CONTROL OF BIODETERIORATION OF SANDSTONE ON THE FISHER FINE ARTS LIBRARY

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To my parents who taught me that nothing was impossible,
encouraged me to follow my dreams,
and supported me in every aspect of my life.
Dad, I wish you could be here to see this dream become a reality.

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Chapter 1. Introduction

1.1 Overview of the Problem

The Fisher Fine Arts Library, on the campus of the University of Pennsylvania, was designed by the Philadelphia firm of Furness, Evans & Co. to serve as the university's main library. The library was constructed between 1888 and 1890 in the Gothic style with Romanesque elements. The apse of the building, located on the north side of the structure, is mostly faced with rough-cut sandstone blocks. The lower courses suffer heavy biocolonization, which is an aesthetical problem while also contributing to the deterioration to the stone. As there is a complete record of past treatments that have been carried out on the building, this will allow to determine when biocolonization first became obvious and to analyze whether and when previous campaigns to eradicate it were carried out.

Biodeterioration of stone is a global phenomenon that plagues monuments and historic sites across the world in a vast array of climates. The concern of biocolonization on stone is more than an aesthetic issue, because it can lead and contribute to the deterioration of the stone. The biocolonization found on the apse can be broadly classified into two groups by their visual appearance: the green or the black colored microorganisms. These are mostly bacteria, fungi and algae and are the precursors for the development of higher organisms, such as lichens, mosses, etc. which may induce even greater deterioration. Therefore is it desirable to control biocolonization at this level. Various means can be used to achieve this end, such as reducing the amount of water that reaches the stone or through the use of biocides.

1.2 Aim of the Study

The aim of this thesis is to test the effectiveness of two commercially available biocides in reducing or eliminating the biocolonization present on the stone. Since two visually distinct groups of colonizing microorganisms can be seen, these will be tested separately with each of the two biocides. For the evaluation of the effectiveness of the two biocides, apart from visual examination and the corresponding photography, a thermal imager will be tested using a simple technique of wetting the stone to determine how the presence of biocolonization affects the absorption/evaporation of water. Changes in water absorption by means of RILEM tube testing will be used to determine changes between the treated and untreated stones.

A large number of studies have been carried out to address the treatment of stone with various types of biocides in order to evaluate their effectiveness. Most of the evaluation methods require sophisticated instrumentation not readily available to architectural conservators. Therefore, this thesis explores the possibility of using a thermal imager following a study where it was used for the evaluation of the effectiveness of water repellent treatments.[1] Since this instrumentation was readily available at the university it was considered an opportunity to test it for evaluating biocides since it is generally use for the inspection of historic buildings.

Documentation on the past history of the Fisher Fine Arts Library was carried out to find out when and how the exterior of the building was cleaned and cared for in the past. This will provide a possible timeline for when the biocolonization currently on the building may have started to grow. The sandstone that was used on the building will be studied to determine its nature, porosity characteristics, and mineralogical composition,

[1] Antonio Sansonetti, M. Casati, and E. Rosin. "Contribution of IR Thermography to the Performance Evaluation of Water Repellent Treatments." *Restoration of Buildings and Monuments* 18.1 (2012): 13-22.

which will provide information on why and how it supports biocolonization and how much this can deteriorate the stone.

A review of the literature on biocolonization of stone helps to understand the deterioration problem. This is complemented with a review of various types of biocides available and their effectiveness. Focus will be given to the two biocides that are to being used in this study, D/2 and BioWash, both of which are US approved for use on buildings.

This thesis will provide new insight into the effectiveness of the two biocides tested on the two types of biocolonizers, green or black, treatment based on thermal imaging. From the results obtained, supplemented with the data pulled from the cleaning records of the apse, suggestions for improved maintenance program for this important part of the building will be suggested.

Chapter 2. Historical Background of Fisher Fine Arts Library

Figure 2.1: University of Pennsylvania Library, 1894. (R. Newell & Sons, University Archives Digital Image Collection)

2.1 Building History

In 1870 the University of Pennsylvania relocated its campus from Center City Philadelphia to the current location in West Philadelphia. During the first two decades at the new campus, the University Library was located in a large and lofty room in College Hall.[2] As the university continued to expand, the library was serving nearly 150 faculty and more than one thousand students in 1885.[3] "Valuable gifts of books could not be even unpacked, but had to be stored by the thousand in garret or cellar."[4] Larger accommodations were urgently needed to properly house the expanding holdings of the

[2] *Proceedings at the Opening of the Library of the University of Pennsylvania 7th of February 1891* (Philadelphia: University of Pennsylvania Press, 1891) http://www.library.upenn.edu/exhibits/pennhistory/library/opening/opening.html

[3] Roger W. Moss, and Tom Crane. *Historic Landmarks of Philadelphia*. (Philadelphia: University of Pennsylvania, 2008) 214

[4] *Proceedings at the Opening of the Library of the University of Pennsylvania*

university's library collection. In response to this situation, Provost William Pepper assembled a committee, headed by Horace Howard Furness, a faculty member of the university, to plan a new library. The Trustees agreed to "erect a building which, while supplying the needs of a Library for a century to come, would afford accommodations also, temporarily at all events, for the fast-growing collections of American, Assyrian, and Egyptian Archaeology."[5]

Horace Howard Furness recommended that his brother, Frank Furness, be the architect for the new library building. Frank Furness' buildings combined the High Victorian Gothic with references to the modern industrial age of his time that characterized his own eclectic style. His buildings were often dramatically over scaled and boldly articulated a variety of forms and materials. Many of his ideas about ornament and pleasuring the senses come from John Ruskin, while the bold geometric and structural expressions were taken from Viollet-le-Duc.[6] Most notably, Furness designed his buildings in a unique personal style that used modern materials in forms that reflected their function.

When designing the University of Pennsylvania Library, Furness took into account the traditional elements of library design to develop a rationally based plan that strictly adhered to the needs of books, staff and readers, in keeping with his principle of function guiding the design.[7] The design for the library also paid homage to the historical and current character of Philadelphia. The fiery red exterior of the building pays tribute to the city's traditional brick building past, while noting the city's current industrial dominance in the buildings overall form. According to Edward Bosley, "the new library was a conflation of towers, chimneys, skylighted rooms and foundry-like clerestoried

[5] Ibid.
[6] Mark Gelernter, *A History of American Architecture: Buildings in Their Cultural and Technological Context*. (Hanover, NH: University of New England, 2001) 173
[7] Edward R. Bosley, *University of Pennsylvania Library: Frank Furness*. (London: Phaidon, 1996.) 12.

halls whose closest sources were the factories of Philadelphia."[8] Furness consulted with two of the leading library theoreticians, Justin Winsor and Melvin Dewey, while designing the library.

