0,07	0,0985	0,058	0	0
1	0	0,033	0,006	0	0,013	0,0275
1	0	0,08	0	0	0,0435	0,001
1	0,013	0	0	0	0,045	0,0085
	OD_{730} Measurements					

Table 4: *In this table OD measurements for NHVL1 in $Ca(ClO_4)_2$ are shown. Negative values are put to zero. Samples lost during measurements are marked with NA. NA = not available*

NHVL1					
$CaCl_2$	**Day**				
Concentration (%)	0	10	20	30	40
0	0,039	0,0625	0,0845	0,1205	0,1545
0	0,0365	0,0695	0,0855	0,1135	0,1525
0	0,047	0,046	0,101	0,111	0,149
0,50	0	0,0665	0,05935	0,138	0,188
0,50	0	0,046	0,052	0,126	0,179
0,50	0	0,03		0,1285	0,187
1	0	0	0,038	0,044	0,1195
1	0	0,0295	0,047	0,1238	0,146
1	0	0,0555	0,0625	0,086	0,1255
	OD_{730}Measurements				

Table 5: *In this table OD measurements for NHVL1 in $CaCl_2$ are shown. Negative values are put to zero.*

Appendix 7

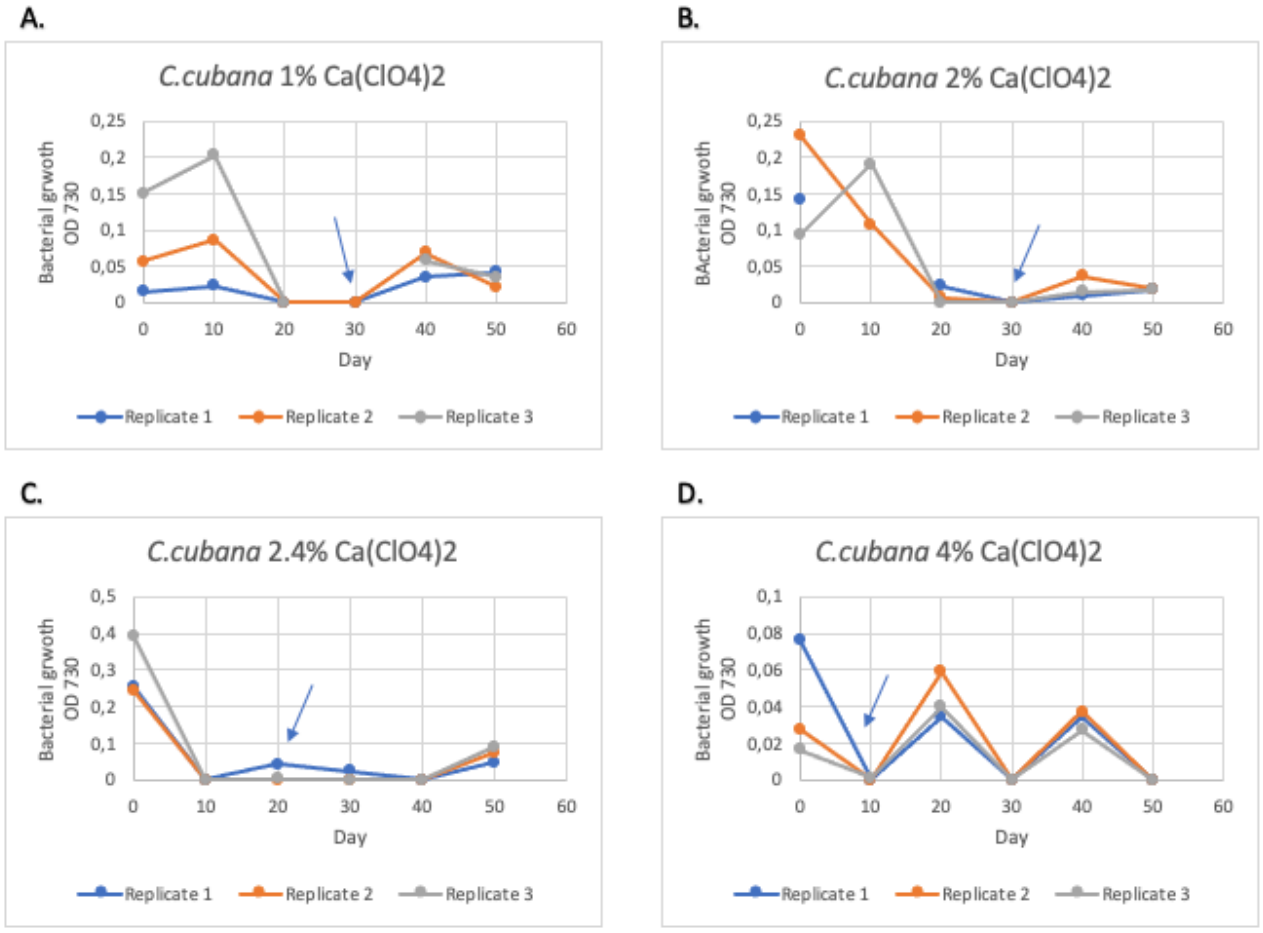

Figure 1: *This figure shows the amount of growth observed in C.cubana 1% (**A**), 2% (**B**), 2.4% (**C**), and 4% (**D**) $Ca(ClO_4)_2$, over 50 days. The arrows in graphs show the bleaching time of C.cubana cells, due to perchlorate. The experiment was performed in triplicates, and each line represents one of the replicates. We can see that there is no significant growth observed in any of the Ca-perchlorate concentrations.*

Appendix 8

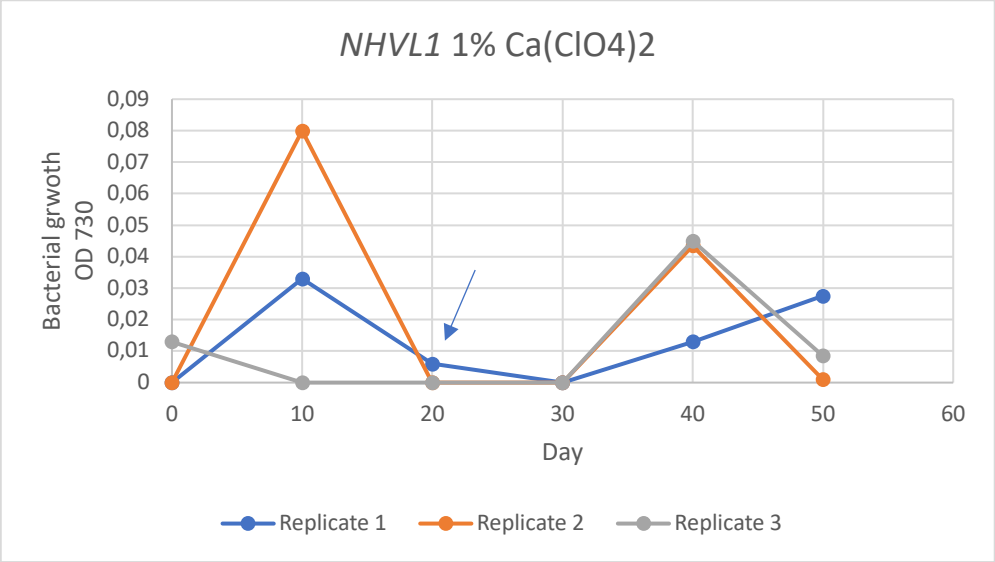

Figure 2: *This figure shows the amount of growth observed in NHVL1 1% $Ca(ClO_4)_2$, over 50 days. The arrow shows the bleaching time of NHVL1 cells, due to perchlorate. The experiment was performed in triplicates, and each line represents one of the replicates. We can see that there is no significant growth observed.*

