Project Number: BB-TCC-1182

CRYPTOBIOTIC CRUSTS OF DESERT ECOSYSTEMS

A Major Qualifying Project Report:

submitted to the Faculty

of the

WORCESTER POLYTECHNIC INSTITUTE

in partial fulfillment of the requirements for the

Degree of Bachelor of Science

by

Jacob Bradley Given

Date: April 28 2005

Approved:

_____

Professor Theodore C. Crusberg, Major Advisor

1. Cryptobiotic Crust
2. Cyanobacteria
3. Growth

Abstract

Cryptobiotic soil (cbs) is a vital component of the desert ecosystem, responsible for carbon and nitrogen fixation, maintaining soil stability, and reducing wind and water erosion. Here the possibility of creating a soil structure that emulates cbs found in the desert southwest based upon a composition dominated by three kinds of cyanobacteria, Nostoc, Microcoleus, and Scytonema was explored. Soil samples experienced cyanobacteria growth indicating that a consistent method of growing cbs may be perfected and utilized by other researchers.

Table of Contents

List of Figures

List of Tables

1 Introduction

Cryptobiotic crust is a soil matrix composed of several species of blue-green algae, cyanobacteria, lichens, mosses, and other bacteria. Certain micro-filamentous species wrap themselves around small soil particles to provide the basic soil structure and a stable environment for the successful colonization of other species.[1]

An important characteristic of the cyanobacteria in the crust is that they are able to dry out and suspend all physiological function until moistened once again.[1] In the desert ecosystem, the crusts will go through cycles of prolonged dry weather followed by eventual wetting from precipitation. Respiration, photosynthesis, and growth occur once the soil has been moistened. The crusts quickly dry out and once again go into stasis.

The micro-filamentous algae are only able to grow approximately 1mm each cycle. Therefore, for the crusts to grow in height, a fine layer of soil must accumulate over the top layer of the forming crust, giving the microfilaments particulates around which they can wrap themselves and grow upwards. This process takes many years, and disturbance of the crusts by trampling from livestock or humans, or recreational or commercial vehicles is detrimental to crustal growth and viability.

These crusts serve many vital ecological functions in the many ecosystems in which they are found. Structurally, they act as a soil stabilizer, preventing erosion from water and wind. They are also capable of fixing atmospheric nitrogen and carbon, which can be leaked into the surrounding soil, thus maintaining soil fertility. Another important aspect is due to the surface structure. Developed crusts are very rough in texture, and, during rain, this texturing slows the speed of water runoff, increasing the amount of

infiltration into the soil.[2] This also helps maintain healthy soil, as vital nutrients are not washed way in heavy runoff.

Many field studies have been done on cryptobiotic crusts, but no one has created a comparable soil structure in the lab. If successful, and a standardized protocol for growing and culturing this type of soil in the lab is determined, then laboratories around the world can conduct research experiments on these uniquely important soil composites without relying on samples taken from the field. It will also give us a much more clear understanding of how these crusts form, the interactions between the different organisms, and potential ways to restore naturally occurring crusts once they have been damaged.

This project aimed to create a soil structure that is similar to desert cryptobiotic crust using three species of cyanobacteria that have been isolated in many areas where crusts are found, *Microcoleus sp., Nostoc sp.,* and *Scytonema sp.*[3, 4] These species are all capable of nitrogen fixation and are especially common in deserts of the western United States.[1]

2 Background

Before attempting this project, an understanding of these diverse and important soil communities was necessary. For years cryptobiotic crusts have been studied and analyzed, and much of the literature on them was studied in order to gain the knowledge necessary to create a lab-grown soil crust. Here, the basic structure, function, and ecological importance of cryptobiotic crusts are discussed, as well as the risks of human behavior and climatic change. Finally, this chapter discusses the potential purpose a standardized protocol for growing cryptobiotic crusts would serve.

2.1 Cyanobacteria

One of the first organisms to evolve that was capable of oxygenic photosynthesis falls under the category of blue-green algae, or cyanobacteria. While they are prokaryotic, the presence of chlorophyll *a* and their utilization of oxygenic photosynthesis is similar to that of eukaryotes.[4] The first instances of cyanobacteria found in the fossil record occur nearly three billion years ago, before the atmosphere contained any substantial levels of oxygen, and evidence indicates that these organisms were responsible for the transition to an atmosphere rich in oxygen.[4]

There has also been strong evidence supporting the endosymbiotic theory of the origin of chloroplasts in eukaryotic photosynthetic organisms. This theory hypothesizes that chloroplasts were once free-living cyanobacteria that became engulfed by early eukaryotes.[4] Evidence for this invovles the similarities in structure and function as well as the presence of circular (bacterial) DNA and chlorophyll *a* and *b* in chloroplasts.

Structurally, cyanobacteria appear in several forms. They form colonies of various shapes and sizes, form long filaments – often in close contact with other strands called trichomes – or appear as single cells among soil particulates.[1, 4] The cells vary in size from 1-10_m, and often appear dark green, or even black due to heavy pigmentation.

Many filamentous species of cyanobacteria are encased in a sticky, mucilaginous sheath which binds soil particles together. During rainfall, the filaments become physiologically active, and begin to photosynthesize, grow, and move through the soil while the sheath remains in place and continues to maintain soil stability.[1] Without the ability for non-living components to hold soil particles together, the crusts would be limited to less than a millimeter below the surface where light can penetrate.

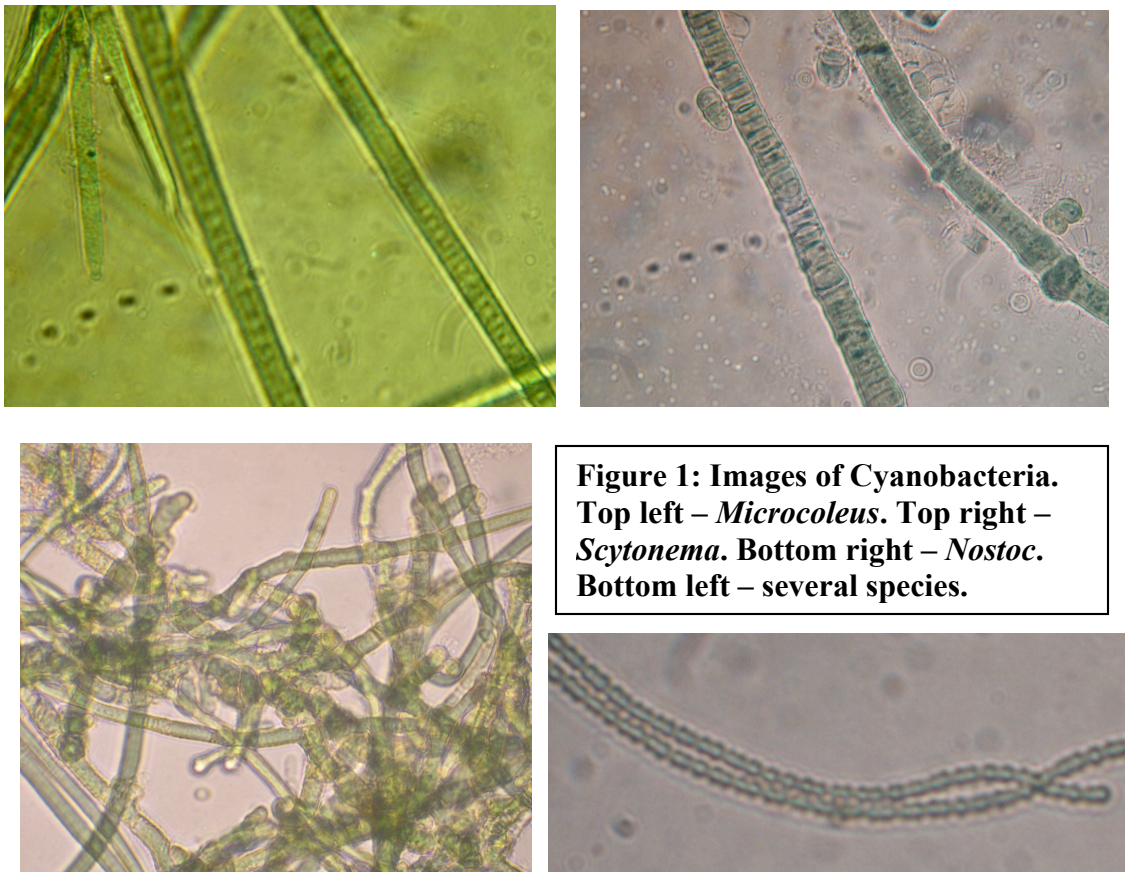


Figure 1: Images of Cyanobacteria. Top left – *Microcoleus*. Top right – *Scytonema*. Bottom right – *Nostoc*. Bottom left – several species.

