

FRAGILE CRYPTOBOTIC CRUSTS

OF DESERT ECOSYSTEMS

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Virginia Elizabeth O'Connell

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Professor Theodore C. Crusberg, Major Advisor

Abstract

Cryptobiotic crusts, found in desert ecosystems, are responsible for fixing carbon and nitrogen and stabilizing soil by reducing erosion. Crusts were grown in a laboratory setting, simulating the temperature, sunlight, and amount of rainfall they would experience in a natural environment. The crusts are composed of combinations of three species of prokaryotic algae: Microcoleus, Scytonema and Nostoc, and were observed using epifluorescent microscopy.

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1 Introduction

Cryptobiotic crusts are found in desert ecosystems throughout the world, in a variety of climates and diverse locations. These crusts which form on desert soils are referred to by a variety of names, including cryptogamic, cryptobiotic, biological, cyanobacterial, and microphytic (Bhatnagar and Bhatnagar 2005, Belnap and Gillette 1998). Cryptobiotic crusts can be composed of a variety of types of microorganisms, including lichens, cyanobacteria, green algae, mosses, bacteria, and fungi (Belnap, Kaltenecker, Rosentreter, *et al.* 2001). Crusts are known to be an important part of desert ecosystems, as they reduce soil erosion, and fix nitrogen and carbon (Belnap 2002, Veluci and Neher 2006). In a natural desert environment, cryptobiotic crusts can take 5-7 years to grow thin layers of vegetation, while a crust that is centimeters deep may take over 100 years to grow (Bhatnagar and Bhatnagar 2005). The microorganisms present in the crusts form a filamentous mesh which entraps soil particles, which protects the soil surface from disturbance (Belnap and Gillette 1997). Unfortunately, this surface protection is vulnerable to compressional disturbances, including foot, livestock, or vehicle traffic (Belnap and Gillette 1998). Since most of the biomass of cyanobacterial cryptobiotic crusts is located in the top 1 mm of soil, any loss of biomass due to disturbance results in greatly increased wind erosion (Hu, Zhang *et al.* 2003, Belnap and Gillette 1997).

Previous study of cryptobiotic crusts has included many field studies (Veluci and Neher 2006; Hu, Zhang *et al.* 2003; Hu, Liu *et al.* 2002; Smith, Halvorson, Bolton 2002; Belnap 2002; Belnap and Gillette 1998, 1997). However, cryptobiotic crusts have not been grown in a laboratory setting. It was the goal of this project to create a standardized

protocol for growing cryptobiotic crusts in a laboratory setting. The growth of crusts grown in the laboratory can be compared to that of wild cryptobiotic crusts maintained under the same conditions. Three species of cyanobacteria were chosen to create these crusts, based on their prominence in the deserts of the American West: *Microcoleus sp.*, *Nostoc sp.*, and *Scytonema sp.* (Belnap, Phillips, Miller 2004). If the growth of crusts utilizing these species is successful, further study can be done on a much wider scope, as researchers will not be restricted by access to field sites. The interaction between the various species that form these crusts can be established, and potential solutions to the problem of wild crust destruction can be determined.

2 Background

A wide body of research is available detailing the properties and function of cryptobiotic crusts in their natural environment. In order to simulate the conditions under which these crusts thrive, it was necessary to review these findings, so that this environment could be recreated in a laboratory setting. Here, the properties and functions of each of the three cyanobacteria species chosen for study are discussed, as well as the properties and functions of the crusts themselves.

2.1 *Cyanobacteria in Desert Crusts*

Cyanobacteria are defined as oxygen producing photosynthetic prokaryotes (Ehling-Schultz and Scherer 1999). These organisms are photosynthetic, and can fix atmospheric nitrogen if they are heterocystic (Belnap, Kaltenecker, Rosentreter, *et al.* 2001). Cyanobacteria are able to convert atmospheric nitrogen into nitrate or ammonia, a form usable by vascular plants or other vegetation. An organism such as *Nostoc*, for example, will release between 5 and 88% of the nitrogen it fixes into the surrounding environment for use by other organisms. Even non-heterocystic cyanobacteria, such as *Microcoleus*, are capable of nitrogen fixation in dark, anaerobic environmental conditions, such as those created due to the layering of filaments within a cryptobiotic crust (Belnap, Kaltenecker, Rosentreter, *et al.* 2001).

A key feature of desert growing cyanobacteria is their ability to dehydrate and suspend respiration without experiencing any negative effects, and then to become photosynthetically active rapidly after hydration – this is known as poikilohydric (Belnap, Kaltenecker, Rosentreter, *et al.* 2001). Since the growth period of cyanobacterial crusts

tends to be infrequent, with few precipitation events, this feature is essential for the organism's survival (Belnap, Phillips, Miller 2004). One of the key features of these cyanobacteria that allows them to exist in a desiccated manner are extracellular polysaccharides, which regulate water uptake and loss, and protect cell walls from damage due to shrinkage and swelling (Potts 1999).

Crusts are able to maintain their structure due to a feature of filamentous cyanobacteria – a sticky gelatinous sheath that surrounds the filaments. As the filaments are moistened during precipitation, the living filaments are able to grow, moving through the soil, as the sheath material is left behind. The discarded sheath material allows for a stabilized soil matrix, although the living organisms only inhabit the upper strata of the crust (Belnap, Kaltenecker, Rosentreter, *et al.* 2001).

As desert organisms, cyanobacteria forming cryptobiotic crusts are exposed to large amounts of solar UV radiation, and have evolved defensive mechanisms to avoid or counteract UV damage. These include a variety of strategies: migrating downward by gliding mechanisms, synthesis of UV absorbing compounds, antioxidants, and extracellular polysaccharides, and repair mechanisms including DNA repair and resynthesis of UV-sensitive proteins (Ehling-Schultz and Scherer 1999).

The three types of cyanobacteria that were studied, *Microcoleus sp.*, *Nostoc sp.*, and *Scytonema sp.*, form what are referred to as “Dark” cryptobiotic crusts, which occur naturally in hot and cool deserts where either precipitation or soil stability limits lichen development, but where disturbance is low. This type of desert corresponds to the deserts of the Southwest United States (Belnap, Phillips, Miller 2004). These three species were chosen due to their prominence in the area local to the collection site of wild

crust samples (Garcia-Pichel, López-Cortéz, Nübel 2001).

2.1.1 *Microcoleus* sp.

Microcoleus is one of the predominant species of cyanobacteria that form cryptobiotic crusts. A filamentous species, *Microcoleus* stabilizes the soil by leaving behind lengths of gelatinous sheath that binds soil particles, forming a soil matrix. Although it is non-heterocystic, *Microcoleus* is capable of fixing nitrogen if it exists in an anaerobic, dark environment (Belnap, Kaltenecker, Rosentreter, *et al.* 2001). Of the three species cultured for this study, *Microcoleus* is known to inhabit soil layers deeper in the crust than the other two species, *Nostoc* and *Scytonema* – on average it is located at the depth of 0.1 to 1.0 mm below the surface (Hu, Zhang *et al.* 2003). Unlike the other two species of cyanobacteria studied, *Microcoleus* has no innate protection against UV radiation, and will die if exposed (Bowker, Reed, Belnap 2002). However, since *Microcoleus* lacks UV protective pigments, the microorganism is capable of movement, able to move upwards into the photosynthetic zone when the soil is wet and return to depth when the soil dries (Belnap, Phillips, Miller 2004).

2.1.2 *Nostoc* sp.

Nostoc is a heterocystic cyanobacteria, located on the surface of a cryptobiotic crust (Garcia-Pichel, López-Cortéz, Nübel 2001). Typically, *Nostoc* is located at the depth of 0.02 to 0.05 mm below the soil surface (Hu, Zhang *et al.* 2003). This is also the only one of the three organisms that is not a filamentous cyanobacteria, in fact, *Nostoc* has a distinctive spherical appearance, resembling a chain of pearls (Potts 1999). In dark

cryptobiotic soil crusts, *Nostoc* is known for its UV blocking ability. Mycosporine amino acids (MAA) are used as a defense mechanism to protect the organism from UV photodamage. The effectiveness of MAAs seems to depend on their location within an organism. When they are located in the cytoplasm, only 10-26% of the UV photons are absorbed. In *Nostoc*, MAAs are located in the extracellular glycan, and 2 out of 3 photons are absorbed before reaching targets within the cell (Liu, Häder, Sommaruga 2004; Ehling-Schultz and Scherer 1999).

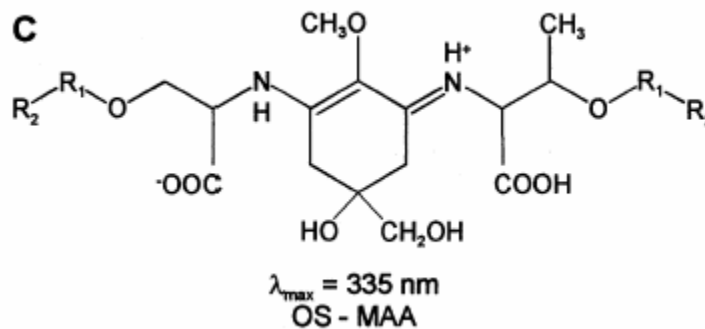


Figure 1: Mycosporine amino acid (MAA) structure found in *Nostoc* (Ehling-Schultz and Scherer 1999)

Nostoc also utilizes carotenoids for their ability to remove toxic oxygen species. The carotenoids myxoxanthophyll and echinenone have been observed acting as outer membrane bound UV-B protectors. Extracellular polysaccharides, are utilized as well, which form a sheath around the cyanobacteria and form a buffer zone between the environment and the cell. Extracellular glycan is increased when *Nostoc* is exposed to UV-B irradiation (Ehling-Schultz and Scherer 1999).

One of the problems that plagues long term plate cultures is the threat of bacterial contamination. *Nostoc* is especially useful in this case, as it has been found to have antimicrobial properties. *Nostoc* was found to exhibit antibacterial activity against both gram positive and gram negative bacteria, by producing an antagonistic phenolic

compound (El-Sheekh *et al.* 2005).

2.1.3 *Scytonema sp.*

Scytonema is a heterocystic cyanobacteria as well, similar to *Nostoc* and is located on the surface of a cryptobiotic crust (Garcia-Pichel, López-Cortéz, Nübel 2001). As a filamentous cyanobacteria, *Scytonema* also displays similar properties to those of *Microcoleus*, with an adhesive sheath which holds the soil in place after the living organism has grown away from that area (Belnap and Gillette 1998). *Scytonemia* is located in the same strata of soil crust that *Nostoc* is, at a depth of 0.02 to 0.05 mm below the soil surface (Hu, Zhang *et al.* 2003). *Scytonema* contains a sunscreen pigment, scytonemin, which serves as a UV shielding pigment that absorbs at 370 nm, and is thought to serve as a UV-A sunscreen (Ehling-Schultz and Scherer 1999; Garcia-Pichel and Castenholtz 1991).

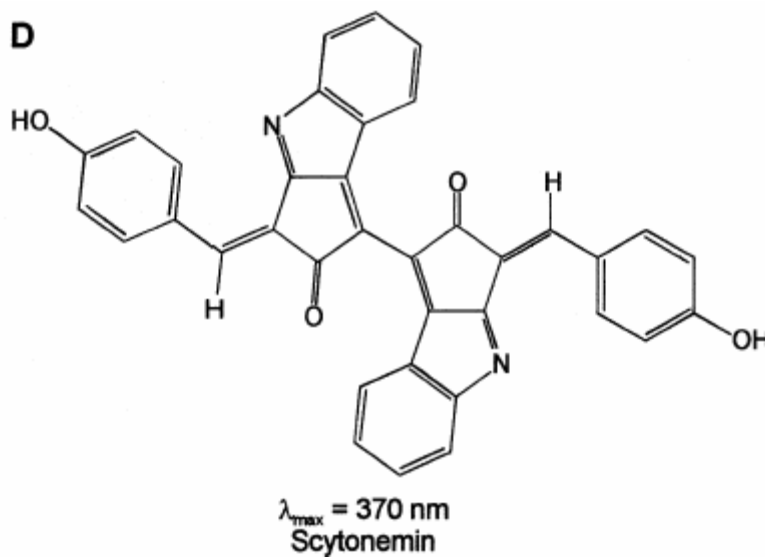


Figure 2: Scytonemin structure (Ehling-Schultz and Scherer 1999)

In addition to *Nostoc*, *Scytonema* species have also been found to have antibacterial properties. *Scytonema hofmanni* produces depsipeptide metabolites that have shown antibacterial activity (Matern *et al.* 2003).

3 Cryptobiotic Crusts

Cryptobiotic crusts in desert environments are dependent on rainfall – their success and subsequent biological activity is determined by the size, frequency, and timing of precipitation events. Crusts are metabolically active only when wet, and physiological functions are dependent on temperature, so if either of these two variables is altered, the function of the organisms will be profoundly affected (Belnap, Phillips, Miller 2004). Concern about the alteration of precipitation events has been brought up in conjunction with discussion about future climate change, which, for the US southwest, is predicted as an increase in temperature and alteration of precipitation timing, intensity, and interannual variability (Belnap, Phillips, Miller 2004). The potential effects of this climate change may disrupt desert ecosystems dramatically due to the fragile nature of the soil. Arid and semi-arid ecosystems represent over 25% of the earth's land area and are increasing in proportion due to desertification (Smith, Halvorson, Bolton 2002). Smith, Halvorson, and Bolton, in their 2002 study designed to predict the effects of climate change on cryptobiotic soil, found that total carbon, nitrogen, and biomass concentrations would decrease, leading to desertification of areas containing cryptobiotic crusts in Washington State if temperature and precipitation projections are accurate.