During the late nineteenth century there were three approaches to institutional library design that were commonly used. The first approach was the ecclesiastical-styled, high-ceilinged reading room, lined with continuous tall book stacks where the reading floor would be lit by either clearstory windows or glass skylights. The second approach consisted of alcoves surrounding a main reading room where each alcove was dedicated to a particular subject. The third library plan consisted of connected reading rooms lined with shelves of books that were easy to reach.[9] Similar to the eclectic style used for the building, Furness fashioned a hybrid of these three approaches while including some of his own ideas to create a new design that was highly functional to all who would be using the library. The main reading room of the library, located in the center of the apse, reflects the ecclesiastical-styled approach, resembling a cathedral with its high ceiling, clearstory windows, and rounded apse. The alcove approach is present in the apse which is lined with separate alcoves that house part of the library's collection. Furness designed a new approach for the book stacks within the library, where the stacks were separate from the main reading room in their own wing. The stacks were designed so that the building could expand to accommodate the library's growing collection and were located in a wing off the south side of the building with a glass ceiling that provided light to the stacks.

The building is constructed out of timber and iron that is clad in brick, red sandstone, decorative terracotta, and Spanish-style ceramic roof tiles. Ground was broken for the new library in August of 1888 and the cornerstone was laid in a masonic ceremony on October 15, 1888. The building took about two years to complete and

[8] Ibid. 4
[9] Ibid, 11-12

during the summer of 1890 most of the books were transferred to the new library. On February 7, 1891 the library was officially dedicated as the Library of the University of Pennsylvania (Figure 2.1). The Duhring Memorial book stacks were added onto the southern wing of the library in 1914-1916. In 1924 construction was completed for the Henry Charles Lea Library and Reading Room along the east elevation of the building. The Horace Howard Furness Memorial Library was the last addition to it in 1931. This final addition was added to the front of the book stacks, west elevation, and is now the Arthur Ross Gallery.

Alterations were also made to the landscape surrounding the library. When the library was completed it rested atop a small hill, elevated above 34th Street (Figure 2.2). Grass surrounded the library with dirt or gravel paths connecting it to other buildings on campus. Images from the 1950's show that shrubs were planted along the apse (Figure 2.3), though exactly when they were planted and subsequently removed has not been recorded. At some point the grass around the building was replaced with brick and the land around the north and west side of the apse was leveled.

Figure 2.2: University of Pennsylvania Library, ca. 1891, 34th Street side. (University Archives Digital Image Collection)

Figure 2.3: University of Pennsylvania Library, ca. 1956, with shrubs along the apse. (Lillian G. Burns, University Archives Digital Image Collection).

At the time it was built, the original design of the library was applauded as functional architecture. However, aesthetically the building went out of fashion almost immediately. Stylistically, the building was not appreciated again until the middle of the twentieth century. At least twice, there were plans to tear the building down; the most recent in 1960, but apparently only the lack of funds prevented this from happening.[10] Once again the university's library collection expanded beyond the capacity of the building where it was housed, and in 1962 the Van Pelt Library was built becaming the new library for the university while the Furness building was turned over to the Graduate School of Fine Arts and was renamed the Furness Library. The building was listed in the National Register of Historic Places in 1972 and was declared a National Historic Landmark in 1985. Between 1987 and 1990 an extensive restoration of the building was carried out and the building was renamed the Fisher Fine Arts Library at a dedication ceremony celebrating its centennial in 1991.

[10] Leslie Mooney, *Frank Furness' Library Building for The University of Pennsylvania, 1891*, (Chapel Hill: 1988) 30

Figure 2.4: Fisher Fine Arts Library, 2011. (J. Focht)

2.2 Past Interventions

For nearly 100 years the exterior of the Fisher Fine Arts Library (Figure 2.4) was exposed to the elements with no intervention to protect its materials. Starting in 1987 the building underwent a full scale renovation under the direction of the firm Venturi, Rauch, Scott Brown, Clio Group, Inc., and Marianna Thomas Architects. The efforts of the restoration mainly focused on the original part of the library and work on the exterior included cleaning, repointing, masonry unit replacement, window repair and replacement, and new roofing systems. According to the report that was produced from this restoration, roof and flashing leaks appeared to be the principal sources of deterioration for the brick, terra cotta, and sandstone walls.[11]

The base of the building, portico and free-standing piers are rusticated Pictou Sandstone, which is the focus of this thesis. The sandstone is coursed with most blocks

[11] Venturi, Rauch, and Scott Brown. *A Master Plan for the Selective Restoration and Continued Use of the Furness Building University of Pennsylvania*. Vol. 3. Building Conditions (Philadelphia: Venturi, Raunch, and Scott Brown, 1986.)

being face bedded and some are naturally bedded. The report from 1986 indicates that the deterioration of the sandstone is due to the presence of moisture within the wall stating:

> The wet/dry and freeze/thaw cycles of Philadelphia's climate are a major source of problems. Black deposits on the stone probably date from the era of coal heat. Spalling stones appear red, as the surfaces with the black deposits have shaled off. The cleaner the surface appears, the more advanced the deterioration. Deterioration apparently started soon after construction.[12]

The stones which are face bedded tend to split along their natural bedding planes causing them to detach from the stone surface and fall off. Through testing it was determined that the black deposit was only on the surface of the sandstone and was carbonaceous in nature and very tenaciously bound to the grains of the stone. Prior to cleaning the entire surface of the building testing was carried out to determine the best product to use on the surface of the stone. A one to one mixture of hydroflouric and phosphoric acid as well as the SureKlean Heavy Duty Restoration Cleaner were both tested on small areas. After testing, the SureKlean Heavy Duty Restoration Cleaner 994 was recommended to be used at full strength in two applications to clean the surface of the sandstone.

The following notes and recommendations were given in regards to the exterior stone surfaces.

- Spalling: install rusticated Dutchman repairs of sandstone (Nova Scotia Pictou or English Corsehill Red Sandstone to match). Basement window jambs are assumed to be repaired thus.
- Shaling: rub surface of sandstone to remove shaling material, in order to leave sound material exposed. Roughly six or seven brackets are affected.
- Treat all spalled stone which remain using specified sandstone consolidation materials and techniques (Wacker stone strengtheners).
- Replace existing concrete coated steps with new cast stone steps.

[12] Ibid.

- Remove existing stone base at location of new door and salvage stone for Dutchman repairs.
- Replace existing exposed portion of rubble stone foundation wall with new cast stone veneer at areas where grade has been lowered. [13]

In 2005 John Milner Architects were hired by the university to do an exterior condition assessment of the Fisher Fine Arts library. During their assessment it was determined that the acidic solution used to remove atmospheric staining and biological growth during the 1980's restoration appears to have had no adverse effects on the sandstone as a whole. The report states that it is difficult to ascertain the performance of the alkoxysilane consolidant that was used, but it appears that there was a long-term ameliorating effect from its application.[14] In 2005, the general condition of the sandstone included open joints, cracking, atmospheric soiling, efflorescence, deteriorating composite patches, biological growth (heavy in areas), and isolated cases of corroded ferrous hardware attachments. However, the most pressing condition with the sandstone is the varying degrees of active deterioration.[15]

Since this 2005 report, the sandstone on the Fisher Fine Arts Library continues to deteriorate. At least, half of the sandstone blocks are covered by biological growth with large areas having moderate to heavy colonization. Portions of the face bedded sandstone continue to detach from the building compromising the rusticated surface of the sandstone that was part of Furness's decoration incorporated into the building design.