As well as being capable of oxygenic photosynthesis, cyanobacteria are capable of atmospheric nitrogen fixation, and in the soil are able to convert nitrogen into nitrate or ammonia, a form that can be utilized by vascular plants and other vegetation which rely on nutrients in the soil.[1,2] Many species of cyanobacteria contain specialized cells used in nitrogen fixation called heterocysts. These cells form when nitrogen sources in the soil are scarce, and in the process of formation develop thicker cell walls, lose their Photosystem II (and so discontinue the production of oxygen), and produce nitrogenase – the enzyme necessary for nitrogen fixation.[4]

Photosystem I is still active in the heterocysts and generates ATP for use in the cell, and nutrients are obtained from adjacent, non-heterocystic cells. Because nitrogenase is quite sensitive to the presence of oxygen, the breakdown of Photosystem II in the heterocysts is necessary to carry out fixation, and the thick cell wall acts to stop diffusion of oxygen into the cell.[4]

Nitrogen fixation does not necessarily require the presence of heterocysts, however. Some species of cyanobacteria, such as Microcoleus, are non-heterocystic and yet are able to carry out fixation of atmospheric nitrogen in dark, anaerobic conditions where oxygen is not present. This situation can occur due to the layering effect the cyanobacterial filaments have within the soil.[1]

Cyanobacteria found in crusts are typically found less than 0.5mm below the surface where sufficient sunlight can penetrate, but ultra-violet radiation – which can be detrimental to viability – is reduced. Certain species, such as *Nostoc* and *Scytonema* are able to produce UV-screening pigments which allow them to persist even on the soil surface.[1, 5] *Microcoleus*, which lacks these protective pigments, must remain below the

surface or else grow in close proximity to other organisms that are resistant to UV radiation.

2.2 Ecological Functions

Cryptobiotic crusts serve many roles in the ecosystem they inhabit, and vary widely based on species composition and regional characteristics such as average precipitation, temperature, elevation, etc.[1] Many functions, though, are common to a large variety of crusts, some of which have been mentioned previously.

2.2.1 Soil Stabilization

Vegetation serves a vital role in any environment by stabilizing the surrounding soil preventing erosion from wind and water. Without such protection, vital nutrients and fertile topsoil will be lost due to these forces. In the hot, arid deserts of southwestern United States, vascular plant vegetation is often sparsely distributed.

The cryptobiotic crusts that grow in the interspaces of the higher plants work to hold loose soil particulates together. The polysaccharides that make up the mucilaginous sheaths bind individual sand particles together. As more soil is bound, the less likely it is that erosion will occur.[1, 2, 4]

By creating a stable surface, the cyanobacterial mat that is characteristic of many crusts also acts as an initial colonizer in desert regions. Once a base has been created, other organisms have a greater rate of success of effectively colonizing the area.

2.2.2 Water Retention

In arid environments, cryptobiotic crusts can have a large impact on water infiltration and retention, however these effects vary significantly based on environmental conditions, soil structure, and crust composition. "Infiltration rates are controlled by the interaction of water-residence time on a soil surface and the permeability of that surface.[1]" Many crusts have rough, uneven surfaces which, during rainfall, act to slow the movement of water increasing the amount that seeps into the soil.[1, 7] As water filters through the soil rather than be washed away as runoff, it carries with it vital nutrients that then remain in the soil.

However, even if the residence time of the water on the surface is high, infiltration will be minimal if the soil is not permeable.[1] In crusts dominated by mucilaginous cyanobacteria, it is also possible that as the filaments absorb water and swell, up to thirteen times their dry volume[1], that any space through which water might flow could be closed.

2.2.3 Carbon and Nitrogen Fixation

Soil fertility is a vital factor in the overall health of any ecosystem. In a desert environment with sparsely distributed vegetation, cryptobiotic crusts play a key role in maintaining the levels of carbon and nitrogen in the soil[1, 8] which maintains healthy, fertile soil in areas lacking vascular plants.

Nitrogen is a vital nutrient needed for the synthesis of amino acids, purines, pyrimidines, and many other substances.[4, 10] The level of nitrogen in the soil, in a form readily available for plants and other organisms (such as nitrates or ammonia), can be a

determining factor in the ecosystem's productivity[1, 9, 10]. Fixation of atmospheric nitrogen by cyanobacteria can occur in both aerobic and anaerobic depending on the formation of heterocysts, and much of it has been shown to leak into surrounding soils as nitrate (NO_3) or ammonium ($N H_4$) ions. *Nostoc*, for example, will deposit between 5 and 88% of its fixed nitrogen into the surrounding environment.[1] This free nitrogen can be utilized by other organisms unable to fix nitrogen themselves.

2.3 Crusts in Danger

Cryptobiotic soils are extremely fragile, especially during the hot summer months when they are dry and brittle. When crusts are trampled by human foot traffic and livestock grazing, or run over with recreational ATV's or commercial vehicles, the soil matrix is broken apart. This leaves the area more susceptible to wind and water erosion, as the loose broken fragments are easily carried away by such forces. Vehicles are especially detrimental, as they leave long, continuous tracks through the crust which channels water away rather than allowing it to infiltrate the soil. Livestock grazing can lead to the removal and death of crust organisms, which results in much slower recovery than if the material were only trampled.[1] See Figure 2 for images of disturbed crusts.

Estimations of rates of recovery have proven highly variable. This variability is not limited to differences in crust composition, environmental conditions, and extent of damage (although recovery does depend on these factors). According to Jayne Belnap, this variability has been in large part due to inefficient methods of quantifying recovery, and has been limited to visual assessment alone. [1]

Figure 2 – Images of cryptobiotic crusts after disturbance. Images taken from Biological Soil Crusts: Ecology and Management. (1)

Another potential future risk for desert crustal ecosystems is the possible affect that climatic changes could have on species composition, and physiological function of cryptobiotic crusts. If temperatures increase as a result of global warming, these crusts which take many years to grow may not be capable of adapting to long term climate changes.

Physiological functions of cryptobiotic crusts occur only when wet, and suspend all functions during dry periods. According to Belnap et al, "Respiration begins less than 3 minutes after wetting, while photosynthesis reaches full activity after 30 minutes or more. Soil oxygen concentrations, a by-product of photosynthesis, reach steady states within 1-2 hours of wetting." In the hot summer months, any precipitation that falls will often evaporate before the crustal organisms are able to reach full physiological capacity, and a net carbon loss results.[1]

An increase in global mean temperature could result in an increase in summer precipitation followed by an even more rapid rate of evaporation. This could lead to long term carbon deficits resulting in the death of crustal organisms.

However, one study done by William Brostoff has shown that as CO_2 levels in the atmosphere increase, there is a linear increase in rates of photosynthesis of the organisms in cryptobiotic crusts. At atmospheric concentrations of CO_2 of 1000ppm, photosynthesis occurred at a rate "several times that of rates at natural CO_2 concentrations."[11] These findings are hopeful, yet more research must be done to ensure the long term viability of these important soil communities.

3 Materials and Methods

The experiment was run using sterilized, screened soil collected from the desert southwest. Three species of cyanobacteria were used to inoculate the soil: *Microcoleus, Nostoc,* and *Scytonema*. The cultures were incubated under the VitaLite full spectrum light setup and were cycled through wet and dry periods. Samples were taken to observe growth. A total of twelve cultures were grown to allow several to remain intact for future use, as sampling damages the intact soil. Aseptic technique was used to maintain sterility of the culture plates.

3.1 Preparing the Soil Samples

Twelve 100mL Petri dishes were obtained for use as culture plates. In the biological safety cabinet, sterile soil was added to a single plate until the entire plate bottom was completely covered. This plate was then weighed using a mass balance and compared to the mass of an empty plate to determine the mass of the soil. This mass was then used to standardize the addition of soil to successive plates.

Pure cultures of *Microcoleus, Nostoc,* and *Scytonema* grown in flasks with BG-11 media were used to inoculate the soil. A sample of each of the three cyanobacteria species was homogenized in a sterile stainless steel 20mL capacity Waring Blender. The container of the blender was sprayed with 70% ethanol and allowed to dry before being flame sterilized. Each sample was then blended for 10 to 15 seconds (until homogenous) before being placed in a single flask. Enough deionized water was added to this inoculation stock to bring the total volume to approximately 100mL.