Direct compression of cryptobiotic crusts due to travel by vehicle or foot greatly decreases the surface resistance of the soil to wind erosion (Belnap, Phillips, Miller

2004). Biomass and activity in crusts is generally concentrated within 3 mm of the soil's surface (Hu, Zhang *et al.* 2003). Removal of this biomass increases wind and water erosion in the area, as well as reducing the fertility of the overall system, one of the definitive aspects of desertification. In a 2004 study done by Belnap, Phillips, and Miller, crusts in 4 locations were disturbed, using both a cow hoof and a four wheel drive vehicle. A wind tunnel was set up to determine the force required to detach soil particles from the surface – this force was decreased by 83% after disturbance by vehicles, with no recovery after one year's time.



Figure 3: Sections of disturbed cryptobiotic soil on the Colorado Plateau (USGS Canyonlands Research Station www.soilcrust.org)

Cryptobiotic crusts grow over time with the accretion of fine layers of sand, often one grain thick, which accumulates over the surface of the crust (Hu, Liu *et al.* 2002). Due to this vertical growth pattern, crusts can take 5-7 years to establish a thin layer of cryptobiotic soil (Bhatnagar and Bhatnagar 2005).

Cryptobiotic crusts provide more than solely soil stabilization - in addition to the atmospheric nitrogen fixation performed by the cyanobacteria of cryptobiotic crusts, the

crusts provide habitat for soil invertebrates, as well as germination sites for vascular plants (Belnap 2002; Yeager *et al.* 2004). In sparsely vegetated areas, cryptobiotic crusts are the primary mechanism for carbon buildup due to crust photosynthesis and thus biomass for these regions (Yeager *et al.* 2004). The rough uneven surface of the crusts enables the retention of nutrients contained in rainfall runoff, as the rough surface slows the runoff and increases the amount that seeps into the soil (Belnap, Kaltenecker, Rosentreter, *et al.* 2001).

4 Materials and Methods

The design of the laboratory methods for this study were intended to replicate in as many ways as possible the environment in which these crusts would have grown in a natural environment, while, of course, maintaining control over their growth. The temperature, lights, soil, and nutrient solution used were all designed to simulate a desert environment. All chemicals used were analytical grade reagent quality and BG-11 was obtained from Sigma (C3061) or made up from individual components.

4.1 Preparation of cryptobiotic soil plates

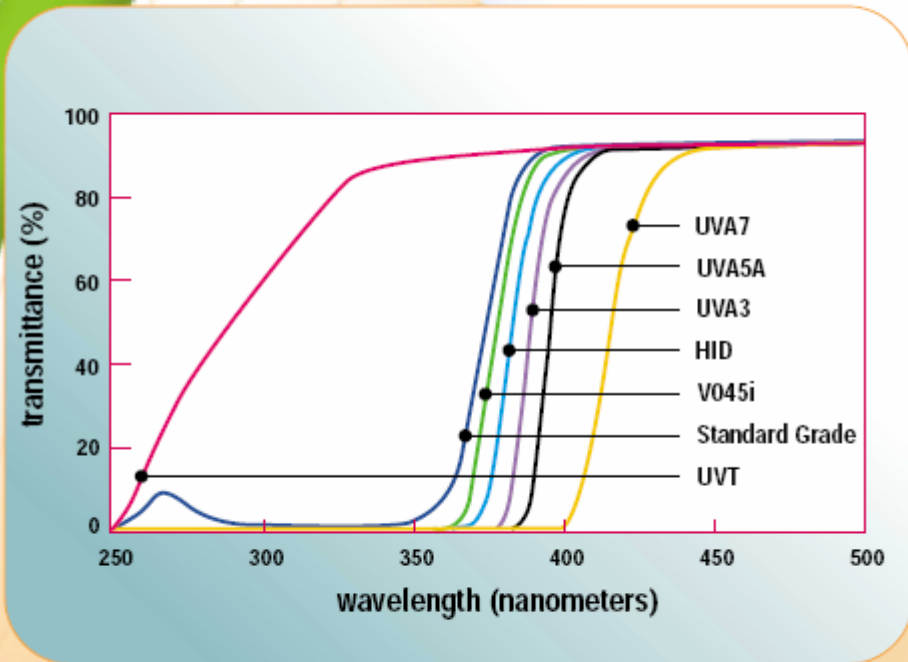
The plates used to grow the samples of cyanobacteria were composed of autoclaved soil collected on site from a desert in the American Southwest, with excess detritus removed. The soil was placed in a 100mL Petri dish, using a mass balance, 20 g of soil was added to each plate.

Microcoleus, *Scytonema*, and *Nostoc* cultures that were grown in flasks with BG-11 medium (as in Hu, Liu *et al.* 2002) were used to inoculate the prepared plates. The samples of cyanobacterial culture were homogenized using a Waring Blender, in order to break down the filamentous species, and this suspension was then placed in a flask with deionized water to make 100 mL. Selected combinations of *Microcoleus*, *Scytonema*, and *Nostoc* (0.5 mL of each) are added to the prepared plates using aseptic technique. The cultures were fed initially with BG-11 medium.

The dishes were covered with a plate cover designed to maximize the full spectrum of light that would reach the crust in a natural environment. Plates that were used involved a standard Petri dish bottom, with a modified Petri dish top. In order to

simulate a desert environment for the organisms, UV rays had to penetrate the cover of the Petri dish – this involved constructing an alternate cover, as standard Petri dish lids are composed of UV blocking plastic. To create this alternate cover, the center was drilled out of a standard Petri dish cover, and a similarly sized disk or square of UVT plastic Plexiglass, which transmits UV rays, was used to cover the dishes. (See Figures 5 and 6) The lids were also raised slightly to allow for evaporation of the cryptobiotic soil medium used to water the samples, by the addition of small pieces of plastic rod attached to the inside rim of the cover. This type of Plexiglass allows for the largest range possible of spectra that transmit through the cover. (See Figure 4)

Figure 5 UV Transmittance Spectra of Plexiglas V-Series Resins (3.2mm thickness)



UV transmittance data is shown for different grades measured at one thickness.

Figure 4: UV transmittance data; UVT plastic transmittance on far left (Atoglas Plexiglass Technology 2001)



Figure 5: Plate setup with UVT cover (round)



Figure 6: Plate setup with UVT cover (square)

4.2 Incubator Setup

The lights used in the incubator were Duro-Test's Vita-Lite full spectrum fluorescent lamps. These transmit both UV-A and UV-B radiation, and simulate natural daylight at 5500K. Duro-Test lights include a covering formulated from three visible halophosphors and an ultraviolet phosphor (Duro-Test Canada, Inc). By simulating natural daylight, the crust samples are placed under more natural conditions than a standard fluorescent light would allow. The spectral power distribution of Vita-Lites is shown in Figure 7 below.

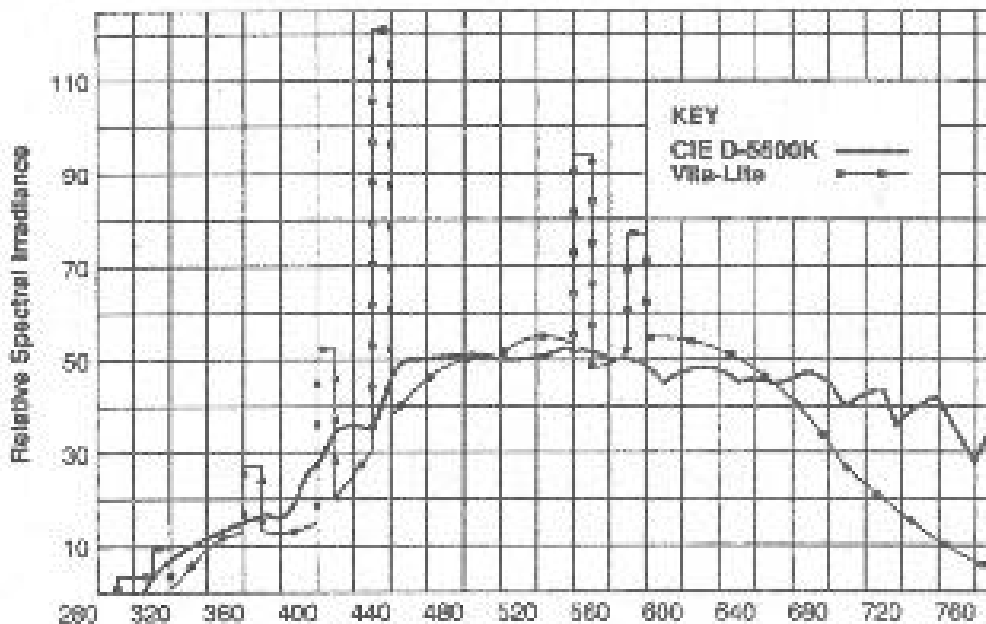


FIGURE 1. Spectral Power Distribution

Figure 7: The spectral power distribution of Duro-Test Vita-Lites (Duro-Test Canada, Inc)

The temperature inside the incubator where the plates are grown was kept constant at 30°C, as per Stradling, Thygerson et.al. (2002). The plates were set up inside an incubator designed to hold approximately 30 standard sized Petri dishes. (See Figure 8)



Figure 8: Incubator setup with plates.

4.3 Cryptobiotic Soil Solution

Cryptobiotic Soil solution, or CBS solution, was used to moisten the cryptobiotic crust sample plates. The composition and amount of this solution used for each watering was determined by analyzing data collected by the National Atmospheric Deposition Program / National Trends Network. This data, collected from the site UT09, was analyzed from 1997-2004. The data for the 1997 year was incomplete, so for the purposes of this study only the data from 1998-2004 was used. Site UT09, or Canyonlands National Park-Island in the Sky is located in San Juan County, Utah. The composition of CBS solution was calculated by determining the deposition of inorganic

nutrients over 1 year from a 7 year average, and the ratios of elements included.

(<http://nadp.sws.uiuc.edu/sites/siteinfo.asp?net=NTN&id=UT09>)

Table 1: Inorganic nutrient precipitation amounts in milligrams per year per plate

		mg/yr/plate
Ca	$6.37/7 = 0.91 \text{ mg}/100\text{cm}^2/\text{yr}$	0.55
Mg	$0.757/7 = 0.108 \text{ mg}/100\text{cm}^2/\text{yr}$	0.066
K	$0.726/7 = 0.104 \text{ mg}/100\text{cm}^2/\text{yr}$	0.063
NH ₄	$3.05/7 = 0.44 \text{ mg}/100\text{cm}^2/\text{yr}$	0.0263
NO ₃	$17.07/7 = 2.44 \text{ mg}/100\text{cm}^2/\text{yr}$	1.484
Cl	$1.56/7 = 0.22 \text{ mg}/100\text{cm}^2/\text{yr}$	0.134
SO ₄	$10.43/7 = 1.49 \text{ mg}/100\text{cm}^2/\text{yr}$	0.072
Na	$0.835/7 = 0.118 \text{ mg}/100\text{cm}^2/\text{yr}$	0.0714

The total precipitation over 7 years was 140 cm, the average of which is 20 cm/yr.

With an average of 33 precipitation events/year, this means that on average 0.606 cm fell in each precipitation event. The cryptobiotic sample plates were watered with 15 mL per week, to simulate a year's worth of precipitation in 11 weeks. With these calculations, approximately 4 years of crust growth can be simulated in one year's time.

The CBS solution is made in several parts, which are combined to form CBS medium (CBSM), in a series of dilutions and additions designed to minimize precipitation of insoluble minerals in the bottle. To make 0.5 L of CBSM, 10 mL of CBS solution part A, 10 mL of CBS solution part B, 500 mL of trace elements for medium 819 (BG-11), and 500 mL of Fe-am-SO₄ 1000x as used in BG-11 are added to a 500mL Gibco bottle, with sterile dH₂O. Tables 1-5 summarize the components of each solution.

Table 2: CBS Solution Part A

CBS Solution Part A

	Molecular weight	Weight (mg)
Ca(NO ₃) ₂	236.15	857
CaCl ₂ (anh)	111.0	60
MgCl ₂ ·7H ₂ O	203.3	93.2
NaCl	58.44	36.2

Table 3: CBS Solution Part B

CBS Solution Part B

	Molecular weight	Weight (mg)
KCl	74.55	38.2
(NH ₄) ₂ SO ₄	132.1	315
MgSO ₄ ·7H ₂ O	246.3	113

Table 4: BG-11 Medium Composition

BG-11 Medium

NaNO ₃	1.5 g
K ₂ HPO ₄	0.04g
MgSO ₄ ·7H ₂ O	0.075g
CaCl ₂ ·2H ₂ O	0.036g
Citric Acid	0.006g
EDTA (Na ₂)	0.001g
Na ₂ CO ₃	0.02g
Trace Metal Mix A5	1.0mL
Fe-am-Citrate	0.006g
Add dH ₂ O to 1.0 L total volume	

Table 5: Trace Mix A5 Composition

<u>Trace Mix A5</u>	
H ₃ BO ₃	2.86g
MnCl ₂ ·4H ₂ O	1.81g
ZnSO ₄ ·7H ₂ O	0.222g
NaMoO ₄ ·2H ₂ O	0.39g
CuSO ₄ ·5H ₂ O	0.079g
Co(NO ₃) ₂ ·6H ₂ O	49.4mg
Add dH ₂ O to 1.0 L total volume	

The medium was prepared by filter sterilization through a 0.22 um filter.