[13] Ibid.
[14] John Milner Architects, INC. *Exterior Condition Assessment, Anne & Jerome Fisher Fine Arts Library, Arthur Ross Gallery, & Duhring Wing*. (Chadds Ford: John Milner Architects, 2005) 9
[15] Ibid. 10

Chapter 3. The Nature of Biocolonization and its Deterioration of Stone

3.1 Introduction

The study of biocolonization and the effect that it has on cultural heritage is a widespread topic that requires a multidisciplinary approach. Biocolonization can be found on a large variety of materials, both organic and inorganic, and has the potential to be destructive to any substrate on which it develops. The presence of biocolonization is not only an issue of aesthetics, but one of deterioration, because microorganisms can alter a material both physically and chemically.

Microorganisms have been present for millennia and play an essential role in the overall ecological balance of the earth. The decomposition of stone is considered normal and even desirable in a natural setting, but in the human environment biodeterioration of monuments, buildings, statues, and grave markers is regarded as a serious problem.[16] The control and prevention of biocolonization on stone has received widespread interest in the field of conservation because a vast number of cultural heritage and monuments are composed of stone.

Conservators have been studying the effect of biocolonization on historic stone for several decades, investigating what conditions are necessary for it to develop and grow, how it deteriorates stone, the best way to mitigate biocolonization, and how to keep microorganisms from recolonizing stone that has been cleaned of it. In general, microbiologists that study biocolonization only look at the biological aspect and do not consider or report how biocolonization interacts with the substrate that it is attached to. Material scientists are the other discipline that study biocolonization, but they study how it affects the material that it is growing on. However, they are not trained to characterize the species of the microorganism(s) that is causing the biodeterioration.

[16] Larry L. St. Clair, and M. R. D. Seaward. *Biodeterioration of Stone Surfaces: Lichens and Biofilms as Weathering Agents of Rocks and Cultural Heritage*. (Dordrecht: Kluwer Academic, 2004). 2

Initial, research into the control and prevention of biocolonization of stone was restricted. Though researchers working to address the complex issues regarding the way biocolonization occurs and the deterioration that it can potentially cause, there was a limited interdisciplinary exchange in studies published in journals and conferences. Nonetheless, these publications are valuable tools for conservators since they include literature reviews, analytical methods used as well as the most recent results from experimental studies.

Collaboration between conservators, microbiologists, and material scientists began through international conferences that brought the various disciplines and specialists together to discuss their shared interest in the preservation of monuments. These conferences provide a forum where the different disciplines can come together to discuss the topic of biocolonization so as to identify the existing gaps between disciplines and determine what direction the study of the control and prevention of biocolonization of cultural heritage should follow.

Biology in the Conservation of Works of Art[17] was one of the first specific books published on biodeterioration of cultural heritage. It provided information about the different types of microorganisms, the various materials that are affected and how they are altered, the contributing factors of growth, and preventive methods. It was designed specifically for conservators without a background in biology and is useful for scientists who do not have an understanding of conservation. This book was an important step forward in the field of biocolonization and biodeterioration because it started to bridge the gap between the different disciplines involved in preservation of cultural heritage by using the same terminology, combining knowledge, and sharing analytical methods.

[17] Giulia Caneva, Maria Pia Nugari, and Ornella Salvadori. *Biology in the Conservation of Works of Art*. (Rome: ICCROM, 1991).

Plant Biology for Cultural Heritage[18] expands on this base of knowledge to include analytical methods and information from a wider variety of sources. *Cultural Heritage and Aerobiology: Methods and Measurement Techniques for Biodeterioration Monitoring*[19] provides a general overview of the application of aerobiology to the conservation of cultural heritage. It explains how to estimate the risks that airborne microorganisms pose to the biodeterioration of artifacts and suggests methods and techniques for aerobiological monitoring. It also lists the microclimatic conditions that attract airborne microorganisms enabling the planning of preventive interventions. A useful aspect is its listing of the most common microorganisms present on cultural heritage describing what conditions they need to grow and how they deteriorate their substrates.

In 2009 the Smithsonian Museum Conservation Institute hosted a workshop entitled, "Biocolonization of Stone: Control and Preventive Methods." Through presentations and discussions the following areas were identified as needing to be further explored. Future research is to be laid out in the field to control and prevent biocolonization while finding a way to mitigate biodeterioration.

Once biocolonization is established, and as it continues to grow, it deteriorates the surface of the stone, breaking it down and changing its properties, permitting it to be more susceptible to other deterioration mechanisms. In order to understand the process of biodeterioration, the conditions under which it will develop needs to be considered. The following questions need to be answered in order to understand biocolonization. What is biocolonization? Under what conditions does it develop? How does biocolonization originate and grow? What kind of damage can it induce? Once these questions are answered the approach for controlling biocolonization can be studied.

[18] Guilia Caneva, M. P. Nugari, and O. Salvadori, eds. *Plant Biology for Cultural Heritage: Biodeterioration and Conservation*. Trans. Helen Gainville. (Los Angeles: Getty Conservation Institute, 2008).

[19] Paolo Mandrioli, Giulia Caneva, and C. Sabbioni, eds. *Cultural Heritage and Aerobiology: Methods and Measurement Techniques for Biodeterioration Monitoring*. (Dordrecht: Kluwer Academic, 2003).

3.2 Biocolonization Mechanism

Biocolonization results from the colonization of a surface by a single species of microorganism or, more frequently, by a community of them.[20] Initial colonization of stone is carried out by microorganisms such as bacteria, cyanobacteria, algae, fungi and lichen. The material surfaces, especially those that exposed to the outdoor environment, become colonized by microorganisms that are normally present in the air, together with dust, and other biological particles, such as fungal and bacterial spores, lichen propagules, algal cells, and pollen grains. These can be deposited on the surface of buildings and other structures and colonization will only occur under favorable environmental and surface conditions.[21]

The most important environmental factors that affect the establishment and growth of biocolonization are water, temperature, and light. The amount of water available will determine what type of microorganisms will colonize a surface and the speed at which growth will occur. Humidity can also affect the growth of microorganisms because some microorganisms can use atmospheric moisture as their water source. An abundance of biocolonization can indicate that there is a continuous supply of water that the microorganisms have access too. Water is perhaps the most important factor contributing to the growth and survival of microorganisms. Light is the main nutritional source for photosynthesizing microorganisms, such as algae. The developments of microorganisms is affected by the quality, quantity, and duration of light that they receive. The effect that temperature has on microorganisms is largely related to its influence on the chemical-physical properties of water, which is the main component of biological structures. For example, below freezing temperatures will cause water to

[20] Ornella Salvadori and A. Elena Charola, "Methods to Prevent Biocolonization and Recolonization: An Overview of Current Research for Architectural and Archaeological Heritage" in *Biocolonization of Stone: Control and Preventive Methods*, ed. A. Elena Charola et al. (Washington D.C.: Smithsonian Institution Scholarly Press, 2011), 42.