Approximately 3 mL of water was necessary to fully saturate a single plate of soil. However, to be sure sufficient biomass was added, 5mL of inoculation stock was added to each plate. The plates were then placed under the VitaLite setup and allowed to dry.

Once the soil cultures were dry, 3mL of BG-11 media were added to each plate in order to ensure sufficient nutrient levels were present in the soil.

The cultures were inoculated on October 12, 2004. After two months, half the cultures' petri dish tops were replaced by a disc of UVT plastic. The petri dish tops do not transmit ultraviolet light, and this may have an effect on the growth rates of the different cyanobacteria. The UVT plastic does transmit ultraviolet light, and so may create more natural conditions in the lab, since desert crusts are exposed to ultraviolet radiation every day.

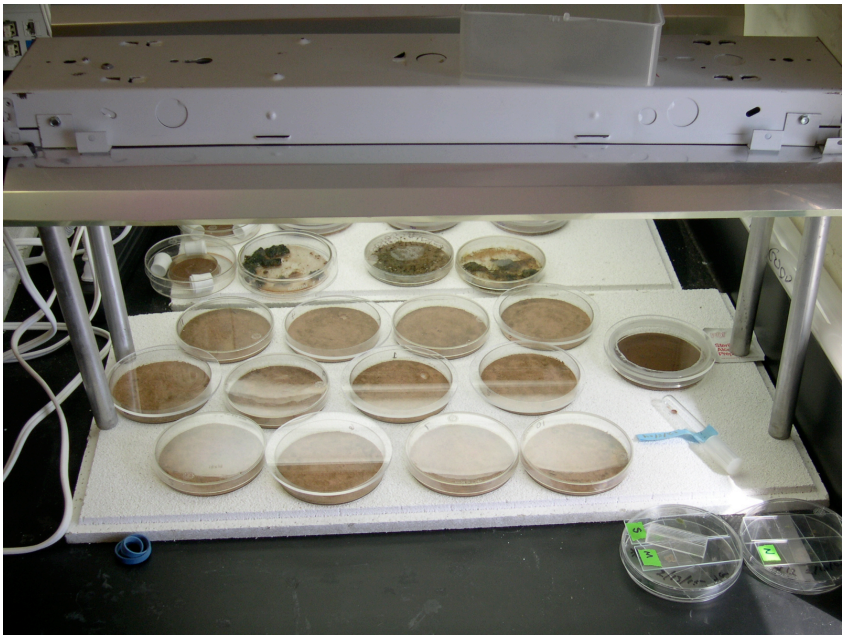


Figure 3: VitaLite setup with 12 soil cultures

3.2 Wet and Dry Cycle

Because all physiological functions of the cyanobacteria cease when the organisms are dry, and are only active when wet, growth can be measured in terms of wetting events. Since precipitation events are very few in number in the southwestern deserts of the United States, a year's worth of growth can be achieved in only a few weeks in the lab.

When all twelve cultures were completely dry, they were again saturated with water to stimulate growth. They were then allowed to dry completely once again, and this cycling was continued throughout the course of the experiment. For the first two months, 3mL of water was added to each plate during the wet cycle, and for the final two months, only 2mL of water was added.

3.3 Soil Addition

The goal of this procedure was to add a fine layer of soil as evenly as possible across the surface of each plate. Several methods were attempted using sample plates of non-sterile soil to determine the amount of soil needed, as well as the most affective procedure in dispensing the soil.

The final outcome was that 1g of soil was added to each plate after wetting, as the fine soil particulates would stick to the moist soil in the plate. Since growth occurs when the soil is wet, this also served to promote growth of cyanobacteria into the newly added soil.

A "foil dispenser" (shown in **Figure 4**) was fashioned that could be used to aseptically transfer the soil evenly across the culture surface. A small square of aluminum

foil (approximately 15cm X 15cm) was shaped about the bottom 5-7cm of a test tube with excess foil pinched into a handle. A flame-sterilized pin was used to puncture a single hole in the bottom of the "foil dispenser." Puncturing from the inside allowed the soil particles to flow through more easily. Using sterile forceps to hold the "foil dispenser," it was then sprayed with 70% ethanol and flamed.

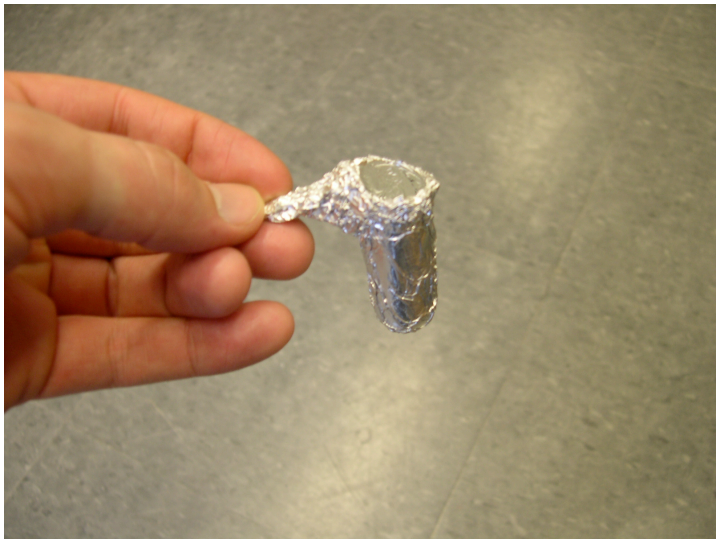


Figure 4: An image of the "foil dispenser."

After measuring 1g of soil on the mass balance, it was poured into the "foil dispenser" which was held above a single culture plate. By holding the handle with the thumb and middle finger, the index finger could be used to gently tap the dispenser while moving it evenly across the surface of the plate to facilitate flow through the pinhole. Once the soil was dispensed, the culture plate was gently tapped several times to spread any loose soil particles more evenly. This procedure was repeated for all twelve cultures.

3.4 Determining Light Transmittance Through Soil

The amount of light capable of passing through the soil is extremely important, as cyanobacteria depend on light as a source of energy in photosynthesis, and if they are too far below the surface, they will be receiving insufficient light energy to maintain their physiological functions. In order for vertical growth to occur, however, some soil must accumulate on the surface.

Spectroscopy was used to determine the transmittance of light in the entire spectrum through one and two layers of soil. The soil particulates were too large to suspend in solution, so a small piece of double sided tape was cut to fit a plastic cuvette. The cuvette was then pressed into a plate of non-sterile soil securing a fine, even layer of soil to the side of the cuvette (see Figure 5).

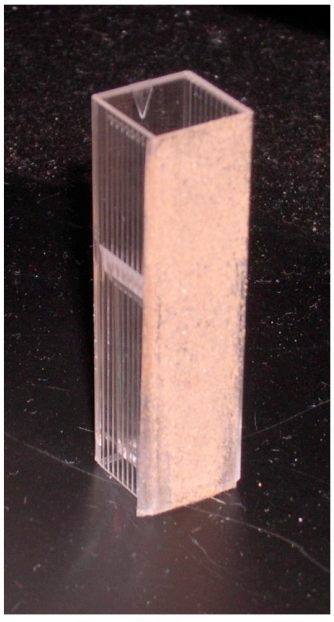


Figure 5: Cuvette with soil used in spectroscopy procedure

Using a cuvette with tape only as a control, transmittance was measured for wavelengths, in increments of 10, ranging from 300nm to 900nm. This was done for a single layer of soil first, and then with two layers. By adding a second piece of tape to the opposite side of the cuvette, and obtaining a second layer of soil, transmittance through two layers could be determined. As a control, a second piece of tape was added to the opposite side of the first control cuvette.

3.5 Microscopy

Before samples were taken from the cultures, pure cultures of each strain of cyanobacteria were observed and photographed under the light microscope for future reference in distinguishing the three species in culture. The camera used was a 4.0 megapixel NIKON Coolpix 4300 digital camera. Pictures were taken without a flash, and using a ten second timer to reduce vibrational distortion.

Observations were also made – and photographs taken – of the Inoculation Stock solution.

After approximately two months after the cultures were inoculated, core samples were periodically taken from plates for observations under a microscope. Again, sampling was done aseptically to avoid contamination of the cultures. Using a spatula, sprayed with 70% ethanol and flame-sterilized, a $1cm^2$ sample was removed from a plate in a dark area where cyanobacteria growth was likely to have occurred. This sample was then transferred to an empty Petri dish.

To separate bound soil particles (resulting from cyanobacteria filaments) from loose, unbound soil, a single drop of water was placed on the sample, and the crust layer could be easily removed and transferred to a microscope slide for observation.