4.4 Cyanobacteria combinations plated

In order to assess the growth of all three cyanobacteria, samples were grown involving various combinations of *Microcoleus*, *Scytonema*, and *Nostoc*. Each of the three alone was grown, as well as all three together. *Microcoleus* and *Nostoc* were plated together, as were *Microcoleus* and *Scytonema*. These organisms are abbreviated: *Microcoleus* (M), *Nostoc* (N), and *Scytonema* (S). Several crusts collected in the Utah Canyonlands National Park were also maintained, to compare growth. (Wild Crusts A and B)

(cyanobacteria type included, date plate was started)

M1/31/06
S 9/29/05
N 9/29/05
N 9/23/05
MN 9/23/05
MS 1/31/06
MS 9/29/05
MS 9/29/05
MNS 1/31/06
MNS 9/23/05
MNS 9/29/05
MNS 11/15/05
Wild crust A
Wild crust B

4.5 Epifluorescence Microscopy

The crusts that were grown were analyzed using a Zeiss® Epifluorescence Microscope. Samples were removed from the plates using a ½” diameter core sampler and utilizing aseptic technique. The removed discs were placed on slides and viewed without further treatment with the epifluorescence microscope. (See Figure 9) The samples were photographed with a Nikon Coolpix 4300 digital camera which included a 3x optical Zoom-Nikkor lens. Ultimately, the magnification of these organisms was 100x, with the magnification of the microscope and the camera zoom factored in.



Figure 9: Slide with disc of crust ready to be viewed with the epifluorescence microscope

4.6 Contamination

One of the problems of a long term culture is the threat of contamination. Unfortunately, as the cryptobiotic crust samples are grown over a period of months, some contamination is unavoidable. Sterile technique was used in an attempt to minimize potential contamination. In addition, as mentioned previously, cyanobacterium utilize an antimicrobial substance, minimizing contamination by gram-positive and negative

bacteria, as well as filamentous fungi (El-Sheekh *et al.* 2006; Matern *et al.* 2003).

5 Results

Results include the microscopy images taken of crust samples under green, blue, and UV excitation.

5.1 *Wild Crust A*



Figure 10: Wild Crust A



Figure 11: Wild Crust A, green excitation, 100x

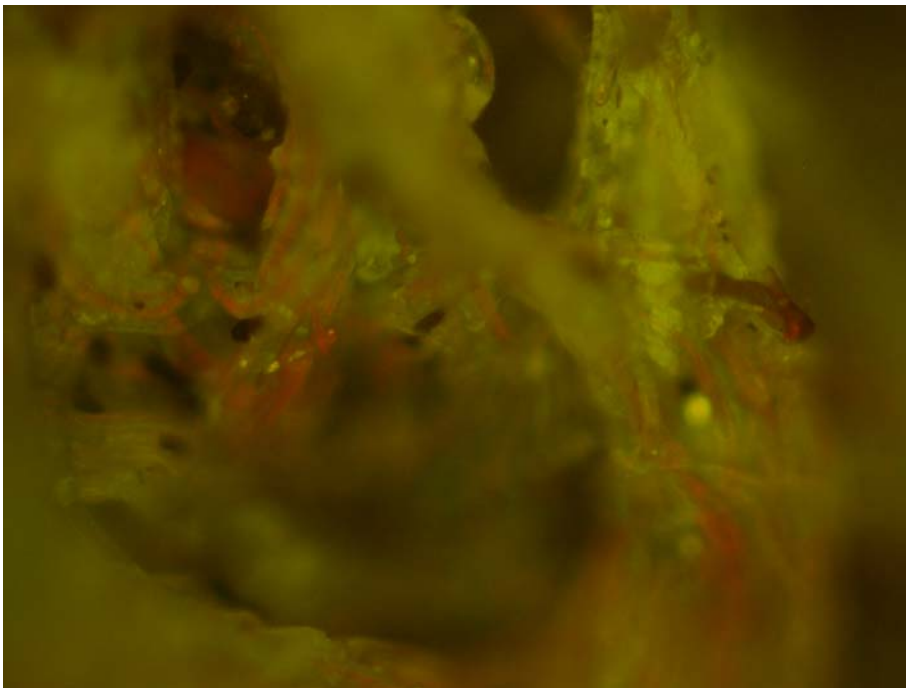


Figure 12: Wild Crust A, blue excitation 100x

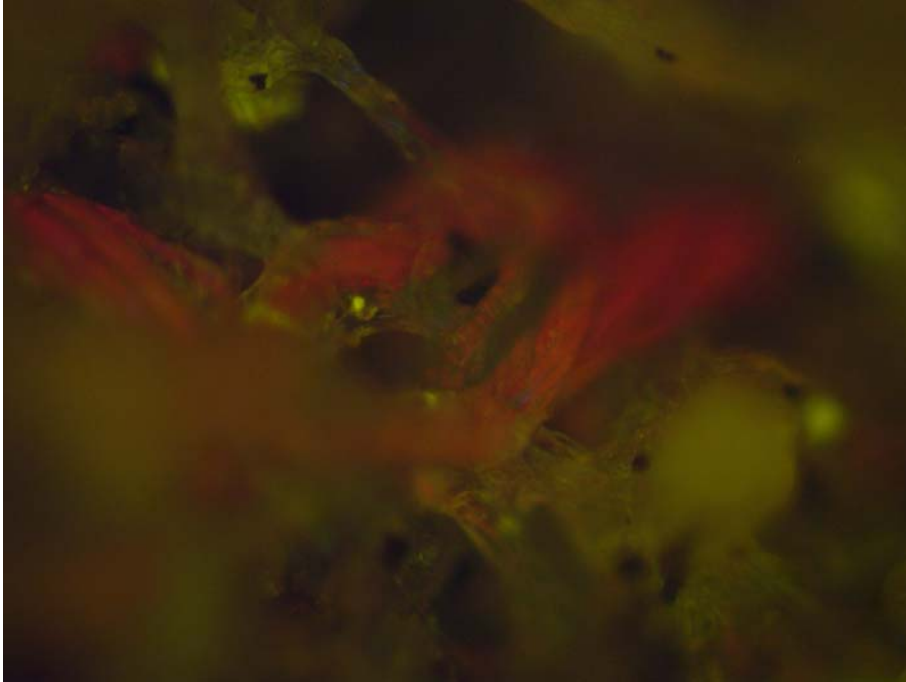


Figure 13: Wild Crust A, blue excitation 100x.

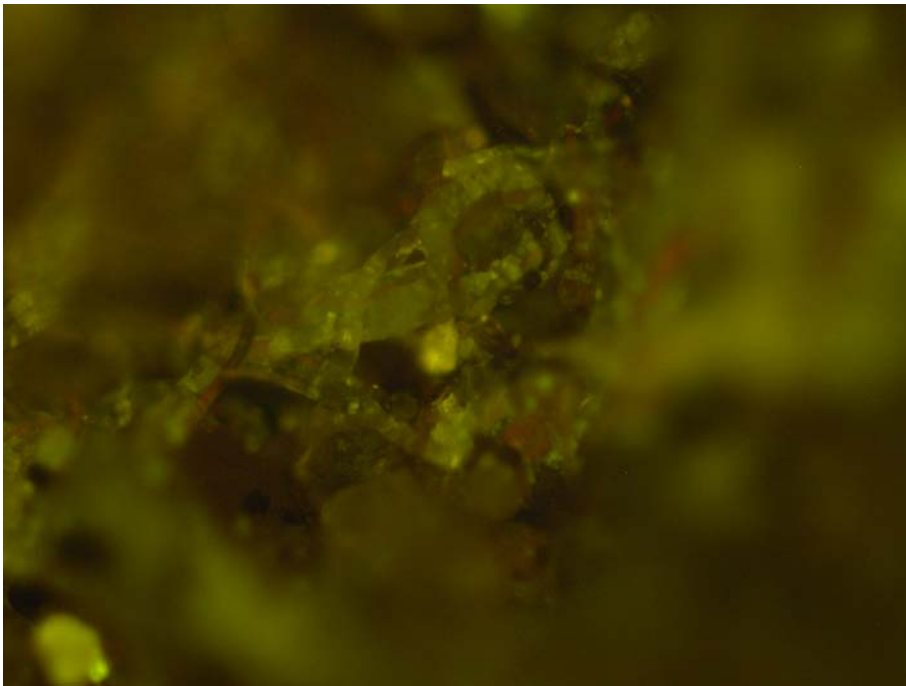


Figure 14: Wild Crust A, blue excitation 100x.

5.2 Wild Crust B



Figure 15: Wild Crust B.

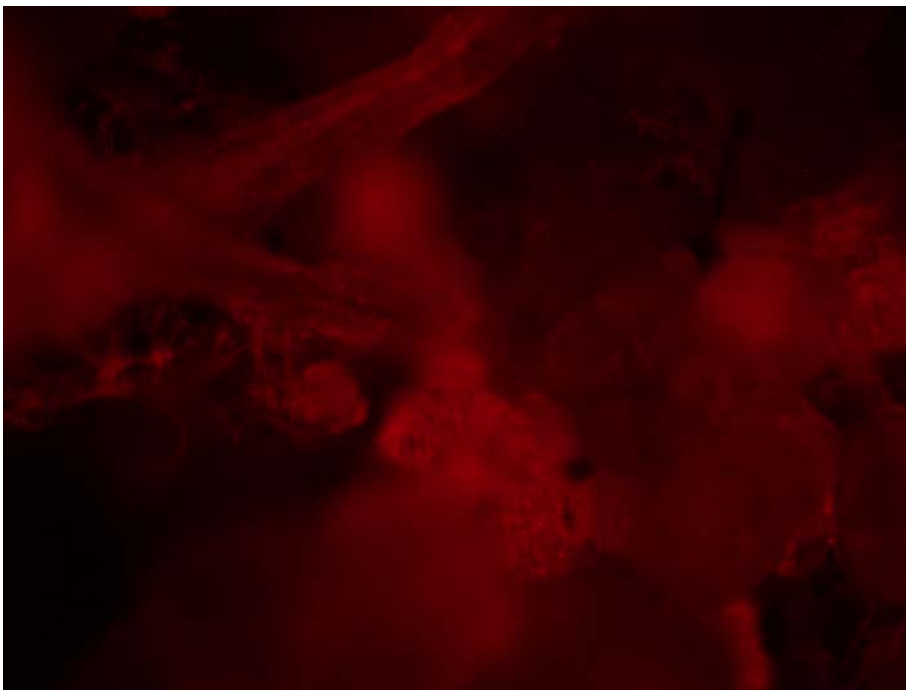


Figure 16: Wild Crust B. Green excitation 100x.

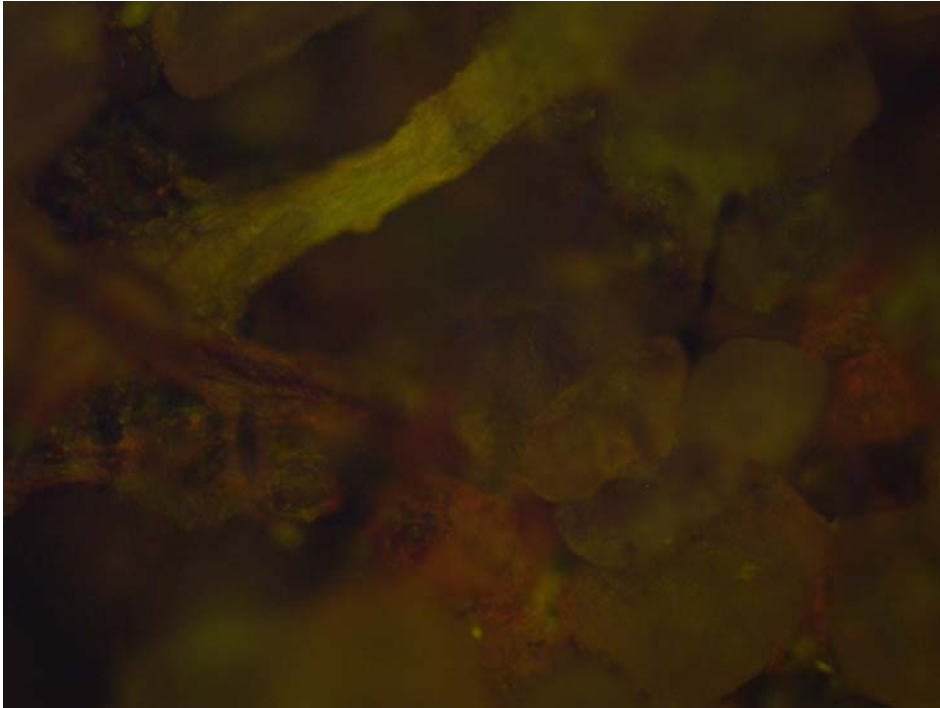


Figure 17: Wild Crust B. Blue excitation 100x.