[21] Mandrioli *et al,. Cultural Heritage and Aerobiology,* 22,23

expand as it torns into ice, rupturing the biological membranes and causing the cell to die.[22]

The surface condition of the substrate can also contribute to the sustainability of microbial life. Porosity, roughness, and chemical composition of the substrate are all factors that come into play when it comes to biocolonization. A substrate with high porosity is more susceptible to germination and development of microorganisms than a smoother one because it can retain more moisture while facilitating the physical establishment for microorganism.[23] The various chemical components of the substrate, such as mineral or salts can provide possible nutrients for the microorganisms.

The microorganisms attach themselves to the surface of the stone, i.e., the substrate, by forming a biofilm which begins with nonspecific reversible reactions that are dependent on the physical and chemical properties of both the cells and the substrate.[24] The biofilm results from the secretion of extracellular polysaccharide substances (EPS) by the microorganism which will enclose and shield the community from desiccation and other environmental factors (Figure 3.1). EPS is composed of carbohydrates, proteins, nucleic acids, lipids/phospholipids, and humic acids forming a hydrogel that contains about 98% water. Within the biofilm microorganisms form microcolonies that are separated by interstitial voids which allows for the circulation of interstitial fluids and nutrients between empty spaces. Physically, the formation of the biofilm is important for the activation and development of alteration processes because it is within the biofilm that retention of fluids and an accumulation of aggressive metabolic compounds takes place. Because of this mechanism, biofilms are able to maintain an environment that can be drastically different from its surrounding environment in terms of pH and chemical composition which can provide better conditions for various microorganisms to

[22] Caneva *et al., Plant Biology.* 44
[23] Ornella Salvadori and A. Elena Charola, "Methods to Prevent Biocolonization and Recolonization", 46.
[24] Caneva *et al*., *Plant Biology,* 18-19.

survive.[25] Biofilms serve as interface micro-habitats that differ from those of the ambient environment.

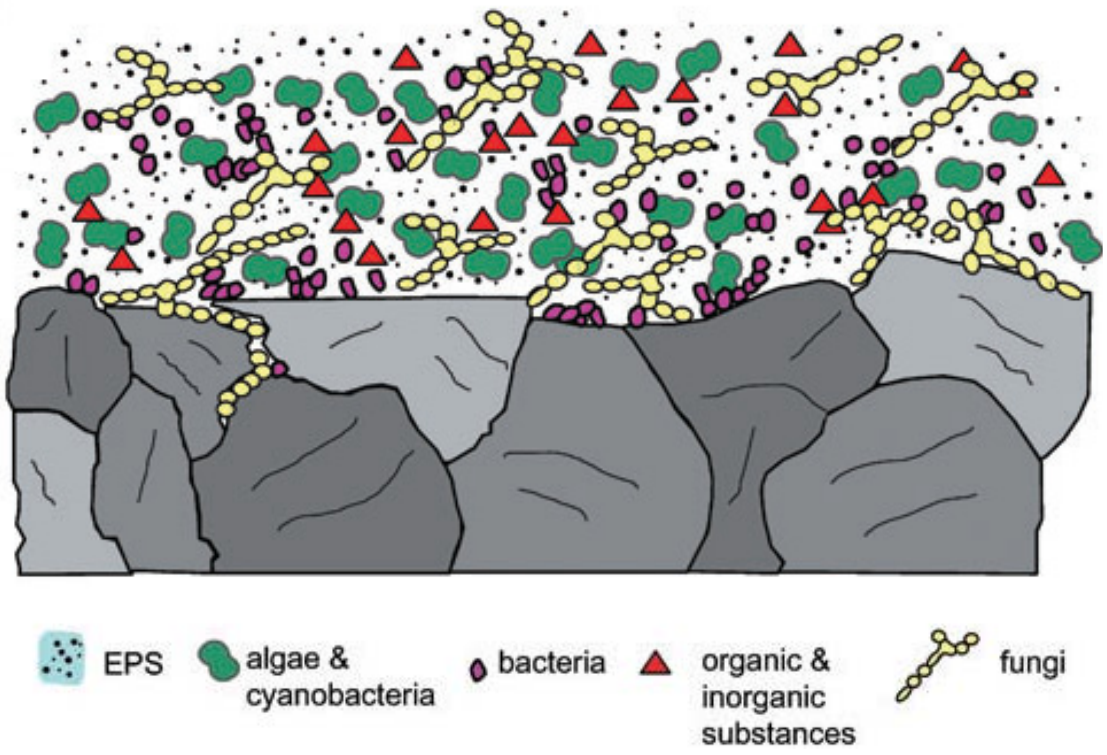

Figure 3.1: Microorganisms embedded in a biofilm. (Gorbushina, "Life on the Rocks")

3.3 Classification of Microorganisms

Microorganisms can divided into two categories, autotrophs and heterotrophs, depending on their source of nutrient. Autotrophic microorganisms are able to manufacture their own source of nourishment because they can synthesize organic molecules through specific metabolic reaction and only rely on the substrate for their support. Among these autothrophic microorganisms are some types of bacteria, algae, lichens, and mosses, that are able to colonize inorganic materials such as stone. Heterotrophic microorganisms must extract organic materials from the substrate in order to survive and therefore are generally found on organic materials. However, they can

[25] Ibid, 19.

also colonize inorganic materials already colonized by autotrophs, using them as their food source. Heterotrophic microorganisms are various bacteria and fungi.[26]

Bacteria are a group of prokaryotic unicellular organisms without a nucleus that exist in three shapes, spherical, rodlike, and spiral. They easily develop on outdoor stone structures and monuments because of their simple ecological and nutritional needs, requiring mineral salts and microelements for their growth and oxygen for respiration. Bacteria exist in both autotrophic and heterotrophic forms. Autotrophic bacteria include, sulfur oxidizing bacteria, nitrifying bacteria, iron bacteria, and hydrogen bacteria. Nitrifying bacteria are known to be among the first colonizers of stone and grow only on specific substrates that contain ammonia and nitrous acid.[27] Heterotrophic bacteria can be cellulolytic bacteria, amylolytic bacteria, lipolytic bacteria, denitrifying bacteria, and actinomycetes. These bacteria normally only colonize organic materials, but can be found on stone if there is organic material present to support them.

Fungi are a group of chemoheterotrophic organisms that are characterized by unicellular or multicellular filamentous hyphae.[28] Hyphae originate from the germination of the fungal spore and are shaped like a narrow tube with a diameter of 2-12 μm. The body of the hyphae forms the mycelium, giving rise to the colony called thallus.[29] Fungi have ridge cell walls that consist of polysaccharides, such as chitin, and lipids, amino sugars, and proteins. They require a source of organic carbon and some essential nutrients, such as nitrogen, phosphorus, potassium, and other mineral salts for their growth. Fungi can adapt to a variety of environment conditions. However, they cannot colonize stone surfaces unless some organic food source is present.