Samples were viewed first using light microscopy, and later by fluorescence microscopy. Again, each species of cyanobacteria was viewed and photographed individually to determine characteristic fluorescence emissions of the separate cyanobacteria when viewed under various wavelengths. The inoculation stock was also observed to gain experience differentiating between species in a mixed culture. Finally the samples previously taken, and observed using light microscopy, were observed using

fluorescence microscopy. Samples were observed and photographed wet as well as dry to determine the most effective method.

4 Results

Data from each section of the entire experiment are documented below and are presented in the order in which they appear in the Materials and Methods chapter.

4.1 Soil Preparation

Approximately 8.2g of sterile soil was sufficient to cover the bottoms of the Petri dishes. However, due to variation in soil particulates, some plates required slightly more soil. To maintain equivalence among all plates, then, 8.5g of soil was used as a base stratum for all 12 plates.

4.2 Wet and Dry Cycle

The cycle period was slightly variable. For the first two months, when 3mL of water was added to each plate at the start of the wet cycle, the period ranged between 7 and 10 days. Between October 12, 2004 and December 16, 2004, the cultures were cycled 5 times.

During the final two months, the plates were moistened with only 2mL of water per cycle. This resulted in a decrease in cycle period ranging from 4 to 7 days.

4.3 Light Transmittance Through Soil

The tape used to hold the soil to the cuvette absorbed most of the light in the UV range, and accurate readings were possible beginning at approximately 290nm. Below in Figure 6 is a chart showing %Transmittance vs. Wavelength for one and two layers of soil.

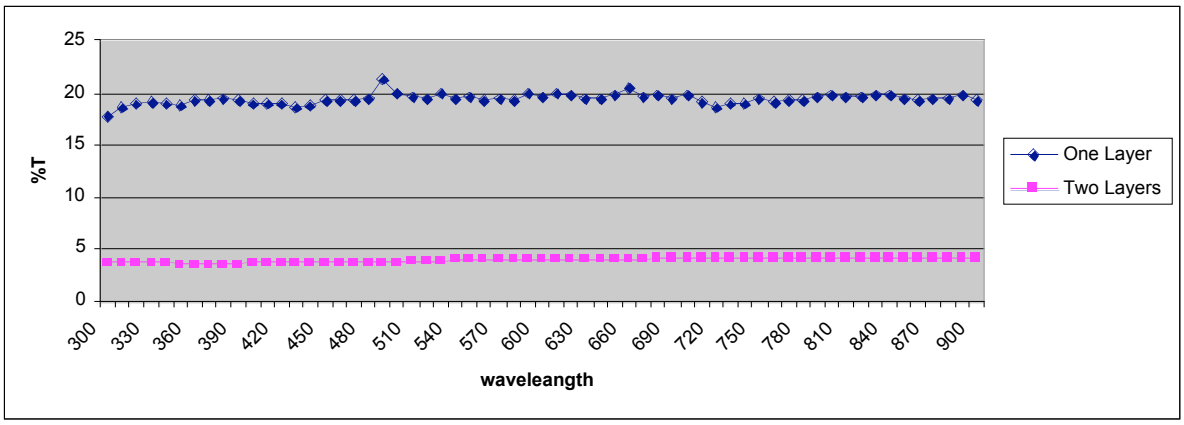


Figure 6: Graph of %Transmittance vs. Wavelength for one and two layers of desert soil.

One layer of soil was shown to have absorbed more than 80% of light over all wavelengths. While transmittance through one soil layer fluctuated slightly over the various wavelengths, transmittance through two layers was much more consistent, and showed a steady increase from 345nm to 900nm, with %T ranging from 3.6 to 4.2.

4.4 Visible Observations of Crust Growth

Cyanobacterial growth was visibly observed in all culture plates. This was characterized by the formation of dark-green/black patches on the soil surface. Several plates demonstrated surface fractionation in areas of heavy growth. These fractured surfaces would, when dry, lift away from the loose soil of the substratum.

Cultured soils also demonstrated greater stability than did non-cultured soil. Gently tapping the side of a culture plate showed no identifiable sign of disrupting the soil, and neither did tipping the plates to an angle of between 45 and 60 degrees.

Figure 7 shows a soil culture after 12 wet cycles which demonstrates the fractionation and lift of the soil surface.

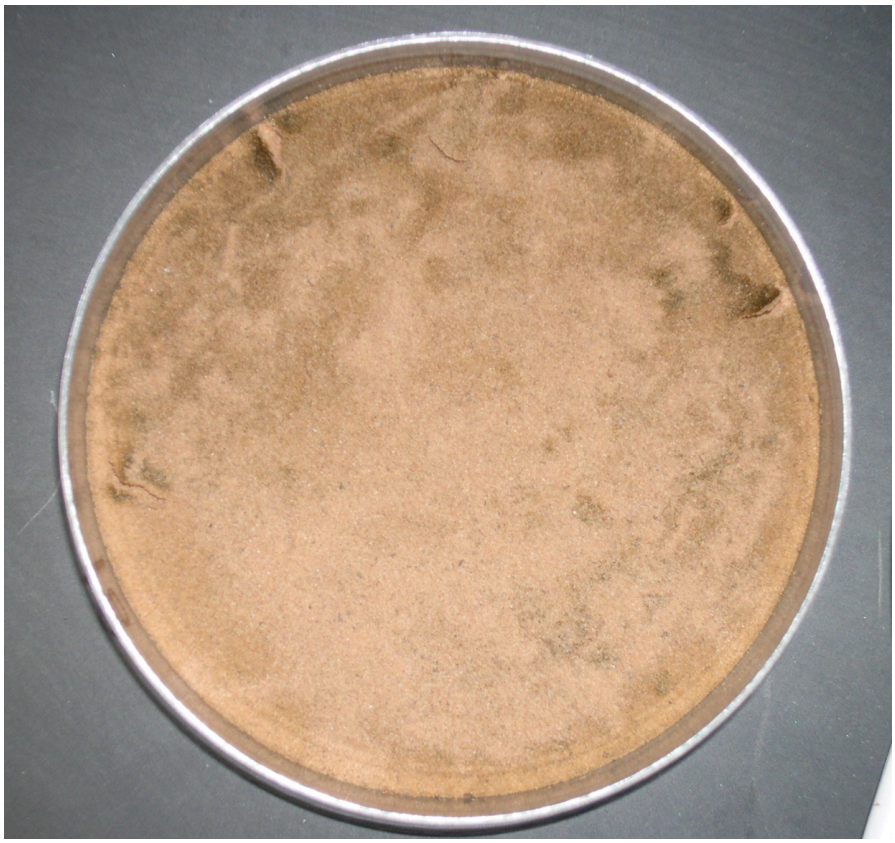


Figure 7: Soil culture after 12 wet cycles. Note the dark patches and the fractured surface. The surface lift is easily seen in the top picture. The bottom shows variation in soil color as a result of cyanobacterial growth.

4.5 Light Microscopy

All cultures sampled for light microscopy demonstrated significant growth, although growth was concentrated in only a few patches in each plate. Because light microscopy requires that light pass through the sample, an image of intact crust layer was not possible, and the crust layer was broken apart to attain any visible image.

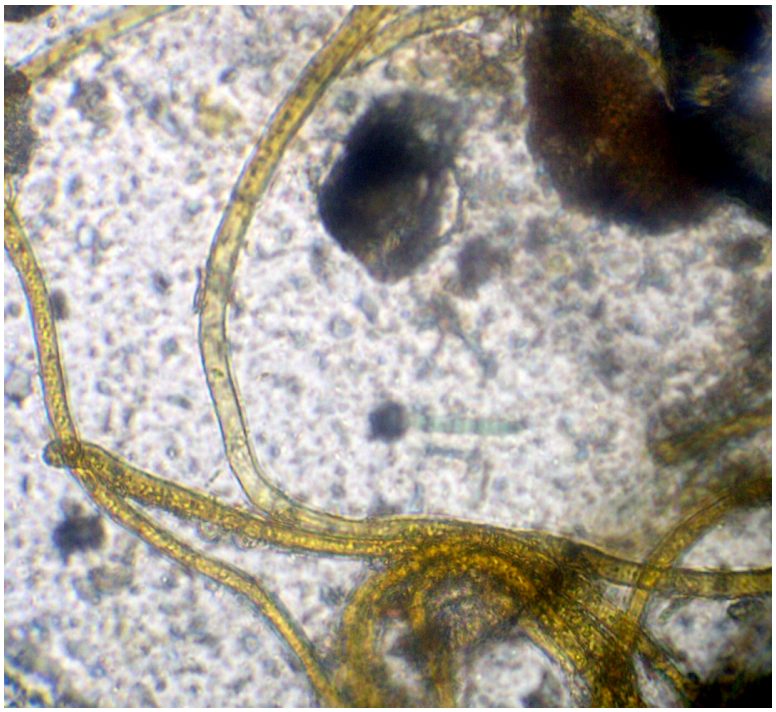


Figure 8: Photograph of soil culture (No UV) Image taken from perimeter of sample.