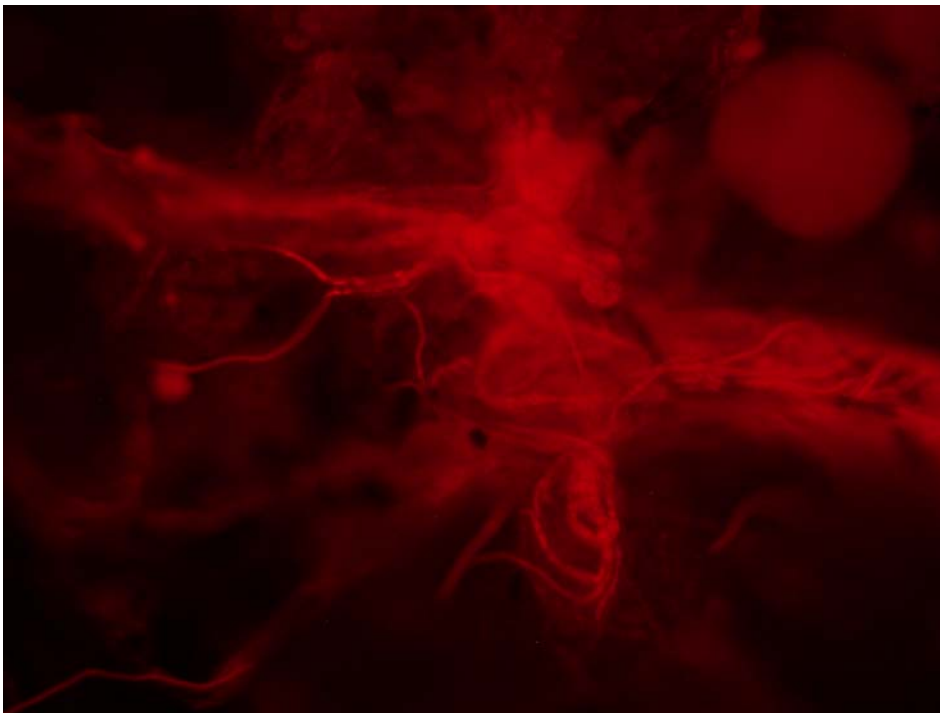


Figure 18: Wild Crust B. Green excitation 100x

5.3 *Microcoleus* 1/31/06



Figure 19: *M* 1/31/06

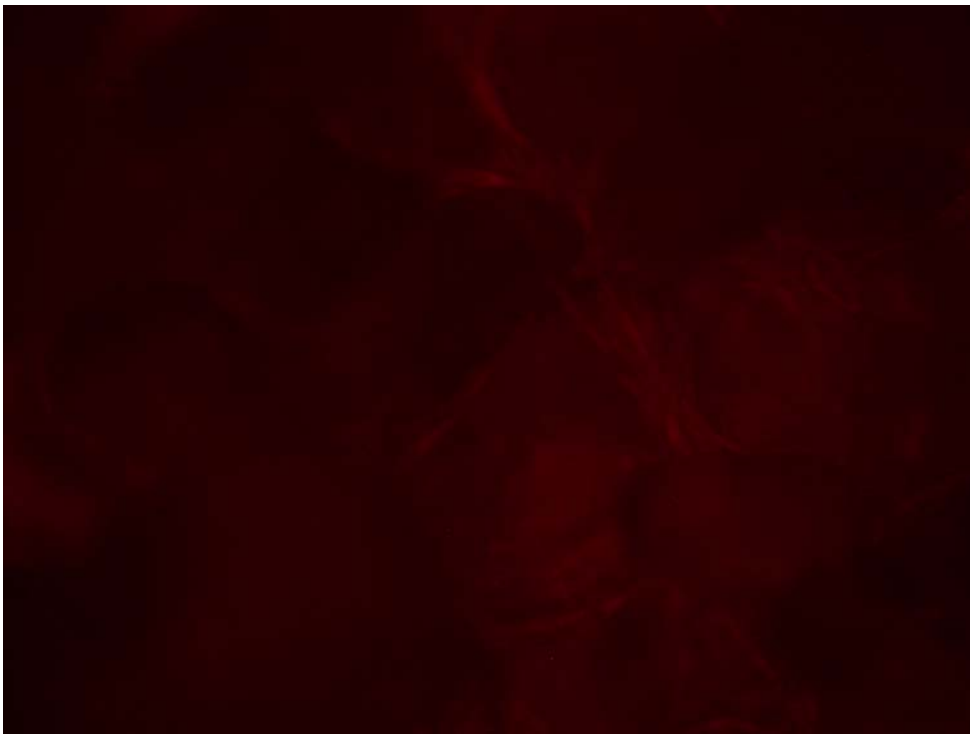


Figure 20: *M* 1/31/06 Green excitation 100x.

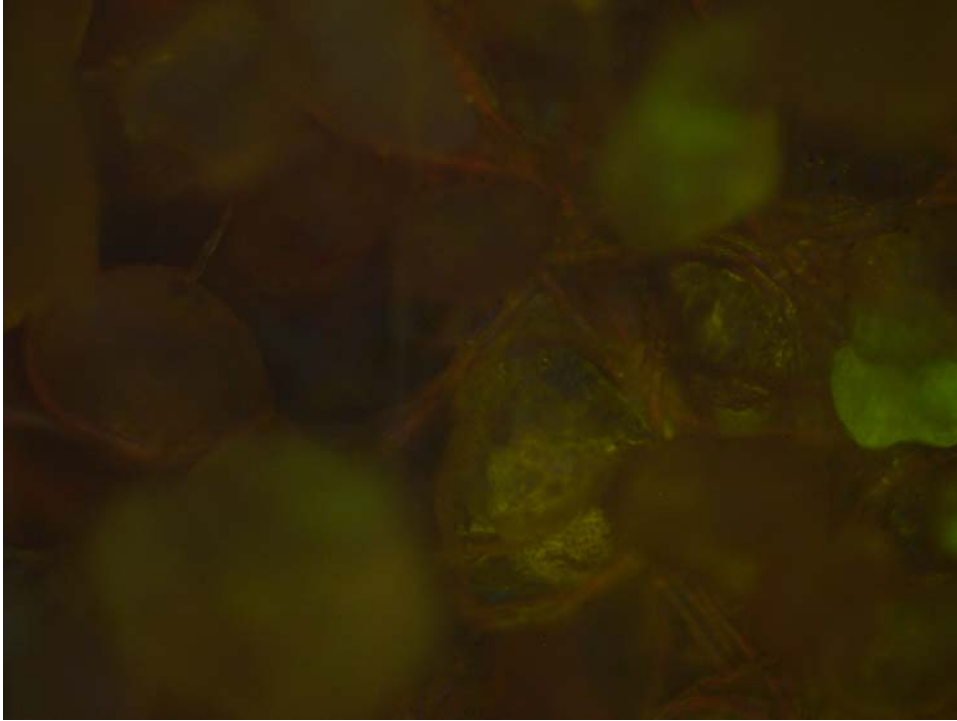


Figure 21: M 1/31/06 Blue excitation 100x.

5.4 Scytonema 9/29/05



Figure 22: S 9/29/05

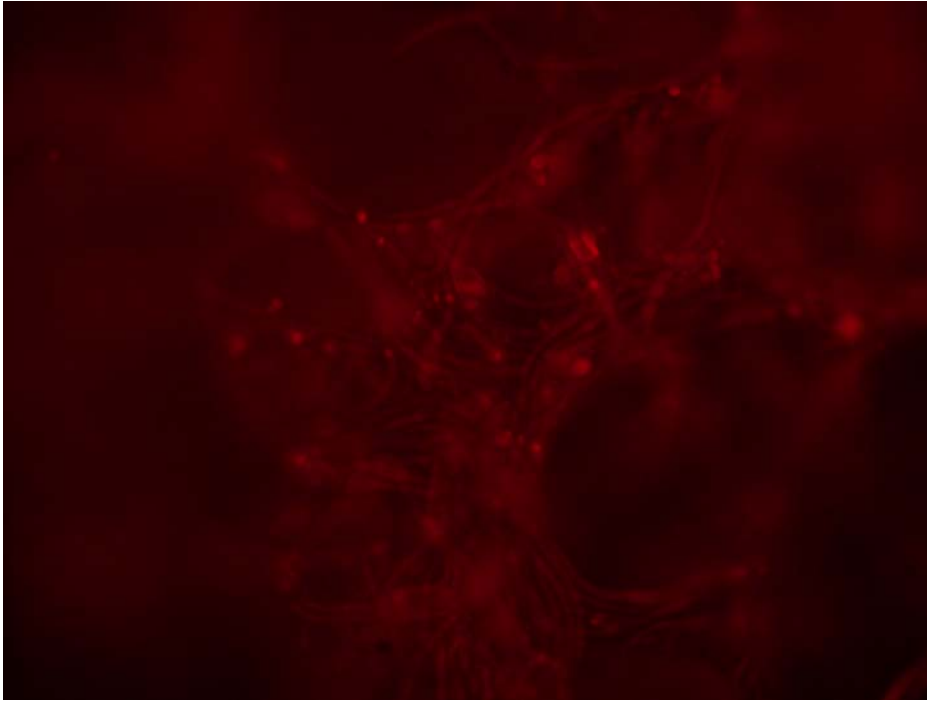


Figure 23: S 9/29/05 Green excitation 100x.

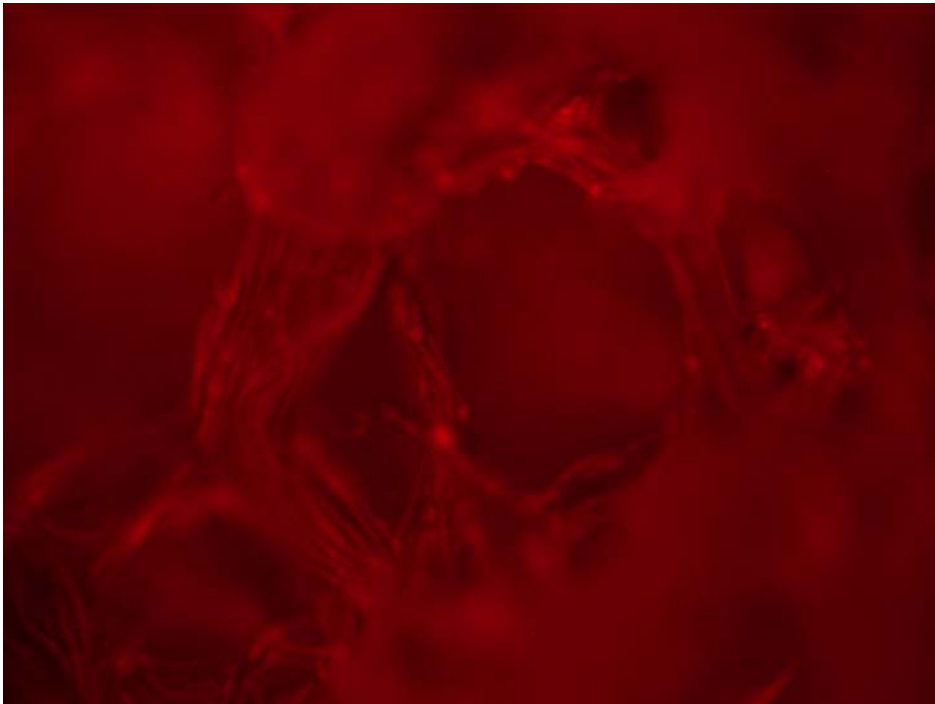


Figure 24: S 9/29/05 Green excitation 100x.

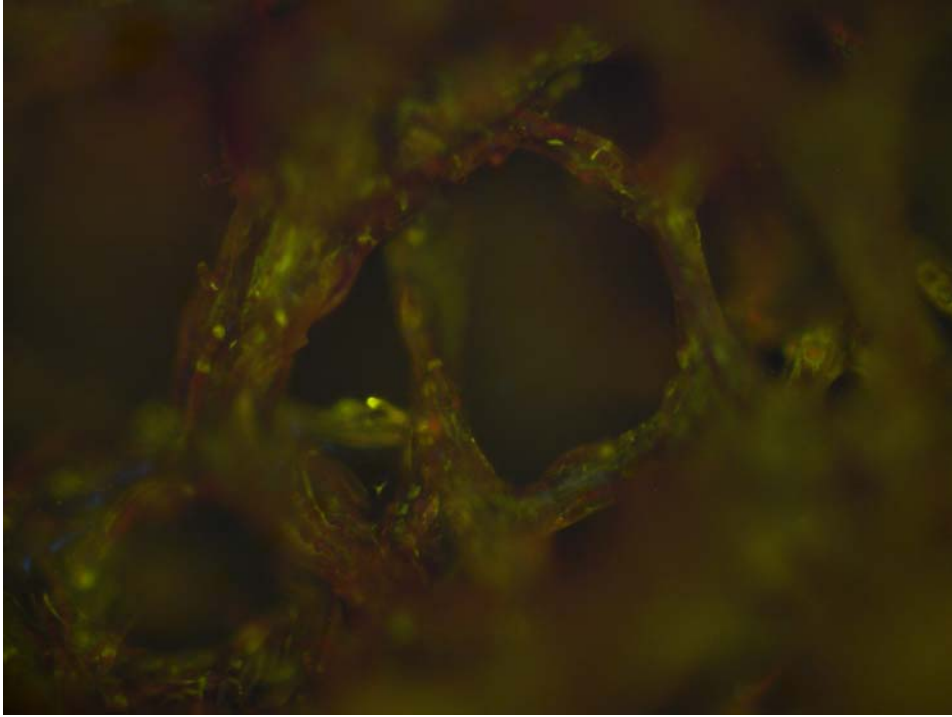


Figure 25: S 9/29/05 Blue excitation 100x.