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[26] Mandrioli *et al,. Cultural Heritage and Aerobiology,* 5.
[27] Ibid. 151, 152.
[28] Rakesh Kumar, and Anuradha V. Kumar. *Biodeterioration of Stone in Tropical Environments: An Overview*. (Los Angeles: Getty Conservation Institute, 1999). 16.
[29] Caneva *et al,. Plant Biology,* 65, 66.

Algae and cyanobacteria are photoautotrophic organisms that are devoid of tissues and organs (Figure 3.2). Algae consist of a diverse group of eukaryotic organisms that can be unicellular or multicellular which contain pigments such as chlorophyll, carotenoids, and xanthophylls.[30] Depending on the type of pigment present, algae can appear in a variety of colors, green, gray, black, orange, yellow, brown, red or violet. Cyanobacteria are prokaryotic organisms which are actually bacteria, but they are commonly referred to as blue-green algae, where each cell is surrounded by a gelatinous pigmented sheath that provides color to the cell and allows for rapid absorption and the slow release of moisture allowing the microorganism to survive in adverse environmental conditions, such as persistent desiccation.[31] The color of cyanobacteria can be golden yellow, brown, red, emerald green, dark blue, violet, and azure.

Figure 3.2: Green biocolonization, mostly algae and some lichens on the Fisher Fine Arts Library, 2012. (J. Focht)

[30] Kumar and Kumer, *Biodeterioration of Stone in Tropical Environments*, 18.
[31] Ibid, 12-13.

Algae and cyanobacteria can also be classified with regard to what part of the stone substrate they colonize. Epilithic algae grow on the exposed surface of the stone while endolithic algae colonize the interior. There are three types of endolithic algae; chasmoendolithic which live in fissures and cavities that are open to the surface, cryptoendolithic which colonize structural cavities within porous substrates, and euendolithic which actively penetrate into the substrate.[32] Algae prefer substrates with the following conditions; damp, warmth, light, and inorganic nutrients.

Lichens are autotrophic organisms that are made up of a vegetative body, resulting from a symbiotic relationship between a fungus and algae and/or cyanobacteria (photobiont) (Figure 3.3). The photobiont carries out the synthesis of carbohydrates while the fungus stores water, mineral salts, and mineral and organic nitrogen while protecting the photobiont from environmental stress.[33] Lichens are composed of a thallus, the vegetative body, which generally develops on the surface of the stone, and

Figure 3.3: Fisher Fine Arts Library, 2012. 1. Lichens. 2. Moss. (J. Focht)

[32] Caneva *et al*. *Plant Biology,* 71.
[33] Mandrioli *et al*., *Cultural Heritage and Aerobiology,* 163.

rhizines or hyphae, which anchor into the substrate. Lichens are typically divided into crustose, folious, and epilithic types, where the thallus is on the surface of the stone, and endolithic, when the thallus is mostly inside the stone.[34] These microorganisms show a remarkable tolerance for environmental stresses, occurring in a wide range of habitats, and are able to grow on most substrates, including glass, plastic, and metals, but are most frequently found on rocks. Together with cyanobacteria they play an important role as pioneer organisms in colonizing rocks.

Mosses are bryophytes and represent a bridge between primitive plants without tissues or organs and evolved plants with differentiated tissues and organs (Figure 3.3). They are simple photoautotrophic organisms that contain pigments and possess rudimentary root-like organs, rhizoids, but no vascular tissues or transport organs.[35] Mosses can be found on the surface of stone and in open cavities and cracks. They usually grow in shaded places that are permanently or frequently wet and frequently occur in association with algae.

When studying the formation of microorganisms on the surface of rocks or stones the term subaerial biofilm (SAB) is used. On rock or stone surfaces microorganisms rarely grow as colonies of single species, rather they form communities that derive their survival success from a collective growth habit. SAB are composed of heterogeneous matrices of microorganisms that are held together and bound to the surface of the stone by EPS.[36] The microorganisms spread and colonize the stone in ways that are characteristic of the various microorganisms that compose the biofilm. Since stones are composed of mineral grains, cementing material and pores, the biofilm tends to spread between the mineral grains filling pores and intergranular spaces causing the biofilm to be more network-like so that the SAB results patchy (Figure 3.4).

[34] Tamara Anson Cartwright, *et al.*, *Illustrated Glossary on Stone Deterioration Patterns*. (Paris: ICOMOS ISCS, 2008). 68.
[35] Kumar and Kumer, *Biodeterioration of Stone in Tropical Environments*, 23.
[36] Anna A. Gorbushina, "Life on the Rocks." *Environmental Microbiology* 9.7 (2007): 1614

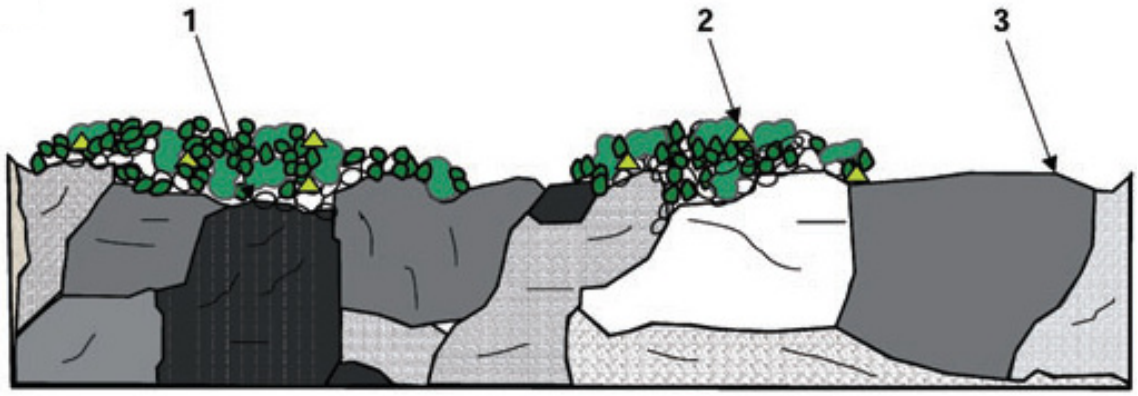

Figure 3.4: Interaction between substrate, microorganisms, and atmosphere in a SAB. 1. inter-organism interaction, 2. biofilm - atmosphere interaction, 3. atmosphere - substrate interaction. (Gorbushina, "Life on the Rocks")

As mentioned above, one of the main functions of a biofilm is the protection of the microorganisms that constitutes it. The surface of stones can be a harsh environment. According to Gorbushina,[37] the surface temperature of rocks can range from -45°C to +60°C. The availability of water may fluctuate from long periods of drought to times when the biofilm is covered by a film of water. Solar irradiation levels range from relatively low radiation doses at night to extremely high levels of infrared and ultraviolet radiation on summer days. Not only do those microorganisms that colonize stone surfaces experience daily changes but they also experience annual and more irregular fluctuations of temperature, humidity, and intense radiation. The other main function of EPS is the retention of water. EPS in SAB does not only protect from the diffusion of water, it also spares microorganisms from water stresses. It does that by:

1. "Retaining water for long periods;
2. Maintaining the viability of the cells, and;
3. Facilitating access to water vapor in the atmosphere."[38]

Because of the way that biofilms interact and form a protective barrier between the atmospheric conditions, the network of microorganisms, and the substrate, the biofilm behaves like a living organism. They differentiate, evolve and replicate.[39]

[37] Ibid.
[38] Ibid. 1615.
[39] Ibid.