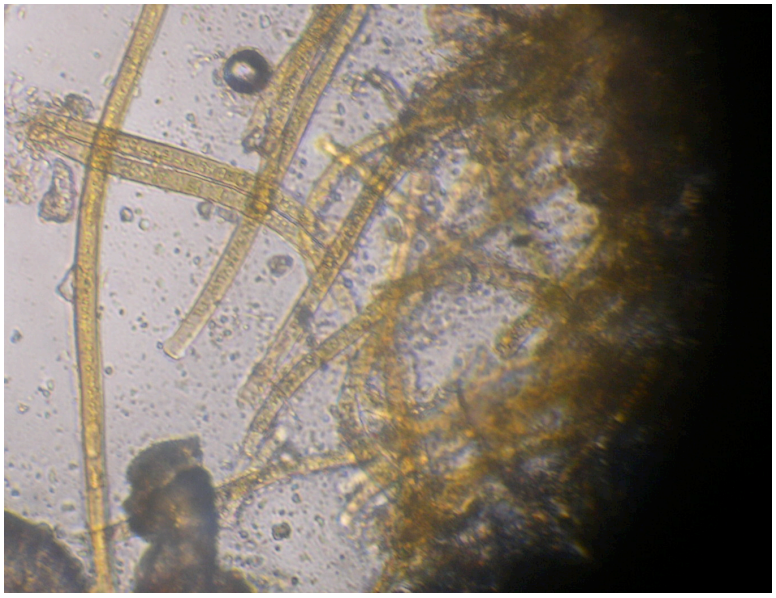


Figure 9: Photograph of soil culture (UVT) Image taken from perimeter of sample. Shaded area at right is dense soil and cyanobacteria

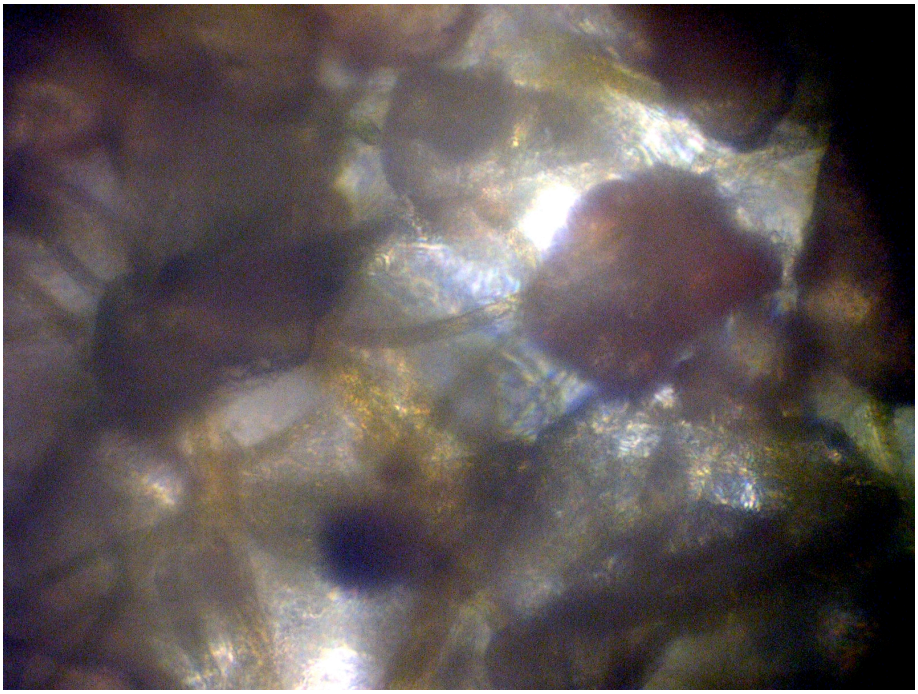


Figure 10: Soil culture (No UV) Taken from interior of crust layer. Note the "blanket" of filaments

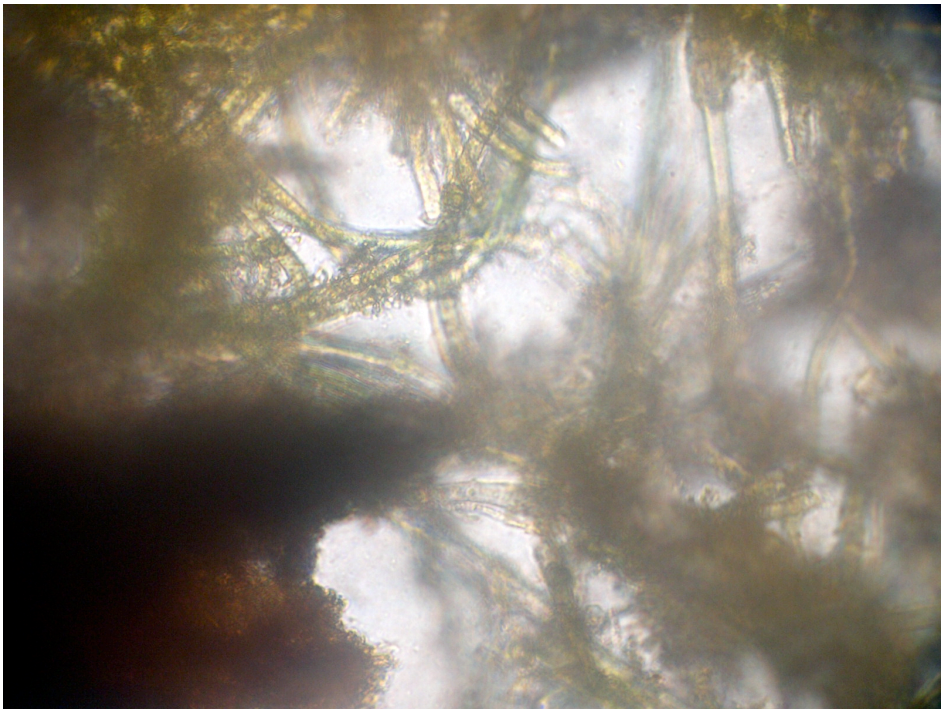


Figure 11: Soil culture (UVT) Interior of crust layer partially visible.

As seen in figures 9-12, cyanobacterial growth was significant in the samples taken. However, photographs of samples from plates with traditional Petri dish tops showed no clear difference between samples from plates with UVT plastic lids. Also, light microscopy did not prove an effective means of distinguishing between different species of cyanobacteria grown in soil. As demonstrated in the photographs, the cyanobacterial filaments turned a brownish-yellow color when grown in the soil, further complicating differentiation.

4.6 Fluorescence Microscopy

All three species of cyanobacteria used were auto-fluorescent, meaning that when exposed to the fluorescent light source, they emitted light without the use of stains. Each species also emitted different signals under different lights and filters. Below are a series of comparisons of the three species under each light condition.

Table 1: Green Light (0-0-0)

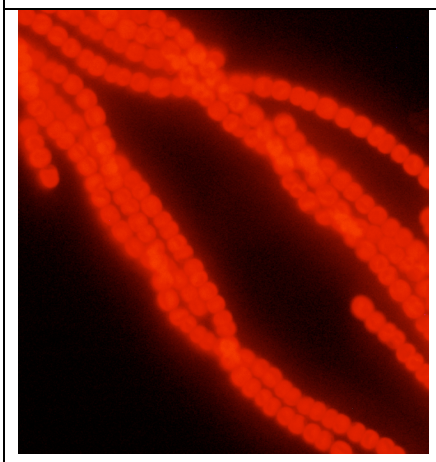
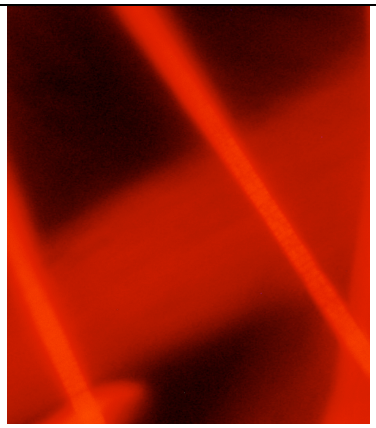
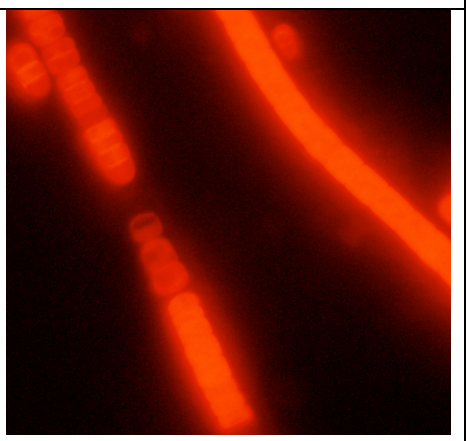
Nostoc	Microcoleus	Scytonema
		