Figure 26: S 9/29/05 UV excitation 100x.

5.5 Nostoc 9/29/05



Figure 27: N 9/29/05

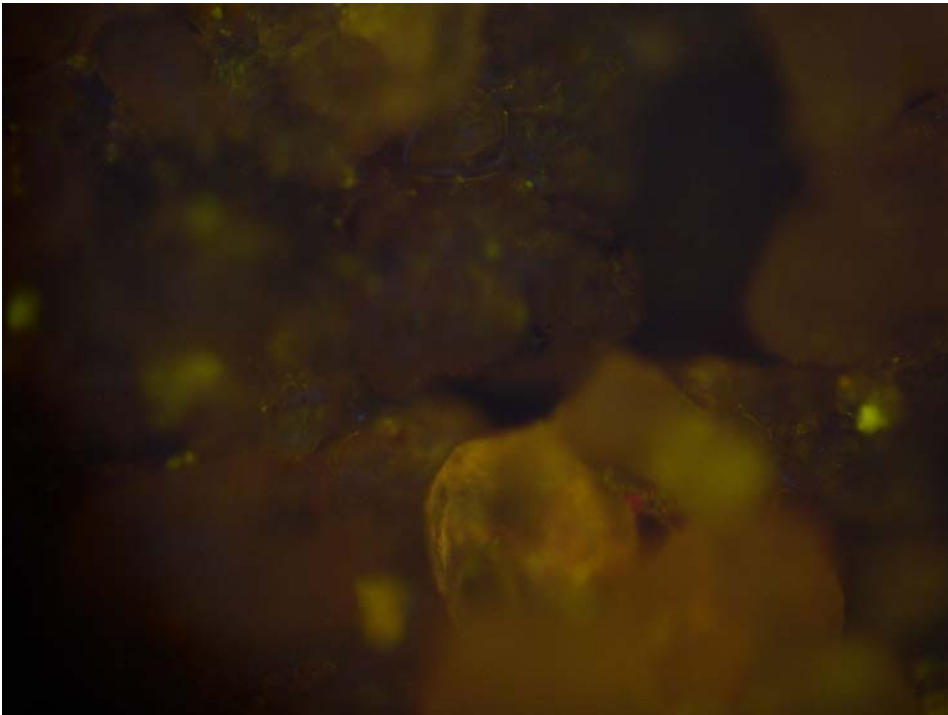


Figure 28: N 9/29/05 Blue excitation 100x

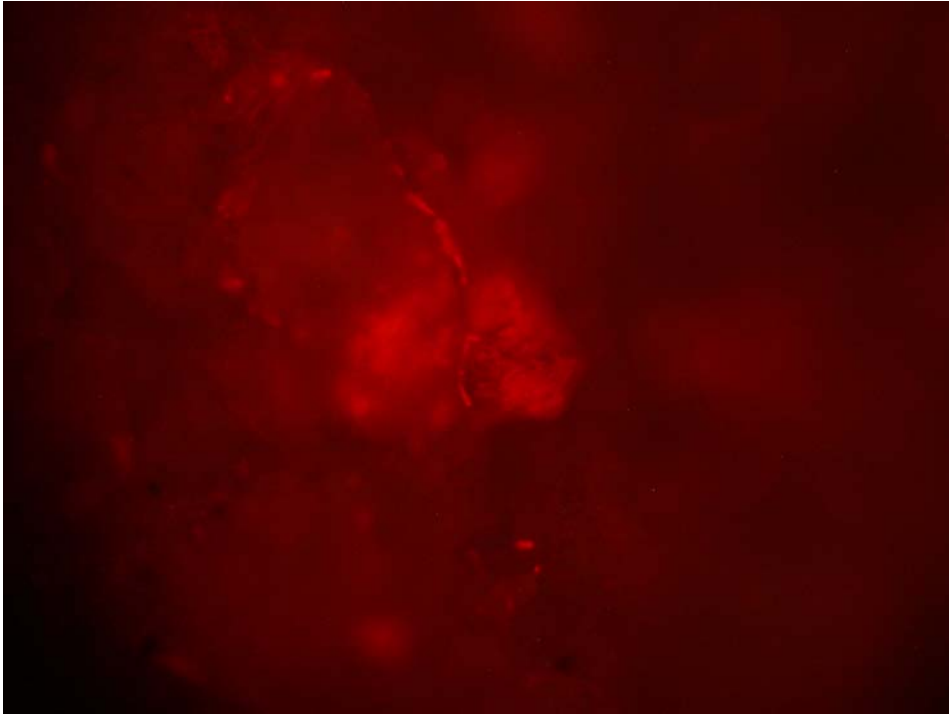


Figure 29: N 9/29/05 Green excitation 100x.

5.6 Microcoleus and Nostoc 9/23/05



Figure 30: MN 9/23/05

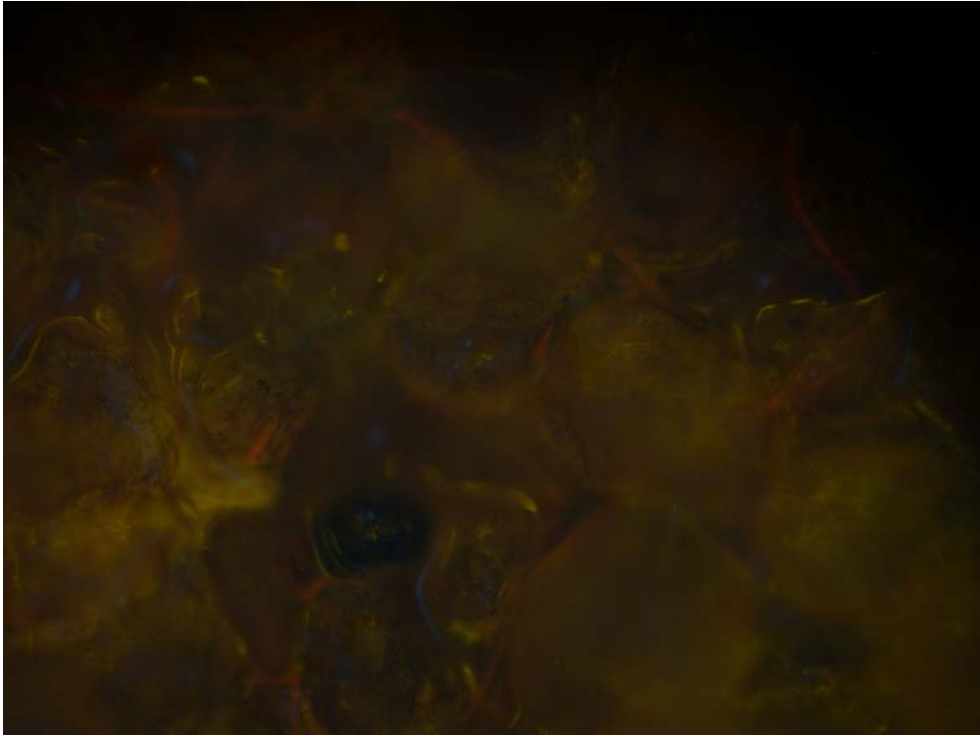


Figure 31: MN 9/23/05 Blue excitation 100x.

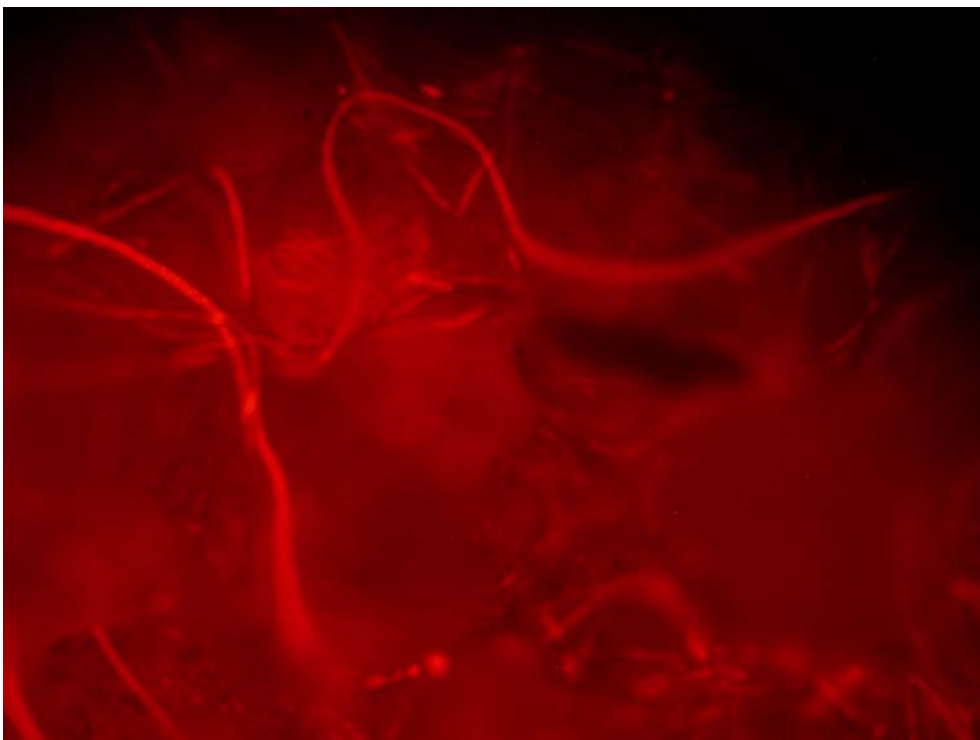


Figure 32: MN 9/23/05 Green excitation 100x

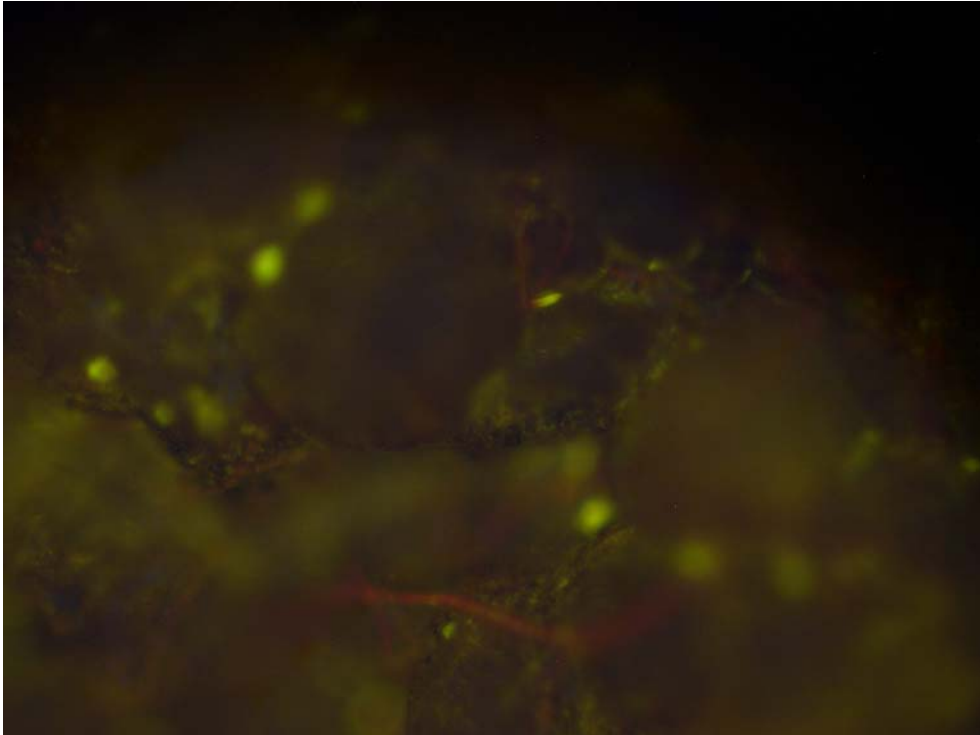


Figure 33: MN 9/23/05 Blue excitation 100x.