Appendix 9

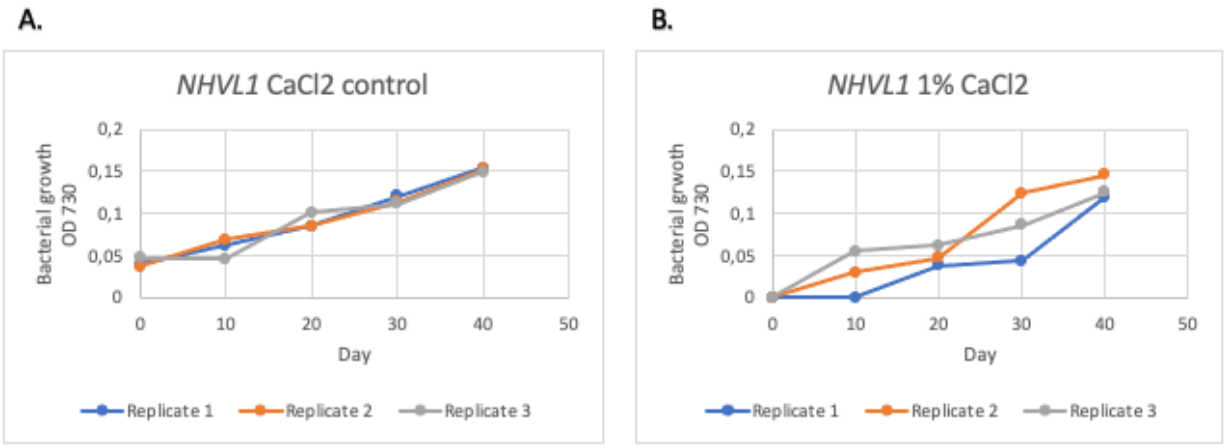

Figure 3: *This figure shows the amount of growth observed in NHVL1 control (**A**) and NHVL1 1% $CaCl_2$ (**B**), over 40 days. The experiment was performed in triplicates, and each line represents one of the replicates. We can see that there is a significant growth seen in both NHVL1 control and in 1% $CaCl_2$.*

Appendix 10

Growth of *Chroococcidiopsis cubana* in different Ca-perchlorate concentrations		
R^2	0.2541	
$Ca(ClO_4)_2$ concentration (%):	Estimated OD_{730}:	P-value
1	-2.7418	1.13e-05 (≈0)
2	-2.1497	0.448
2.4	-2.1730	0.496
4	-3.9315	0.148
	Estimated growth rate:	
1	-0.0096	0.573
2	-0.0447	0.153
2.4	-0.0134	0.881
4	0.0129	0.450

Table 6: *This table compares the estimated starting point of C.cubana in 2, 2.4, and 4% Ca-perchlorate, to Chroococcidiopsis cubana grown in 1% $Ca(ClO_4)_2$. It also shows the calculated growth rates of C.cubana exposed to each Ca-perchlorate concentration. The associated p-values show that there is no significant difference in growth rates found, when compared to 1% Ca-perchlorate exposure. It also shows that there is no significant difference in the estimated starting points. P-values are shown. This model underlines that C.cubana has a small perchlorate threshold.*

Appendix 11

NHVL1 in $CaCl_2$		
R^2	0.9352	
$Ca(ClO_4)_2$ concentration (%):	Estimated OD_{730}:	P-value
Control	-3.163	≈ 0
0.5	-3.661	0.0175
1	-3.746	0.0102
	Estimated growth rate:	
Control	0.033	7.81e-09 (≈ 0)
0.5	0.050	0.029
1	0.041	0.32

Table 7: *This table compares the estimated starting point of 0.5 and 1% $CaCl_2$ to NHVL1 control (no $CaCl_2$ exposure), and if they differ significantly from each other. P-values are shown. It also shows the calculated growth rates of NHVL1 in the different concentrations of $CaCl_2$. The associated p-values show if there is a significant difference in growth seen in 0.5 and 1% $CaCl_2$ compared to control. points.*

Appendix 12

Chroococcidiopsis cubana

Ca-perchlorate concentration (%):	**Day of bleaching**
4	21.03.2022, after 7 days cells were bleached.
2.4	24.03.2022, top layer of cells was bleached. (10days) 03.04.2022, almost all cells bleached. (20days)
2	13.04.2022, most cells were bleached, but there is still some yellow colour to be observed. (30 days)
1	13.04.2022, bleaching observed in top layer of cells (20days) 23.04.2022, total amount of cells bleached. (40 days)
0.5	13.04.2022, bleaching observed in some cells. (30 days) 03.05.2022, cells were bleached compared to control but there was still a greenish colour to be observed. (50 days)
Control	No bleaching observed

Table 8: *This table shows the exact dates for when bleaching was first observed in Chroococcidiopsis cubana.*

Nostoc sp. NHVL1

Ca-perchlorate concentration (%):	**Day of bleaching**
1	03.04.2022, bleached (20 days)
0.5	13.04.2022, some bleaching observed (30 days) 23.04.2022, fully bleached (40days)
Control	No bleaching observed

Table 9: *This table shows the exact dates for when bleaching was first observed in NHVL1.*

Appendix 13

Nostoc sp. NHVL1 UV exposure time (min)	CO_2 consumption ($\mu gCO_2/d$)	LN transformed CO_2 consumption
0 (control)	72,27	4,28
0 (control)	64,85	4,17
0 (control)	66,11	4,19
0,5	57,75	4,06
0,5	56,30	4,03
0,5	54,87	4,01
1	53,89	3,99
1	35,55	3,57
1	43,40	3,77
5	21,86	3,08
5	19,31	2,96
5	17,37	2,85

Table 10: *This table shows the amount of CO_2 consumed ($\mu gCO_2/d$) by NHVL1 under UV exposure. Together with the LN transformed rates.*