Table 2: Green Light (65-0-0)

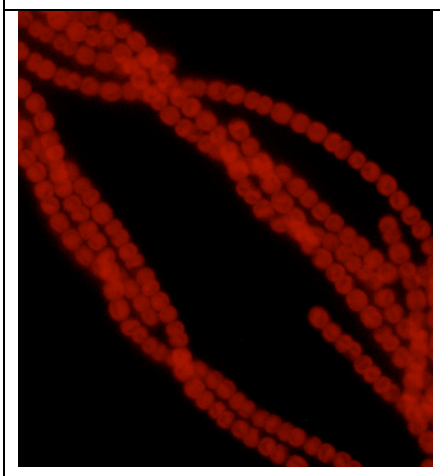
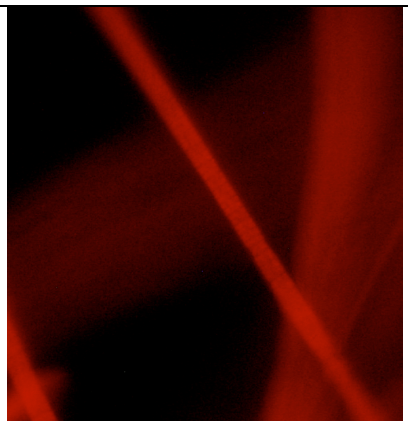
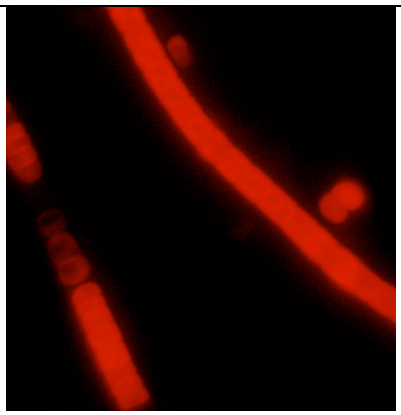
Nostoc	Microcoleus	Scytonema
		

Table 3: Blue Light (0-0-0)

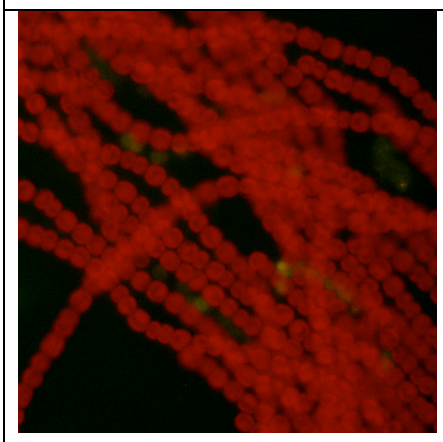
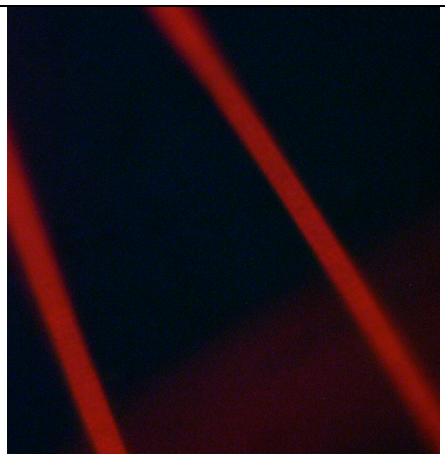
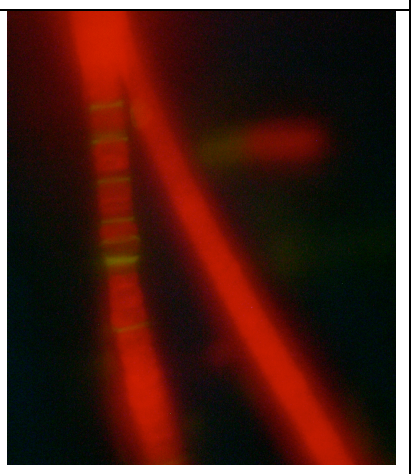
Nostoc	Microcoleus	Scytonema
		

Table 4: Blue Light (65-0-0)

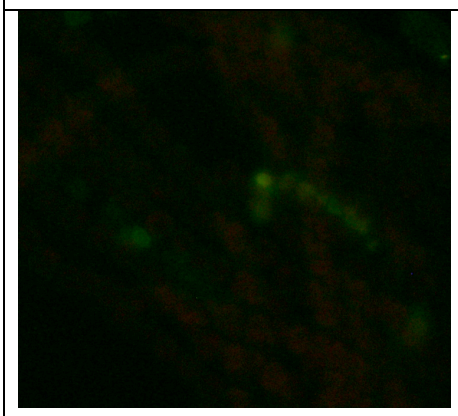
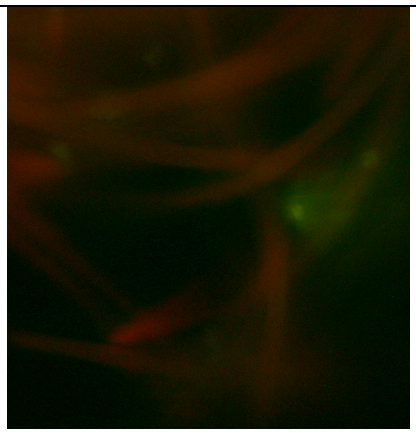
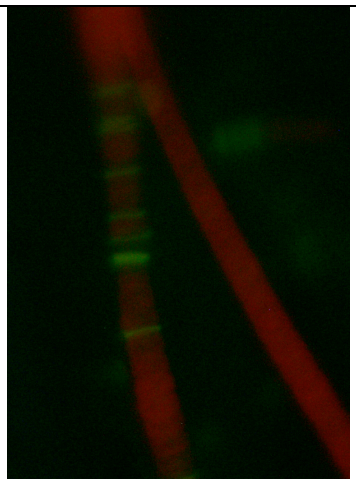
Nostoc	Microcoleus	Scytonema
		

Table 5: Ultra Violet Light (0-0-0)