5.7 *Microcoleus* and *Scytonema* 9/29/05



Figure 34: MS 9/29/05

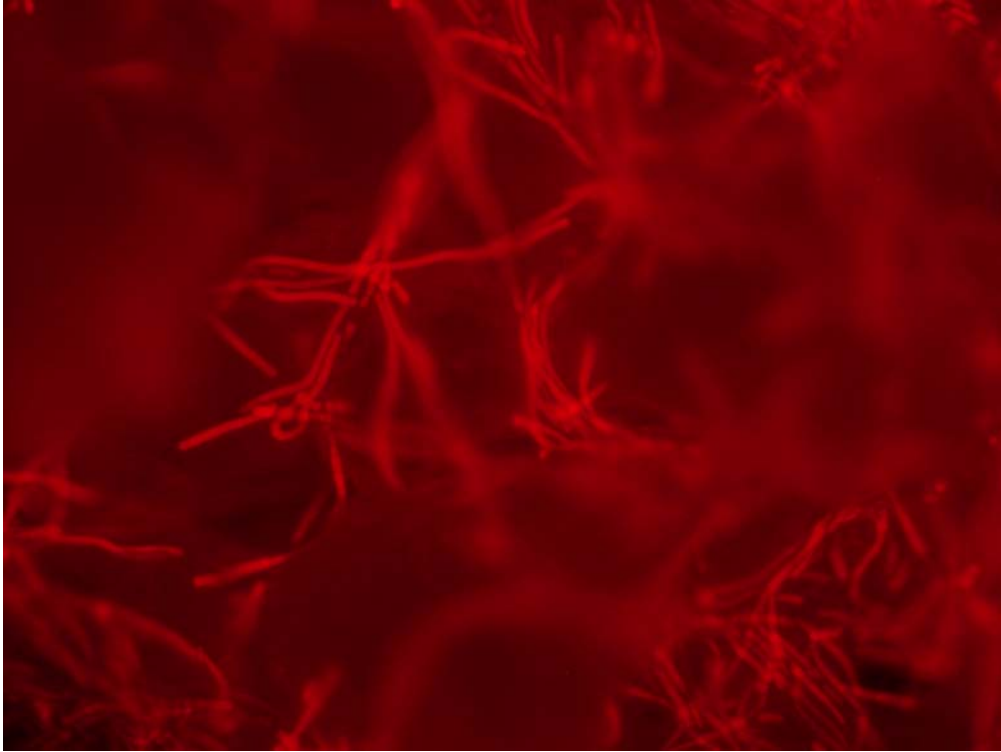


Figure 35: MS 9/29/05 Green excitation 100x.



Figure 36: MS 9/29/05 Green excitation 100x.

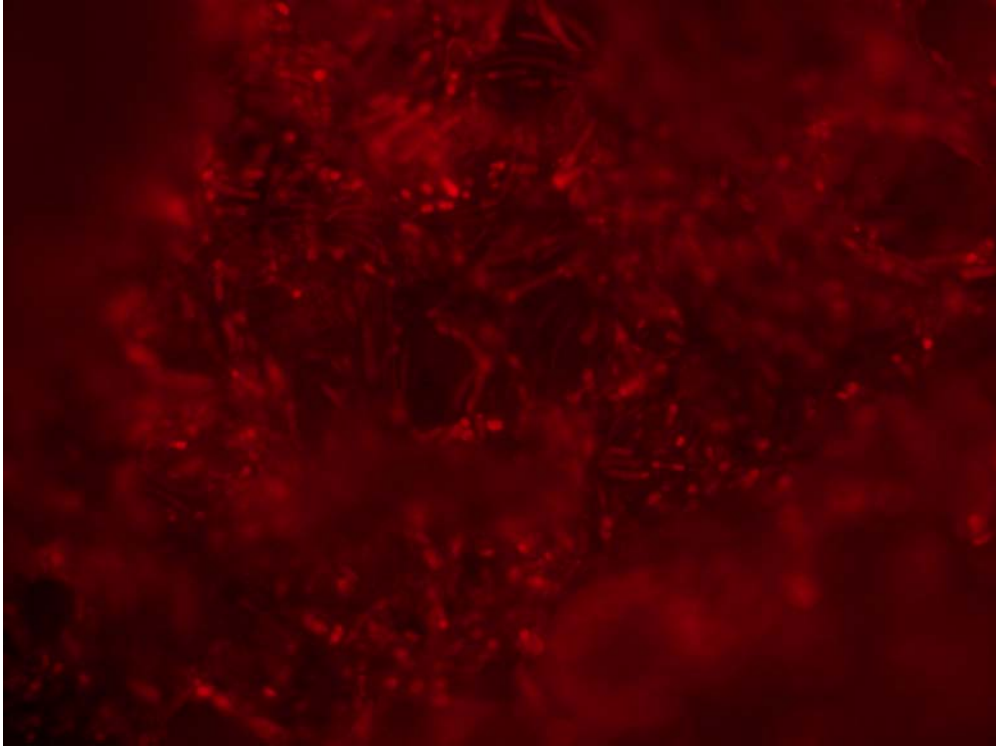


Figure 37: MS 9/29/05 Green excitation 100x.

5.8 *Microcoleus* and *Scytonema* 1/31/06



Figure 38: MS 1/31/06

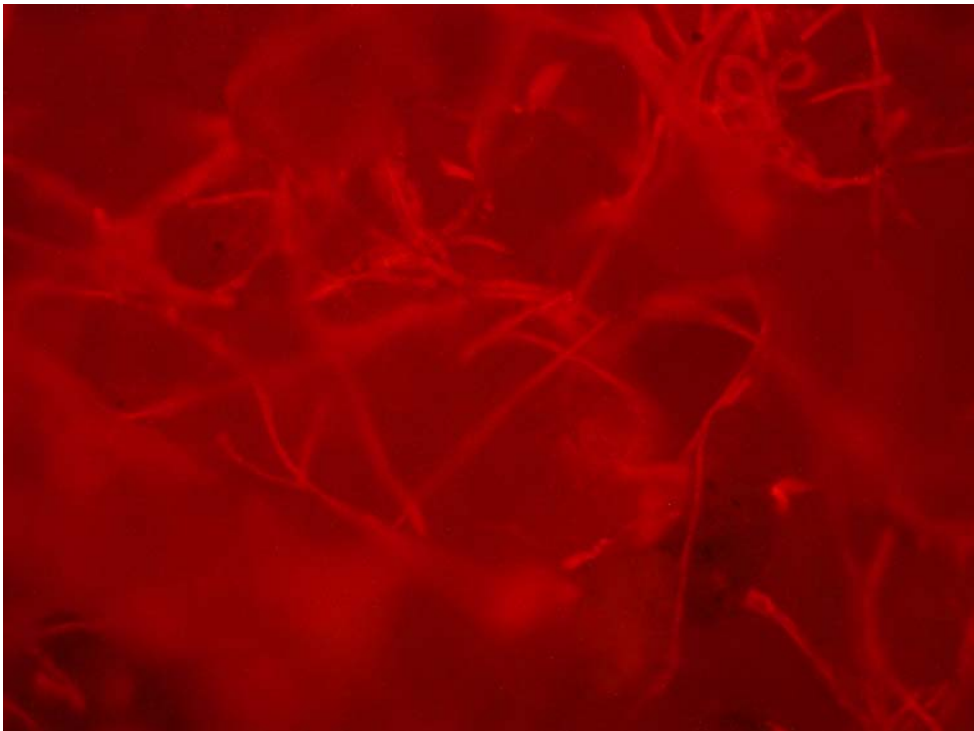


Figure 39: MS 1/31/06 Green excitation 100x.

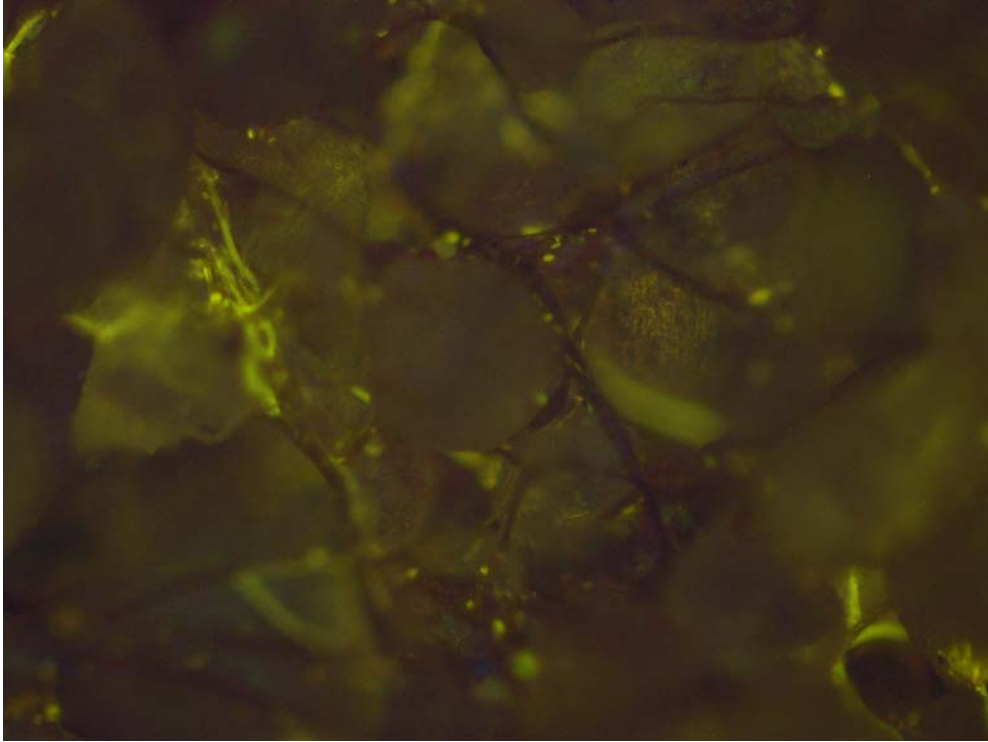


Figure 40: MS 1/31/06 Blue excitation 100x.

5.9 Microcoleus, Nostoc and Scytonema 9/23/05



Figure 41: MNS 9/23/05

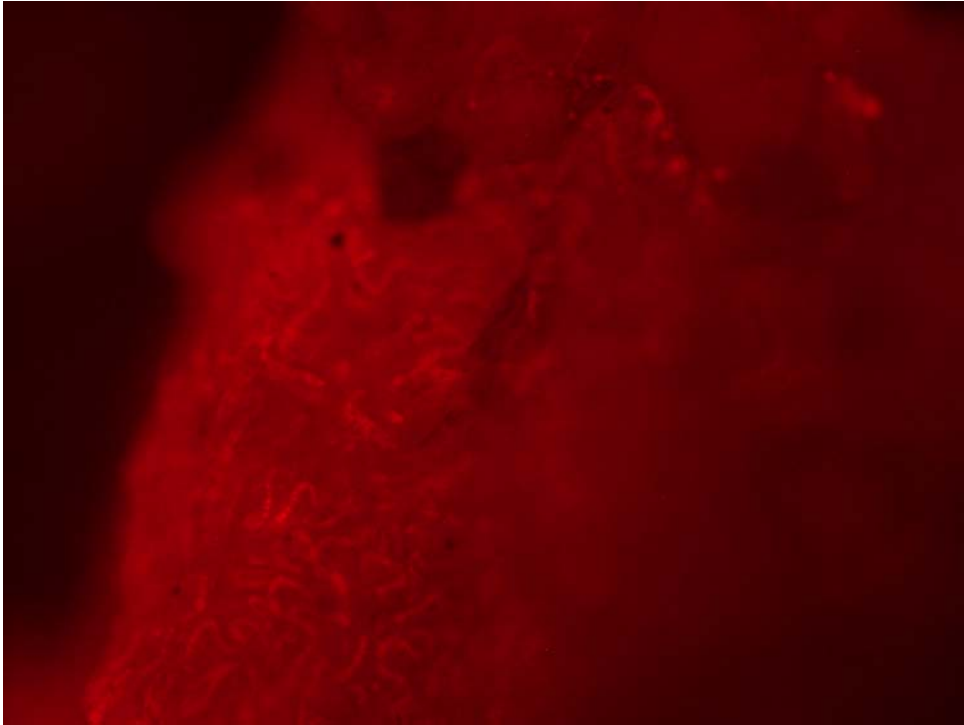


Figure 42: MNS 9/23/05 Green excitation 100x.

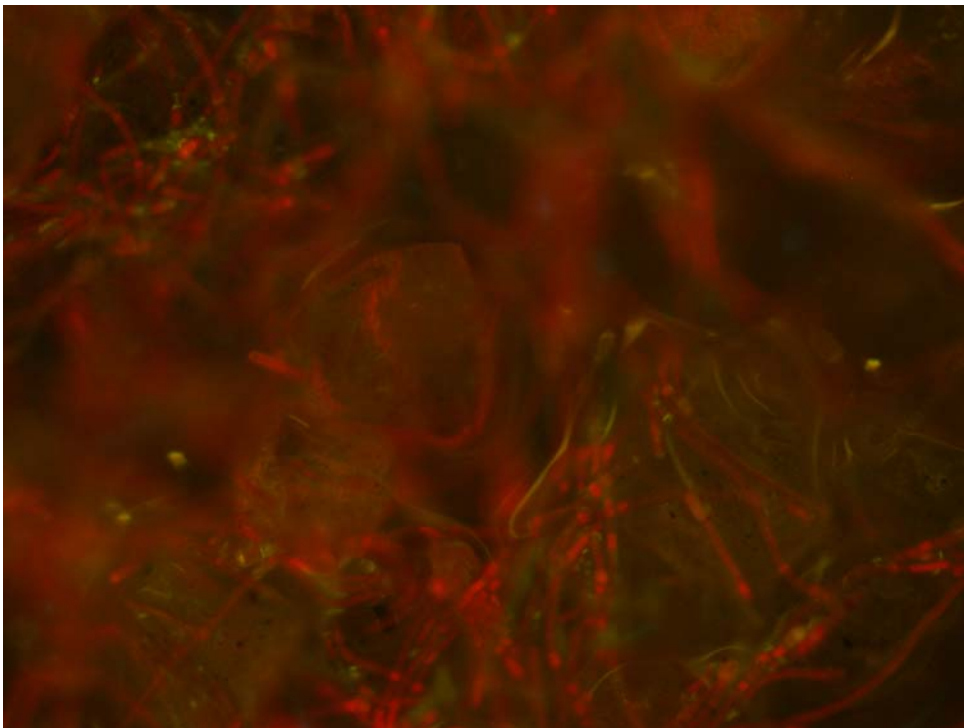


Figure 43: MNS 9/23/05 Blue excitation 100x.