***C.cubana* in 2% $Ca(ClO_4)_2$** UV exposure time (min)	CO_2 consumption ($\mu gCO_2/d$)	LN transformed CO_2 consumption
0 (control)	220,62	5,40
0 (control)	195,66	5,28
0 (control)	200,65	5,30
0,5	163,06	5,09
0,5	221,16	5,40
0,5	213,29	5,36
1	215,75	5,37
1	218,10	5,38
1	250,58	5,52
5	94,13	4,54
5	101,11	4,62
5	108,66	4,69
10	84,78	4,44
10	83,95	4,43
10	75,31	4,32
30	32,80	3,49
30	29,35	3,38
30	35,36	3,57

Table 11: *This table shows the amount of CO_2 consumed ($\mu gCO_2/d$) by Chroococcidiopsis cubana exposed to 2% Ca-perchlorate prior and under UV exposure. Together with the LN transformed rates.*

C.cubana		
UV exposure time (min)	**CO_2 consumption ($\mu gCO_2/d$)**	**LN transformed CO_2 consumption**
0 (control)	202,16	5,31
0 (control)	209,74	5,35
0 (control)	192,96	5,26
0,5	188,74	5,24
0,5	222,01	5,40
0,5	210,46	5,35
1	205,47	5,33
1	240,23	5,48
1	233,62	5,45
5	100,09	4,61
5	99,73	4,60
5	98,73	4,59
10	121,14	4,80
10	115,51	4,75
10	111,21	4,71
30	55,26	4,01
30	56,42	4,03
30	54,66	4,00

Table 12: *This table shows the amount of CO_2 consumed ($\mu gCO_2/d$) by Chroococcidiopsis cubana under UV exposure. Together with the LN transformed rates.*

Appendix 14

Cyanobacteria	P-value for slope
Chroococcidiopsis cubana	1.83e-07 (≈ 0)
Chroococcidiopsis cubana 2% $Ca(ClO_4)_2$	1.27e-09 (≈ 0)
NHVL1	3.00e-07 (≈ 0)

Table 13: *This table shows the calculated p-values for the slope of C.cbana, C.cubana 2% $Ca(ClO_4)_2$, and NHVL1.*

Appendix 15

Chroococcidiopsis cubana 2% $Ca(ClO_4)_2$		
R^2	0.9868	
UVC time exposure (min):	Estimated CO_2 consumption ($\mu gCO_2/d$):	P-value:
Control	5.33	≈ 0
0.5	5.29	0.628
1	5.43	0.221
5	4.62	1.23e-06 (≈ 0)
10	4.40	6.65e-08 (≈ 0)
30	3.48	2.43e-11 (≈ 0)

Table 14: *This table shows, the estimated rate off CO_2 consumed by C.cubana exposed to 2% Ca-perchlorate. Over longer periods of UVC irradiation, a change in CO_2 consumed by C.cubana is to be observed. The p-values shown, tell us if there is a significant difference observed in CO_2 consumed by cells over longer periods of UV exposure compared to control.*

NHVL1		
R^2	0.9575	
UVC time exposure (min):	Estimated CO_2 consumption ($\mu gCO_2/d$):	P-value:
Control	4.22	≈ 0
0.5	4.03	0.10431
1	3.78	0.00239
5	2.97	1.64e-06 (≈ 0)

Table 15: *This table shows, the estimated rate off CO_2 consumed by Nostoc sp. NHVL1. A change in CO_2 consumed by NHVL1 is to be observe, over longer periods of UVC irradiation.. The p-values shown, tell us if there is a significant difference observed in CO_2 consumed by cells over longer periods of UV exposure compared to control.*

Appendix 16

Comparing *C.cubana, C.cubana 2% $Ca(ClO_4)_2$,* and *NHVL1*		
R^2	0.9329	
	Estimated CO_2 consumption ($\mu gCO_2/d$):	P-value:
C.cubana	5.241	≈0
C.cubana 2% $Ca(ClO_4)_2$	5.239	0.98238
NHVL1	4.133	2.65e-14 (≈0)
	Growth rate:	
C.cubana	-0.044	6.84e-12 (≈0)
C.cubana 2% $Ca(ClO_4)_2$	-0.065	0.00585
NHVL1	-0.238	1.16e-07 (≈0)

Table 16: *In this table linear regression of C.cubana exposed to 2% Ca-perchlorate and NHVL1 are compared to C.cubana. We can see the estimated starting points of our cyanobacteria, and if there is a significant difference observed compared to control. P-values are shown. It also shows if there is a significant difference observed in CO_2 consumed over time in C.cubana exposed to 2% Ca-perchlorate and NHVL1 compared to C.cubana. Associated p-values are shown.*