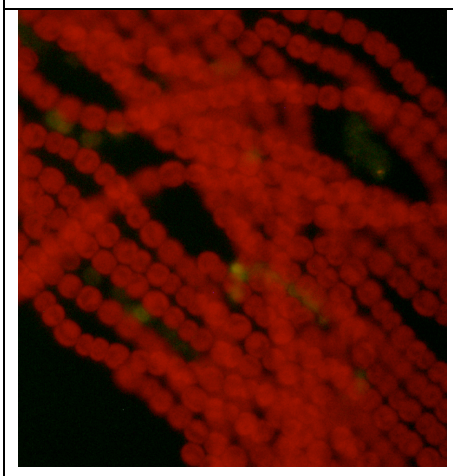
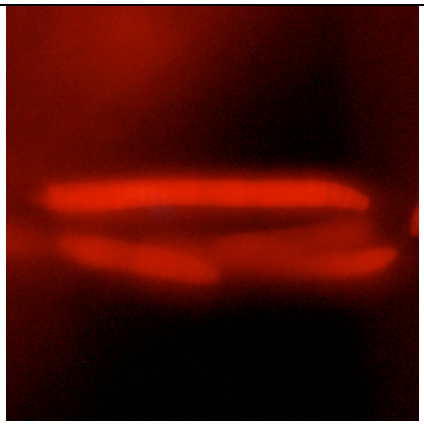
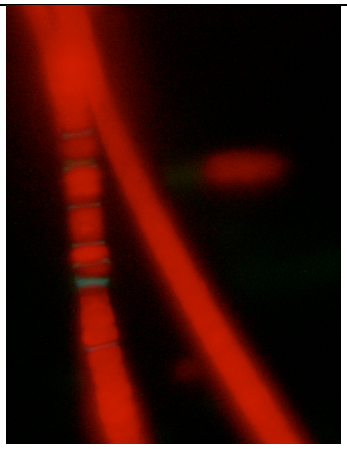
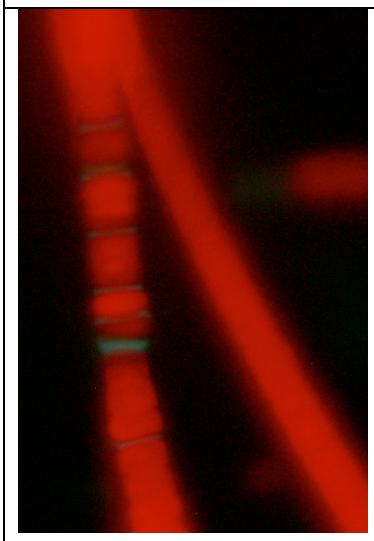
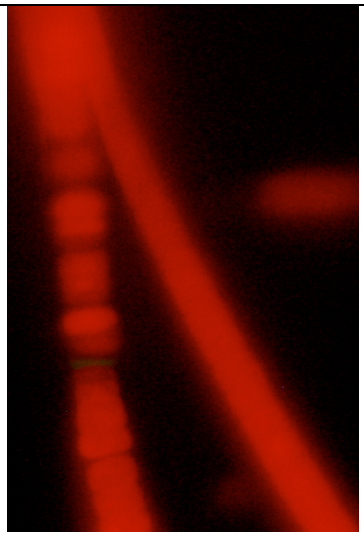
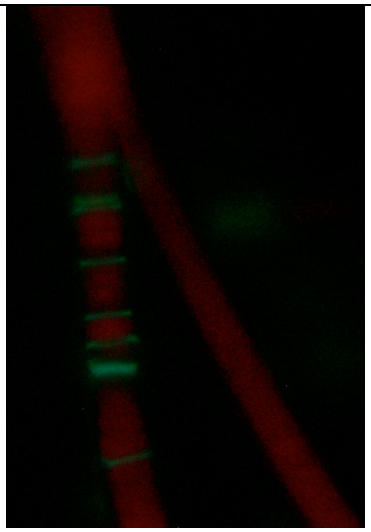
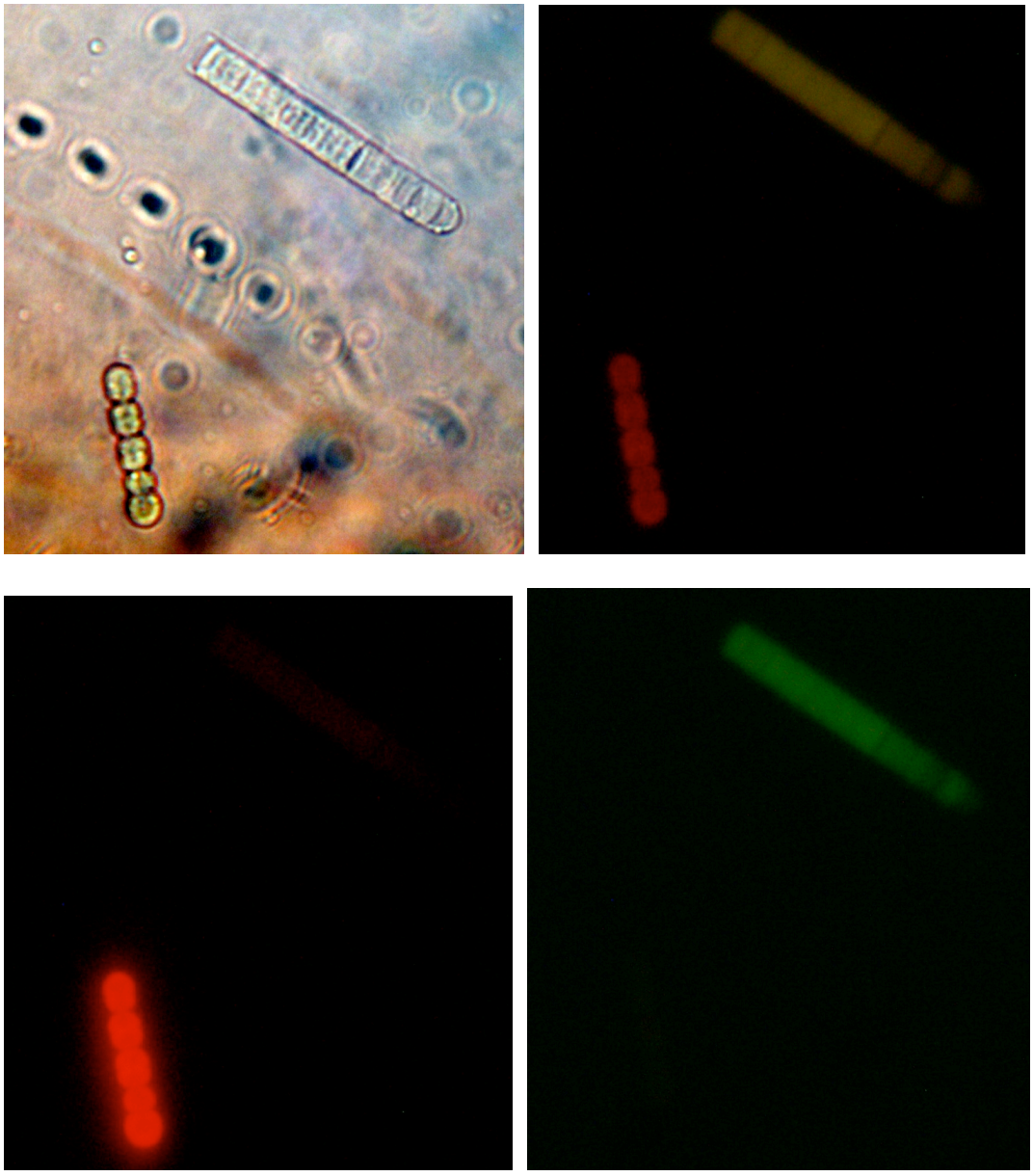
Nostoc	Microcoleus	Scytonema
		

Table 6: Scytonema Under UV using 3 Filter Settings

(0-0-0)	(53-0-0)	(65-0-0)
		

The images taken of the inoculation stock containing all three species, while showing more variation in fluorescence signals, still demonstrated distinguishable characteristics between species. Figure 13 shows the same field of vision using visible light, and three different settings of fluorescent light.

Figure 12: Inoculation Stock viewed under four light settings



These results seemed to indicate that differentiating between the different species in soil cultures would be possible through fluorescence microscopy. However, the images taken from soil cultures showed much more variation than did the single species stocks. Figure 14 shows several images taken from the soil cultures demonstrating signals drastically different than the separate species alone.

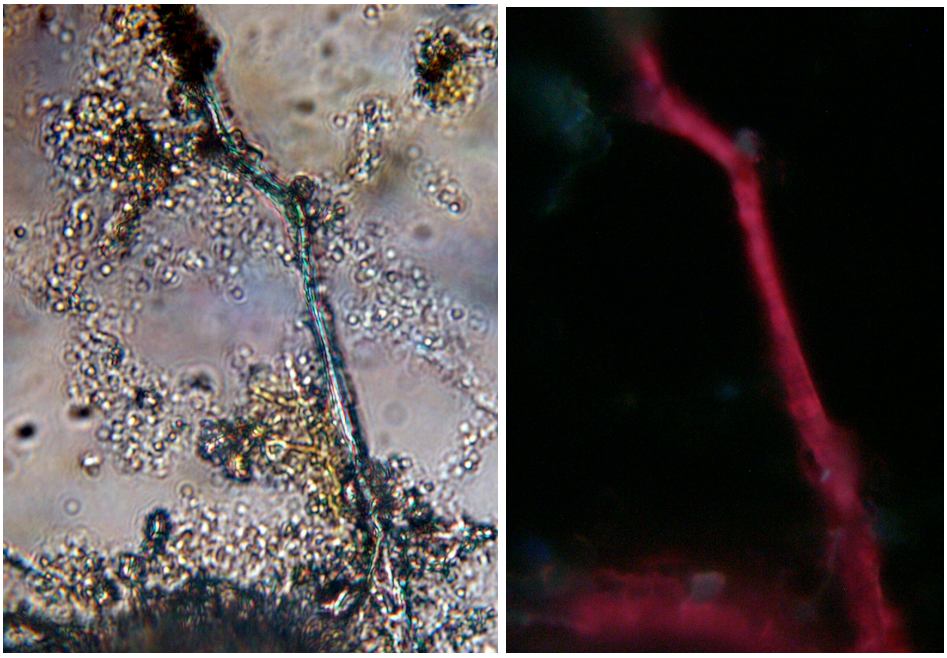
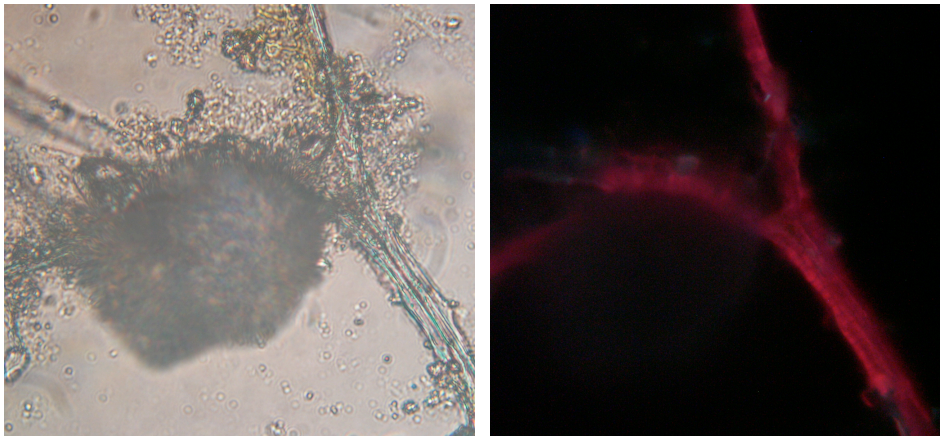