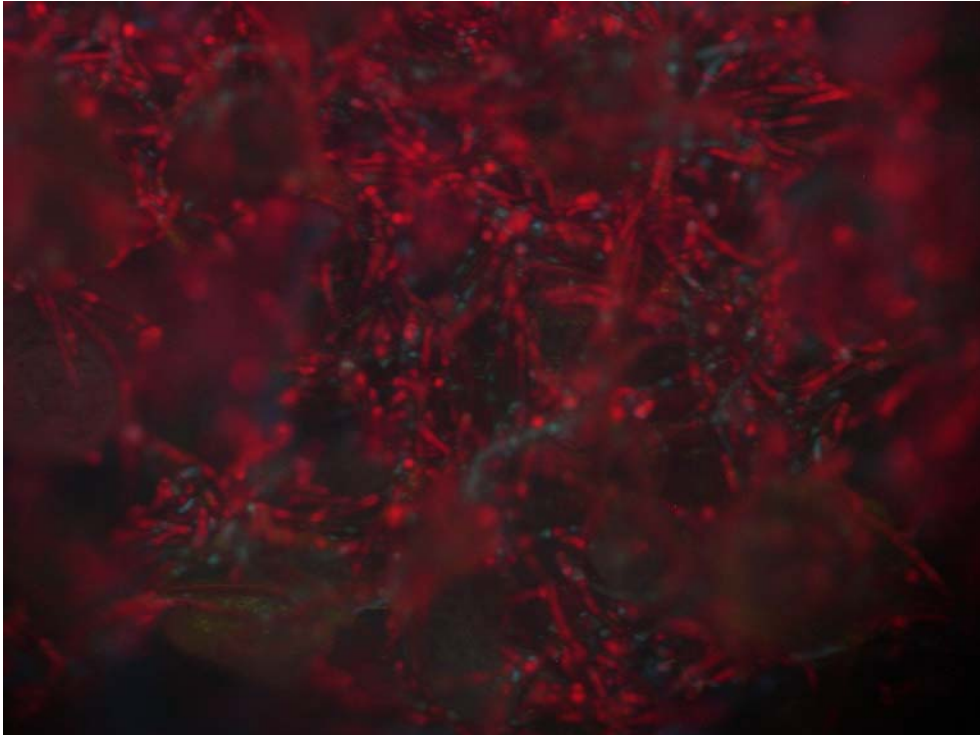


Figure 44: MNS 9/23/05 UV excitation 100x.

5.10 Microcoleus, Nostoc and Scytonema 9/29/05



Figure 45: MNS 9/29/05

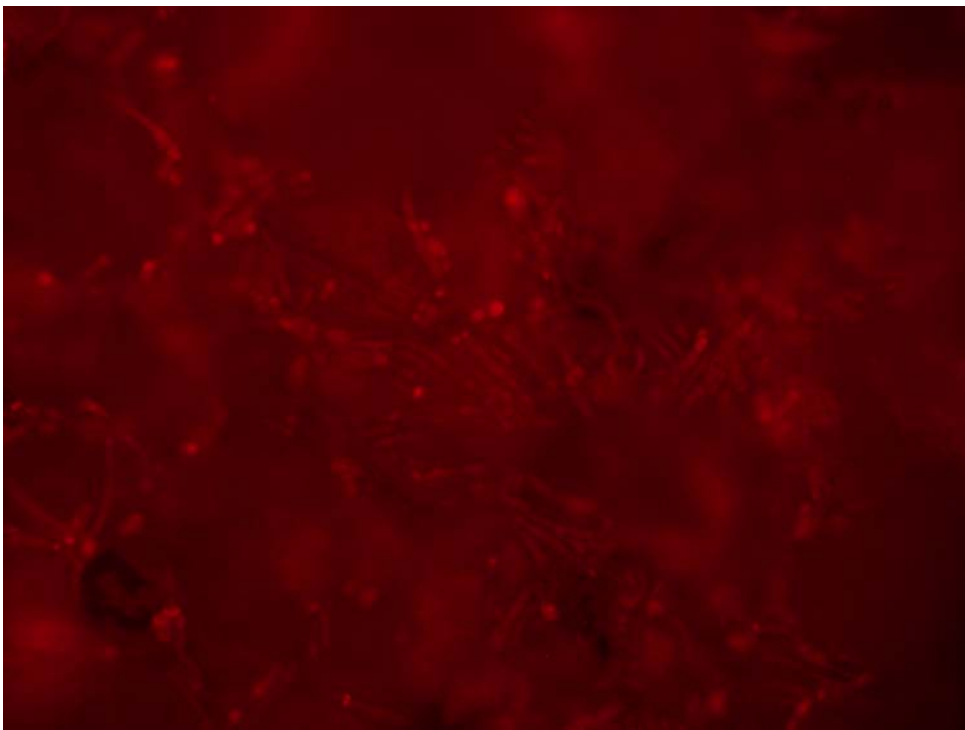


Figure 46: MNS 9/29/05 Green excitation 100x.

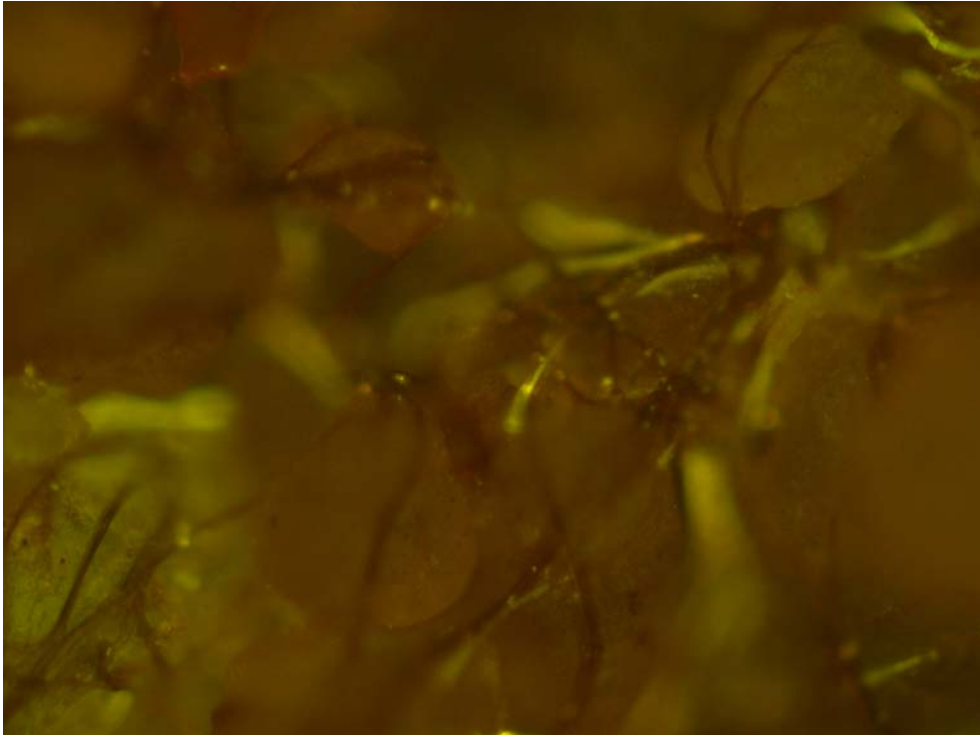


Figure 47: MNS 9/29/05 Blue excitation 100x.



Figure 48: MNS 9/29/05 UV excitation 100x

5.11 Microcoleus, Nostoc and Scytonema 11/15/05



Figure 49: MNS 11/15/05, after watering.

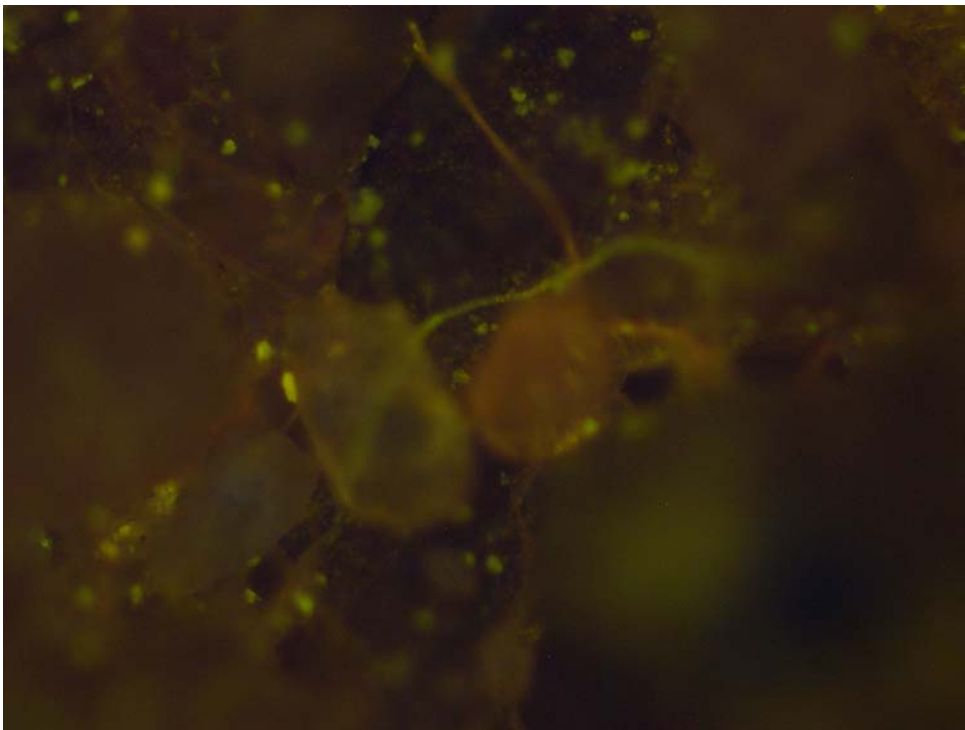


Figure 50: MNS 11/15/05 Blue excitation 100x.