3.4 Biodeterioration Mechanism

The deterioration of stone occurs through a complex interaction of physical, chemical and biological weathering. Clarification of the role of microorganisms in the overall deterioration process may further our understanding of weathering of stone in natural environments. According to H. J. Hueck the definition of biodeterioration is "any undesirable change in the properties of a material caused by the vital activities of organisms."[40] Various types of mechanisms result in the biodeterioration of stone. Physical or mechanical processes lead to the loss of cohesion, rupturing, or disaggregation. Chemical processes lead to the transformation, degradation or decomposition of the stone. Physical and chemical processes caused by microorganisms usually occur simultaneously; however, one type can predominate over another depending on the substrate, the biotic community, and the environmental conditions. When referring to physical and chemical alterations caused by microorganisms the terms biophysical and biochemical deterioration should be used,

Biophysical deterioration of stone can occur due to the pressure exerted on the substrate during the growth or movement of microorganisms. The attachment devices, such as hyphae and rhizines, penetrate into the stone through preexisting cracks and crevices causing mechanical stress to the substrate that can result in the loss of cohesion of mineral grains and can lead to disaggregation. As microorganisms expand and contract following the cycling of moist and dry periods, they can exert a considerable amount of force on the stone substrate, which through time will eventually loosen mineral grains.[41] The growth of endolithic microorganisms can result in the detachment and lifting of scales from the stone surface. The presence of colored patinas formed by microorganisms can be another source of physical stress on the substrate. These patinas can induce a rise in temperature, a change in thermohydric expansion, and

[40] Caneva *et al.*, *Plant Biology,* 15.
[41] Kumar and Kumar, *Biodeterioration of Stone in Tropical Environments,* 15.

increase the water retention resulting in the loss of material.[42] Biophysical deterioration generally may fragment the stone surface, and the increased surface area allows other deterioration agents, such as rain, wind, freeze-thaw cycles and pollutants, as well as other microorganisms, to further degrade the stone.

Biochemical deterioration of stone is a result of chemical alterations due to the effects of the metabolic processes of the microorganisms that are present. The chemical transformation of the substrate can be caused by the excretion of intermediate metabolic products or as the results of assimilatory processes and their production of extracellular enzymes, which result from microorganisms using the substrate for nutritional purposes.[43] The principle process of biochemical deterioration is the production of: inorganic and organic acids, CO_2, alkalis, enzymes, pigments, chelating agents, cationic exchanges, and selective mobilization and/or accumulation of elements.[44] Chemical alterations of the stone caused by the interaction of microorganisms with the substrate can lead to physical deterioration. Acids can decompose some minerals producing salts, and chelation of elements may introduce changes in the stone pore system resulting in the formation of cracks. They may also precipitate and concentrate new compounds on the stone surface creating a crust.

The microorganisms that are commonly found on stone and the damage they cause to the substrate are listed in Table 3.1 Biological Alteration of Stone. This table was taken from *Cultural Heritage and Aerobiology: Methods and Measurement Techniques for Biodeterioration Monitoring*,[45] and lists the different types of microorganisms and the general damage they can cause to stone. It also provides the commonly involved genera of each type of microorganism.

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[42] Caneva *et al*., *Plant Biology,* 21.
[43] Ibid.
[44] Ibid.
[45] Mandrioli *et al*., *Cultural Heritage and Aerobiology,* 19

Table 3.1: Biological Alteration of Stone (Mandridi et al.)

GROUPS	INVOLVED GENERA	DAMAGES CAUSED
Autotrophic bacteria	*Thiobacillus,Desulfovibrio, Nitrosomonas, Nitrosovibrio, Nitrosococcus, Nitrosospora, Nitrobacter, Nitrococcus, Nitrospira*	Black crusts, patinas, exfoliation, pulverization
Heterotrophic bacteria and Actinomycetes	*Bacillus, Nocardia, Streptomyces*	Black crusts, patinas, exfoliation, pulverization
Fungi: Deuteromycetes	*Cladosporium, Alternaria, Stachybotrys, Aspergillus, Penicillium, Phoma*	Patinas, spots, pitting
Algae: Chlorophyceae, Cyanobacteria	*Chlorella, Chlorococcum, Haematococcus, Scenedesums, Stichococcus, Ulothrix, Chroococcus, Gloeocapsa, Lyngbya, Nostoc, Oscillatoria, Scytonema, Myxosarcina*	Patinas and films of various colors and consistences
Lichens	*Acarospora, Aspicilia, Caloplaca, Candelariella, Diploschistes, Lecanora, Lecidea, Verrucaria, Xanthoria*	Encrustation, exfoliation, pitting
Mosses and higher plants	*Eurrhinchium, Eucladium, Parietaria, Hedera, Ficus, Capparis, Cymbalaria, Sonchus, Anthirrinum, Ailanthus, Ulmus, Robinia, Rubus*	Encrustation, erosion of surfaces, breakage, detachment

Microorganisms damage stone through physical and chemical alterations which result from the interaction of the microorganisms with the substrate. Environmental conditions for the establishment of microorganisms need to be favorable and are dependent on water, temperature, and light. However, once biocolonization is established and a biofilm is formed the biological community is protected from most atmospheric conditions. The formation of biocolonization on historic structures and monuments does not only devalue it aesthetically but the damage that is caused is permanent and in most cases irreversible. The loss of stone material can change the way a building or monument

is perceived because the finer details are normally the first to disappear. In order to reduce the risks involved with biodeterioration, biocolonization needs to be remediated, controlled, and prevented.

Chapter 4. Control and Preventive Methods of Biocolonization

4.1 Biocides

The most used method to control and prevent biocolonization is the application of biocides. Biocides are chemicals with a toxic effect on living organisms.[46] Because of their toxicity, some biocides are not approved for use in the United States. They can be divided into various categories according to their chemical nature, the presence of characteristic functional groups, the type of formulation, and their action on bioorganisms.[47] The two main categories of biocides are those that act through contact and those that inhibit certain specific metabolic activities of microorganisms. Biocides can range from chemicals, to metallic ions, to naturally occurring antifouling agents.