Figure 14: Soil Cultures viewed under white light (top left and bottom left) and blue fluorescent light (top right and bottom right)



5 Discussion

The workings of a standardized protocol for growing cryptobiotic crust in the lab have been determined in this MQP. Crusts clearly demonstrated growth of the cyanobacteria during the course of the experiment. The methods of observation, while proving relatively useful, resulted in more questions than answers. The protocol also has much room for improvement in later experiments.

5.1 Possible Improvements to Protocol

Although the soil cultures experienced significant cyanobacterial growth, this growth was isolated to only a few small patches per plate, indicated by dark green coloration. It is possible that the cultures simply need more time and more wet/dry cycling to further develop and grow, although modifications and improvements on the experimental procedure seem likely to enhance crustal development, as well as improve efficacy of observations. Below are several modifications which, if incorporated into the experimental design, could potentially aid future work.

1) *Begin the experiment using UVT plastic*

Since there did not seem to be any indication that the ultra violet light to which half the cultures were exposed made any difference in growth and development of the cyanobacteria, it may be that after two months (when six Petri dish tops were replaced with UVT plastic) the cyanobacteria had already been sufficiently established, and *Microcoleus* was already protected by the UV screening pigments of *Nostoc* and *Scytonema*. Beginning the experiment with UVT plastic lids would establish a more clear

experimental condition and will provide a more reliable account of the relationship between ultra violet light and crust formation.

2) *Apply additional soil layers more frequently*

During this experiment, additional soil was added only once, after approximately 2 months after inoculation. Because the cyanobacteria depend on additional soil to grow upward, there was very little vertical growth in any of the 12 cultures. However, much care must be used in determining the timing for each consecutive layer added, because if too little growth occurs between additions, cyanobacteria buried too deeply by new soil will not receive sufficient sunlight and die.

The light transmittance experiment demonstrated that a single layer of soil (one granule thick) allows only about 20% transmittance of light, so after 2 or 3 layers of soil have been added without sufficient growth, the cyanobacteria will be receiving as little as < 1% of the light hitting the surface, which could be potentially fatal for the cyanobacteria.

3) *Multiple Colonization Events*

It is likely that crust formation will occur more quickly and cover more surface area if growth is not solely reliant upon the initial inoculation's ability to grow and spread across the entire soil surface. By periodically transferring more inoculation stock to the cultures, it is very likely to increase growth rates, both vertically and horizontally, especially if optimal intervals of soil addition are determined. This would be a more

accurate representation of how these crusts form if free-living cyanobacteria can be carried via wind currents and set down again.

However, even if this is not the case in the desert, if multiple colonization events do increase cyanobacterial growth rates and total surface area covered in the lab, it could prove an effective restoration procedure for desert crusts damaged by livestock or vehicles. By spraying a damaged area with a mixture of cyanobacteria, the recovery rates, which, despite the variation in estimations, are very slow, could be drastically increased.

4) *Grow different cyanobacteria in separate soil cultures*

While the three species of cyanobacteria displayed distinct fluorescence signals in pure cultures, differentiation became quite difficult in soil culture samples. Fluorescence signals were present that were not present in the separate stocks. There are several reasons this could have occurred.

First, it could be linked to the color change observed in the cyanobacterial filaments in soil. The cyanobacteria, which had been distinctly green when observed in separate cultures, had turned a yellowish-brown color when grown in the soil. This could be due to bleaching and loss of pigmentation. It could also be that the mucilaginous sheaths encasing the cyanobacteria, while holding loose soil particles together, could also have accumulated various minerals and salts (from BG-11 media) present in the soil. These could have an effect on the wavelengths emitted by the sample.

Other possible reasons for the variation in signals are that the cells in the culture were dead, or water molecules in the sample were altering the signals. One way to help determine the cause is to grow each species of cyanobacteria separately in soil cultures.

By observing only one strain in the soil, differentiation between various species in a single culture may be more accurate.

5.2 Future Experiments

This project leaves much room for continued research, both in perfecting the protocol, and in comparing lab grown crust to naturally occurring crust to determine how accurately the natural crust is replicated.

One way to determine comparability is to use a gas chromatograph to separate and identify the gases emitted by the physiological processes of the crust. If an accurately emulated soil crust can be grown, a doorway will be opened to new research across the globe, in areas where acquiring cryptobiotic soil crusts is very difficult.

Future work could be done to verify Brostoff's results[10] to determine the potential of cryptobiotic crusts to photosynthesize more, thus reducing atmospheric CO_2, when concentrations of CO_2 are higher. Because cryptobiotic crusts make up such a large percentage of total biomass in many desert areas, this might suggest that cryptobiotic crusts will prove to be extremely helpful in reducing the effects of global warming.

If a standardized protocol for growing cryptobiotic crust in the lab can be perfected, the possibilities for future research are great. It would also increase our understanding of how these complex communities live, function and die, allowing for many previously studied experiments to be re-examined with more aptitude.

References

1. Belnap J, Kaltenecker J.H, Rosentreter R, et al. 2001. Biological Soil Crusts: Ecology and Management. USDI, BLM, National Science and Technology Center, BLM/ID/ST-001+1730.

2. Eldridge, D.J., and R.S.B. Greene. 1994. Microbiotic Soil Crusts - A Review of Their Roles in Soil and Ecological Processes in the Rangelands of Australia. Australian Journal of Soil Research. 32: 389-415.

3. Hawkes C.V., Flechtner V.R. 2002. Biological Soil Crusts in a Xeric Shrubland: Composition, Abundance, and Spatial Heterogeneity of Crusts with Different Disturbance Histories. Microbial Ecology. 43: 1-12.

4. Prescott L.M., Harley J.P., Klein D.A. Microbiology 6th Edition. McGraw-Hill, New York, NY. 2005.

5. UNEP, Environmental Effects of Ozone Depletion: 1998 Assessment. United Nations Environment Programme, 205pp, 1998. ISBN 92-807-1724-3

6. Veluci R.M., Neher D.A. 2003. Fixation and Nitrogen Leaching by Biological Soil Crust Communities in Mesic Temperate Soils. Microbial Ecology. Submitted Oct. 2003.

7. Hawkes C.V. 2004. Effects of biological soil crusts on seed germination of four endangered herbs in a xeric Florida shrubland during drought. Plant Ecology. 170: 121-134.

8. Belnap J. 2003. The world at your feet: desert biological soil crusts. Frontiers in Ecology and the Environment. Vol. 1, No. 4, pp. 181-189.

9. Ettershank G, Ettershank J, Bryant M, Whitford W. 1978. Effects of nitrogen fertilization on primary production in a Chihuahuan desert ecosystem. Journal of Arid Environments. 1:135-139.

10. Deacon J. The Microbial World: The Nitrogen cycle and Nitrogen fixation. Institute of Cell and Molecular Biology, University of Edinburgh. http://helios.bto.ed.ac.uk/bto/microbes/nitrogen.htm

11. Brostoff W.N., Sharifi M.R., Rundel P.W. 2002. Photosynthesis of cryptobiotic crusts in a seasonally inundated system of pans and dunes at Edwards Air Force Base, western Mojave Desert, California: Lab studies.