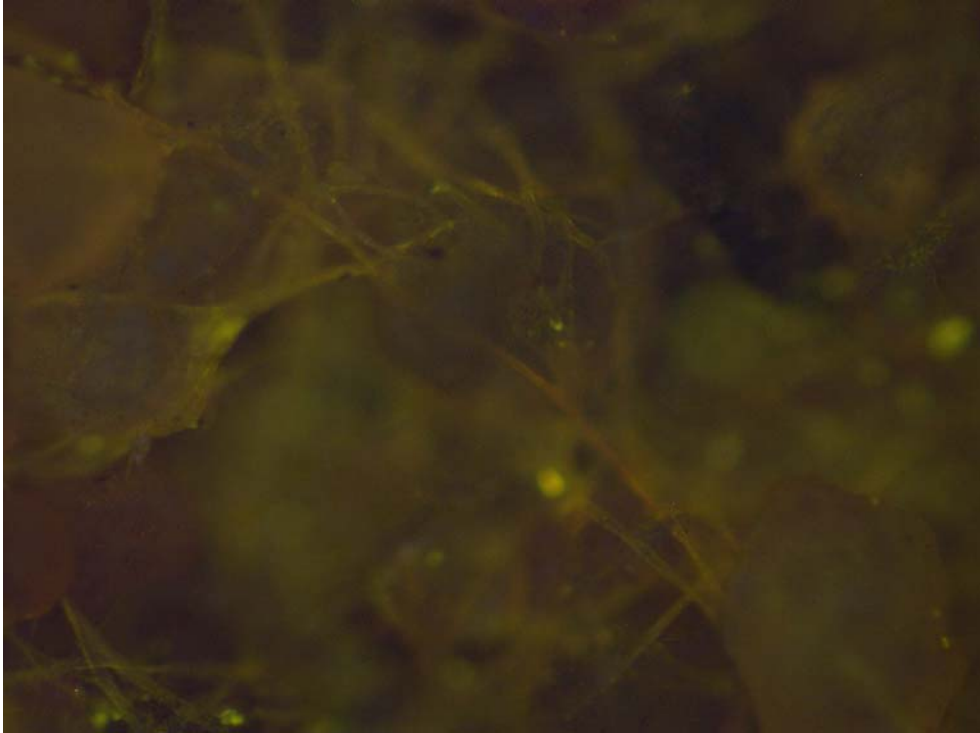


Figure 51: MNS 11/15/05 Blue excitation 100x

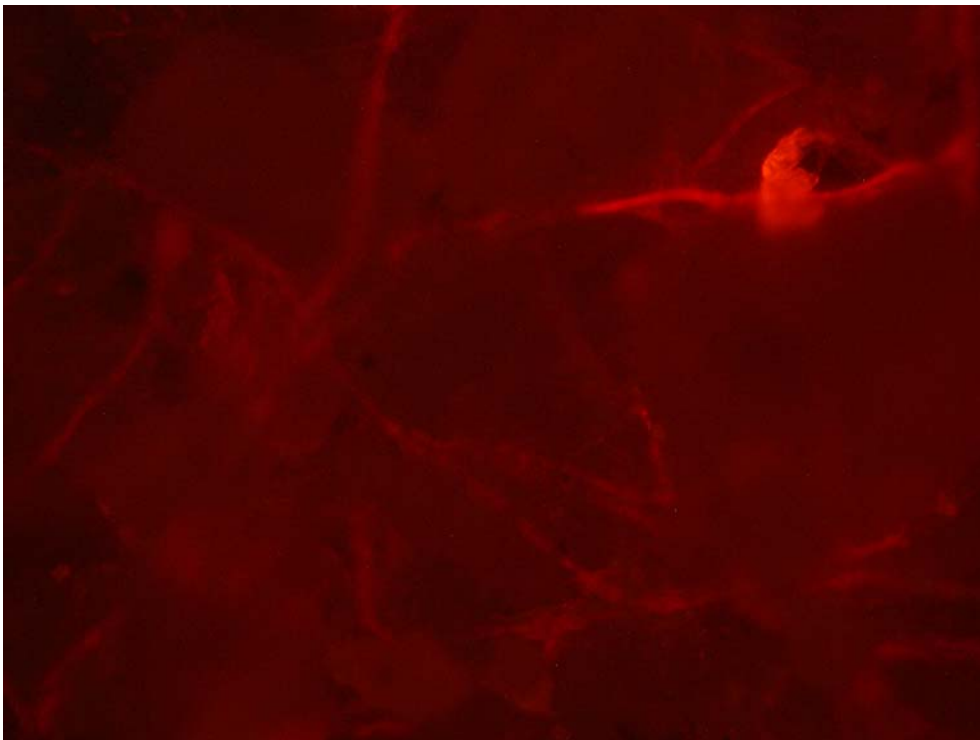


Figure 52: MNS 11/15/05 Green excitation 100x.

5.12 Microcoleus, Nostoc and Scytonema 1/31/06



Figure 53: MNS 1/31/06

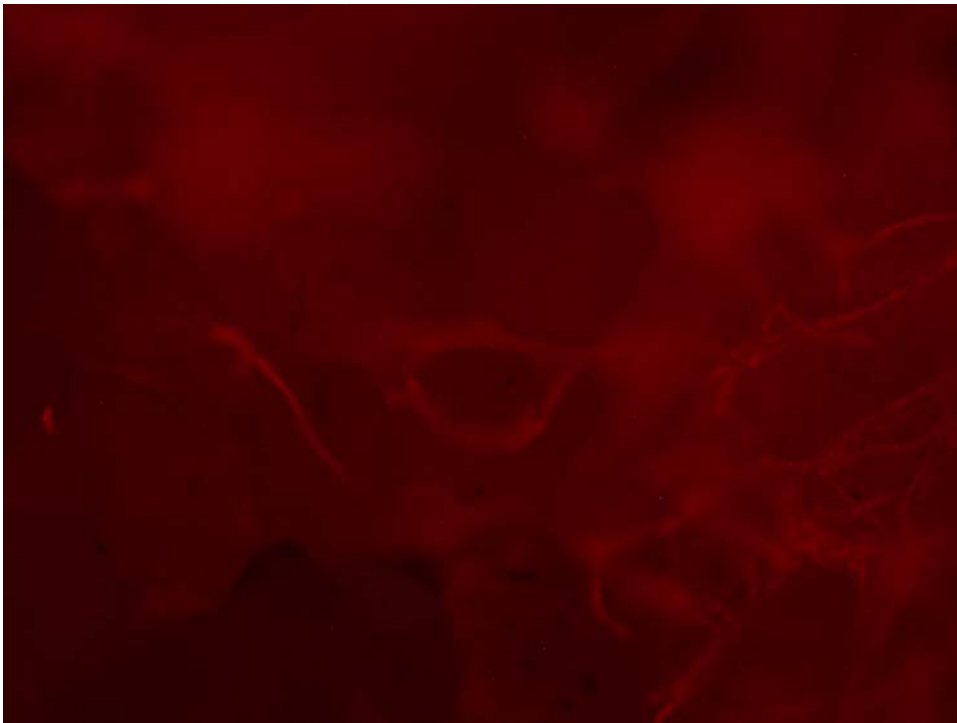


Figure 54: MNS 1/31/06 Green excitation 100x.

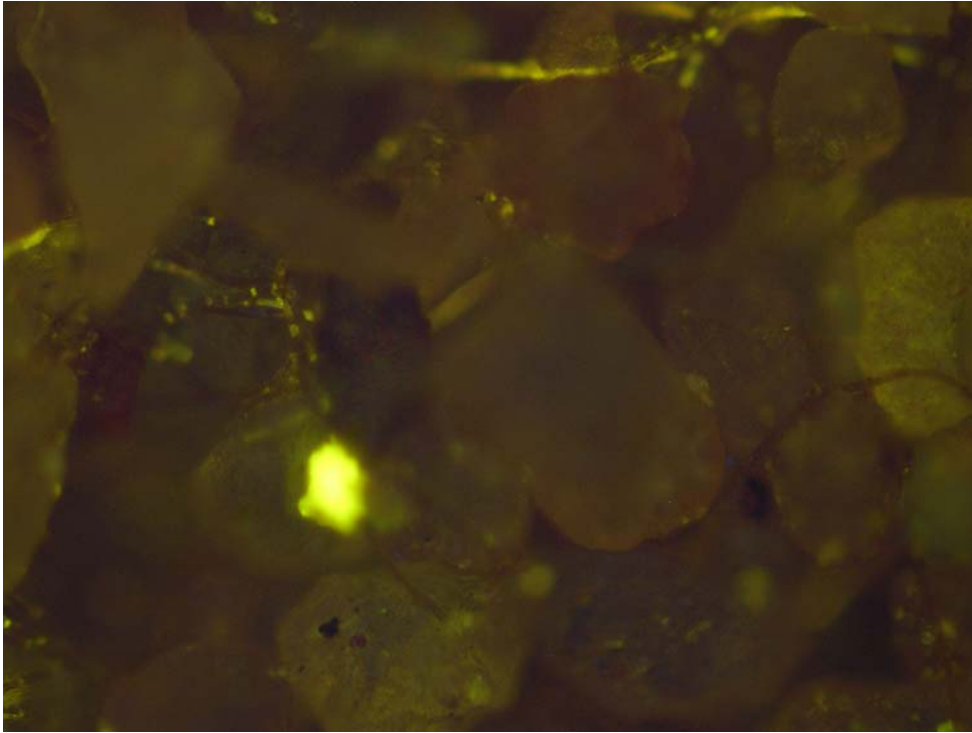


Figure 55: MNS 1/31/06 Blue excitation 100x

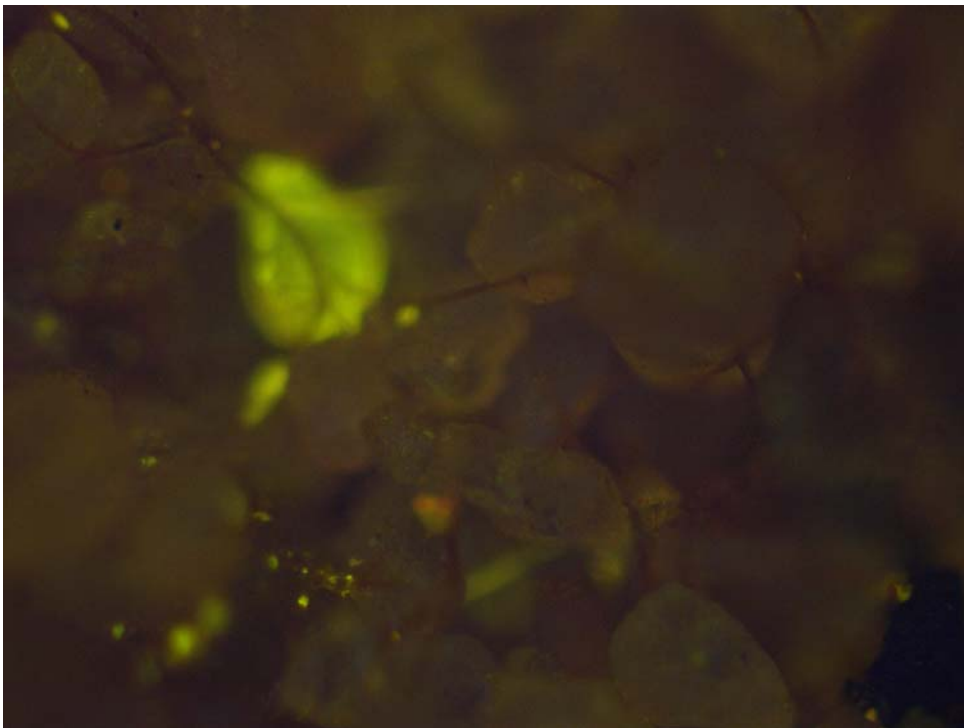


Figure 56: MNS 1/31/06 Blue excitation 100x.

5.13 Plates with UV protection



Figure 57: *Microcoleus* on the left, MNS on the right



Figure 58: *Scytonema* on the left, *Nostoc* on the right

6 Discussion

Although significant growth was observed in the plates that were cultured, not all combinations of cyanobacteria were as successful as could be hoped. *Microcoleus*, for instance, when grown individually, exhibited little to no growth in early plates, as the literature would suggest (Bowker, Reed, Belnap 2002). A subsequent plate of *Microcoleus* was created, although there was still little visible growth, as can be seen in Figures 20 and 21. The plates that had solely *Nostoc* and *Scytonema* both exhibited growth, yet Figure 22 (*Scytonema*) and Figure 27 (*Nostoc*) show clearly how neither of these species is capable of stabilizing the underlying soil. *Nostoc* was the most difficult of the three microorganisms to photograph clearly using the epifluorescence microscope – a bit of *Nostoc*'s distinctive shape can be seen in Figure 42, but otherwise it was elusive in the pictures of more than one cyanobacteria. It can be conjectured that *Nostoc* did not respond to the excitation spectra of the epifluorescence microscope that were used. The edges of *Scytonema* curl so dramatically that only a portion of the crust is touching the soil underneath when the crust is dry (Figure 22, 58). The crusts that seemed to be the most successful were those that combined all three species of cyanobacteria and these seemed, from microscopic observation (Figure 44), to come closest to displaying the depth and complexity of the Wild crust samples (Figures 11-14, 16-18). It was also clear from observation that the Wild crust samples were significantly more developed and complex than the lab grown crusts, a feature which can be determined by observing the many strands of filamentous cyanobacteria intertwined with one another (Figures 11-14, 16-18). It is not clear from this microscopy the scope of the developmental difference between the Wild crust samples and the lab grown samples, as their exact relationship in terms of age was not known at the start. However, it is possible to observe developmental

differences corresponding with age in the crust samples that were lab grown. For instance: the plates including all three cyanobacteria species that were started at different times show increased growth corresponding with increased age, Figure 46 displays the oldest, which is significantly more complex than either Figures 52 or 54, the younger crust samples.

6.1 Potential Difficulties and Future Studies

One of the things that was changed most dramatically in the environment in which lab cultured crusts were grown versus the development of wild cryptobiotic crusts is the aseptic environment in which they are kept. As wild crusts have no protection from the elements, it is highly likely that they come into contact with bacteria on a constant basis. This might even be the reason why some of them have developed antibacterial properties (El-Sheekh *et al.* 2006; Matern *et al.* 2003). By growing crusts in an aseptic laboratory environment, opportunities for natural development are restricted.

Another aspect of laboratory crust growth which might become problematic is the accelerated growth rate due to the increase of precipitation events per year. If the ultimate goal of study of these cyanobacteria is to reintroduce them into areas where wild crusts have been destroyed, the crusts with an accelerated growth rate might not be able to adapt, given the scope of time it takes for wild crusts to develop (Bhatnagar and Bhatnagar 2005). This would be an excellent topic for a future study involving laboratory grown crusts.

Given that it is impossible to recreate the full experience of a wild crust's desert environment in a laboratory setting, it must be considered that lab grown crusts can never

be an exact replica of their wild counterparts. However, future study using lab grown crusts could be used to examine these differences in more detail.

6.2 Recommendations for Future Studies

Although the unique properties of each species of cyanobacteria can be observed when they are cultured and grown as individual crusts, for future studies, it is recommended that all three of these species, *Microcoleus*, *Nostoc*, and *Scytonema* be grown in conjunction with one another. Clearly from the work here, cultures of only one or two species cannot be used to emulate their growth in the natural environment.

Since it has been established that cryptobiotic crusts grow by the addition of fine layers of soil particles to the upper surface (Hu, Liu *et al.* 2002), it can be seen as a challenge for future studies to design a viable method of reintroduction to areas with destroyed crusts.

Future study might also address the extent to which the growth of these cryptobiotic crusts can be accelerated through frequent precipitation events. A different type of crust may be grown utilizing a variety of other species of cyanobacteria that occur in natural cryptobiotic crusts.

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