There is debate on which are the best biocides to use. Some believe that organic and chloride containing biocides should be avoided because of their toxicity and possible nutritious values for biocolonization.[48] Some biocides have been known to stain the surface of the substrate they are applied to and can induce negative physical changes in some stone.[49] However, biocides used in the field of conservation should be highly effective in eliminating biodeteriogens, should not interfere with the original material or substrate, have low toxicity for human health, and a low risk of environmental pollution.[50] The most frequently used biocides in stone conservation, along with the type of microorganisms they eradicate can be found in Table 4.1.

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[46] Francesca Cappitelli, Federica Villa, and Claudia Sorlini, "New Environmentally Friendly Approaches against Biodeterioration of Outdoor Cultural Heritage" in *Biocolonization of Stone: Control and Preventive Methods*, ed. A. Elena Charola et al. (Washington D.C.: Smithsonian Institution Scholarly Press, 2011), 52.

[47] Caneva *et al*., *Plant Biology,* 318.

[48] Thomas Warscheid and Hans Leisen, "Microbiological Studies on Stone Deterioration and Development of Conservation Measures at Angkor Wat." in *Biocolonization of Stone: Control and Preventive Methods*, ed. A. Elena Charola et al. (Washington D.C.: Smithsonian Institution Scholarly Press, 2011), 14.

[49] St. Clair *et al*., *Biodeterioration of Stone Surfaces,* 116.

[50] Caneva *et al*., *Plant Biology,* 318.

Table 4.1: Most frequently used biocides in the conservation of stone (modified from Caneva 2008)

Chemical Classification	Chemical Composition	Commercial Name	BF	CA	L
Inorganic compounds	Sodium and potassium hypochlorite		•	•	•
	Lithium hypochlorite				•
	Sodium sulphite				•
	Hydrogen peroxide		•	•	•
	Sodium octaborate	Polybor		•	
Phosphoorganic compounds	Glyphosate	Roundup, Spasor, Rodeo			•
Alcohols	Ethanol		•		
Phenol derivatives	Thymol		•		
	o-phenyl-phenol	Lysol	•		•
	p-chloro-m-cresol		•		
	Chlorinated and phenolic compounds	Panacide, Halophane, Thaltox C		•	•
	Sodium pentachlorophenate			•	•
Nitroorganic compounds (ureic and carbamates)	Diuron	Karmex, Diuron	•	•	•
	Chlobromuron	Maloran			•
	Fluometuron	Lito 3			•
Quaternary ammonium salts	Alkyl-benzyl-dimethyl-ammonium chloride	Preventol R50	•	•	
		Preventol R80	•	•	•
		Neo Desogen	•	•	•
		Hyamine 3500	•	•	•
		BAC	•	•	•
		Catamin AB		•	•
	Benzyl-dodecyl-bis(2-hydroxyethyl)-ammonium chloride	Bradophen	•		
	(Diisobutylphenoxyethoxyethyl)dimethyl-benzyl-ammonium chloride	Hyamine 1622		•	•
	Dodecyl-benzyl-trimethyl-ammonium chloride	Gloquat C		•	
	Lauryl-dimethyl-benzyl-ammonium chloride	Cequartyl		•	•
Organic metal salts	Tri-n-butyl tin oxide	TBTO	•	•	•
		Thaltox		•	•
	Tri-n-butyl tin naphthenate	Metatin N58-10	•		•
Pyridine	2,3,5,6 tetrachloro-4-methyl sulfonyl pyridine	Algophase		•	•
Heterocyclic compounds (diazines and triazines)	bromacil	Hyvar X			•
	hexazinone	Velpar, Velpar L		•	•
	terbutryn	Igran			•
Mixtures	quaternary ammonium salt + tri-n-butyl tin naphthenate	Metatin N58-10/101	•	•	•
	quaternary ammonium salt + tri-n-butyl tin oxide	Thaltox Q		•	•
		Thaltox 20, Murasol 20			•
	Dimethyl-thio sodium carbamate + 2-mercaptobenzothiazole	Vancide 51	•	•	•

Key: B = bacteria; F = fungi; C= cyanobacteria; A = algae; L = lichens

Biocides require a certain amount of time to complete their action, depending on the type and concentration of the biocide applied and the nature and vegetative state of the colonizing species. The surroundings of the treated area should also be taken into account, because biocides may be harmful to nearby plants. Other factors that may influence the efficacy of biocides include the physical and mineralogical properties of the substrate, the presence of organic material or pollutants, and meteorological conditions.[51] When choosing a biocide it is important to keep in mind whether other treatments had been carried out on the stone because they may reduce the effectiveness of the biocide. Research has been done on the effects of biocide(s) applied to stones that had been consolidated or had a water repellent applied.[52] However, more research needs to be done to determine the long term effects of combined conservation treatments and to determine how the order of treatment application affects the efficiency of one or both products.

Recent research has focused on finding environmentally friendly biocides. The effects of synthetic analogues of capsaicin and zosteric acid have been tested in the laboratory as antifouling agents. According to this research zosteric acid has proven to be an effective biocide on certain species of microorganisms when tested on biocolonized cultures on glass slides. The effects of capsaicin were not reported.[53] Another area of research regarding environmentally friendly biocides focuses on identifying naturally occurring viruses that affect algae. Researchers have proven that in principle, viruses can inhibit algal types that are commonly found on stone, however, testing has only been carried out under controlled conditions in the laboratory.[54] Enzymes, which are naturally occurring, have successfully been used as a non-toxic and low impact cleaning

[51] Ibid. 328.
[52] Ornella Salvadori and A. Elena Charola, "Methods to Prevent Biocolonization and Recolonization: An Overview of Current Research for Architectural and Archaeological Heritage" 43-45.
[53] Francesca Cappitelli, Federica Villa, and Claudia Sorlini, "New Environmentally Friendly Approaches against Biodeterioration of Outdoor Cultural Heritage." 54.
[54] Eric May, Dania Zamarreno, Sarah Hotchkiss, Julian Mitchell, and Robert Inkpen, "Bioremediation of Algal Contamination on Stone" in *Biocolonization of Stone: Control and Preventive Methods*, ed. A. Elena Charola et al. (Washington D.C.: Smithsonian Institution Scholarly Press, 2011), 60.

agent alternative for lichen elimination. They have been found to essentially liquefy the lichen biomass and dissolve their subsurface elements, allowing them to be removed with little mechanical stress to the stone.[55] More research is needed on most of these environmentally friendly biocides before they are ready to be tested in the field, but they appear to be promising based on the laboratory tests.

4.2 Quaternary Ammonium Salts

Quaternary ammonium salts are within the category of biocides that act through contact in order to eradicate microorganisms. They are the most widely used class of products for the control of biocolonization in the field of conservation. They can be classified as surfactants, a vast group of substances that combine a detergent action and wide-range efficacy with a middle-to-low level of toxicity.[56] While many have been tested for possible use in the field, alkyl-dimethyl-benzyl-ammonium chloride is the most commonly used and has produced good results as a bactericide, algicide, fungicide, and lichenicide. Alkyl-dimethyl-benzyl-ammonium chloride is incompatible with anionic surfactants, nitrates, hydrogen peroxides, and many other substances.[57] Hence, it is important to know what products had been previously applied to the surface that is to be treated with a quaternary ammonium based biocide because they could render the biocide ineffective. In the past, quaternary ammonium had been used in formulations with other biocides, such as organotin compounds, however, this has been banned in many countries because of its high toxicity levels.[58]

According to *Plant Biology for Cultural Heritage*[59] the biocidal action of quaternary ammonium does not have a residual action over time. However, testing has

[55] St. Clair *et al*., *Biodeterioration of Stone Surfaces,* 125.
[56] Ceneva *et al*., *Plant Biology,* 333.
[57] Ceneva *et al*., *Plant Biology,* 328.
[58] Kumar and Kumar. *Biodeterioration of Stone in Tropical Environments.* 33.
[59] Ceneva *et al*., *Plant Biology,* 333.

shown that there has been an absence of recolonization for a more or less extended period of time, where quaternary ammonium has been used. Six years after the completion of the National Museum of the American Indian in Washington D.C. black biocolonization was present on the building. Testing was carried out on surplus stone blocks from the building, using D/2 Biological Solutions. Nearly a year after the biocide was applied it was still effective and the surface of the stone was cleaner.[60] In the gardens of the National Palace of Queluz in Portugal, the biocide Preventol R80, based on a quaternary ammonium salt, was applied to some of the stone elements in the garden. Prior to application the stone elements were brushed to remove any surface debris. After six months most of the biocolonization had disappeared and the dead remains were brushed cleaned. Two years after the Preventol R80 was applied, no recolonization of the stone elements had been observed, even in shady and damp areas.[61]

4.3 Preventive Methods

Routine or even periodic cleaning schedule for the exterior of buildings and monuments can reduce the soiling from biocolonization. Dust, deposits of organic substances, bird droppings, and unsuitable restoration materials on the surface of stone can all serve as nutrients for microorganisms. Cleaning the surface of the stone removes most of these and any other dirt, spores, or seeds that may have been deposited on the stone. However, once a stone building or monument has been cleaned of biocolonization it is important that it remain free of biological growth in order to prevent further biodeterioration. Preventive methods for recolonization are aimed to inhibit biological

[60] A. Elena Charola, Melvin Wachowiak, E. Keats Webb, Carol A. Grissom, Edward P. Vicenzi, Wang Chong, Hanna Szezepanowska, and Paula DePriest. "Developing a Methodology to Evaluate the Effectiveness of a Biocide." *12th International Conference on Stone Deterioration and Conservation* (2012)

[61] A. E. Charola, M. Vale Anjos, J. Delgado Rodrigues, and A. Barreito. "Developing a Maintenance Plan for the Stone Sculptures and Decorative Elements in the Gardens of the National Palace of Queluz, Portugal." *Restoration of Buildings and Monuments* 13.6 (2007): 377-88.

attack by modifying, where possible, the environmental conditions and physicochemical parameters of a stone surface so they become unfavorable for biological growth.[62] These methods include routine maintenance, design solutions, and the use of metallic strips.

Since water is one of the main factors needed to support biocolonization, keeping stone dry from unnecessary sources of water is important. Performing routine maintenance to roofs, gutters, and other water-shedding system, as well as improvements to the drainage system around the building or monument can reduce or possibly eliminate the source of water that the building stone has access to. Also re-designing how water sheds over the building can reduce the amount of water that the stone receives. The landscape design around a monument or building may be used as a preventive method. Vegetation within the landscape may help modify the microclimate enough to change the conditions that microorganisms need to survive, making the microenvironment unfavorable for the continued growth of microorganisms. Suitable chosen vegetation may lower the water table, minimize evaporation, reduce air salinity and pollution, and reduce erosion.[63]

The use of metallic strips, copper, bronze, zinc, or lead, to control biocolonization has long been acknowledged, but implementing this method is not always easy. This method relies on the slow leaching of the metal ions from the metal strip by rainwater that flows over the surface that is to be cleaned of biocolonization. The metal ions act as a long-term biocide to eliminate existing and to prevent new biocolonization from occurring.[64] On a wall at the San Ignacio Mini Jesuit-Guarani Mission in Misiones, Argentina, three metals were tested to determine how they would perform as a biocide and as a preventive method. The metals used were lead strips, zinc mesh, and brass mesh

[62] Kumar and Kumar. *Biodeterioration of Stone in Tropical Environments*, 28.
[63] Ibid. 29.
[64] Marcelo L. Magadan, Gisela M. A. Korth, Marcela L. Cedrola, A. Elena Charola, and Jose L. Pozzobon, "Case Study: Biocontrol Testing at the San ignacio Mini Jesuit –Guarani Mission, Misiones, Argentina" in *Biocolonization of Stone: Control and Preventive Methods*, ed. A. Elena Charola et al. (Washington D.C.: Smithsonian Institution Scholarly Press, 2011), 91, 92.

and strips (58% copper, 40% zinc, and 2% lead).[65] These metals were placed on top of a wall where the test area for each metal was divided into two sections, an uncleaned as a control and a cleaned, where a biocide was applied to remove the biocolonization. After 16 months no new biocolonization was visible, except for some algae on the cleaned lead section, and on the uncleaned areas some vegetation seemed to have disappeared. Twenty-one months after the metal strips were installed the cleaned areas had no obvious recolonization.[66]

In 1997, zinc strips were fitted onto the ridge of the roof of the Stanford Mausoleum, in California, after it was pressure washed to remove any soiling and an application of Heavy Duty Restoration Cleaner by PROSOCO to remove lichens.[67] Twelve years after the zinc strips were installed, the roof was inspected to evaluate their effectiveness and no biocolonization was evident. The zinc strips had prevented recolonization during these years and are expected to do so for many more. Metallic strips as a preventive method for biocolonization are most effective when the object they are applied to has a regular shape and design that ensures even distribution of rain water over the surface.[68] When choosing what type of metal to use it is important to keep in mind the surrounding area of the monument or building because of the toxicity of the metal as well as any negative effect that the metal may have on the stone such as staining, as is the case for brass, bronze and copper strips.[69]

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[65] Ibid. 93.
[66] Ibid. 96.
[67] David P. Wessel, "Case Study: Field Observation on the Effectiveness of Zinc Strips to control Biocolonization of Stone" in *Biocolonization of Stone: Control and Preventive Methods*, ed. A. Elena Charola et al. (Washington D.C.: Smithsonian Institution Scholarly Press, 2011), 109.
[68] Ibid. 112.
[69] Ornella Salvadori and A. Elena Charola, "Methods to Prevent Biocolonization and Recolonization: An Overview of Current Research for Architectural and Archaeological Heritage" 42.

4.4 Final Remarks

The study of biocolonization and how it affects historic stone is still being continued by conservators, biologist, and material scientists. New analytical methods to determine the growth and amount of biocolonization present on the surface of stones are being developed. Advances in understanding the action of specific biocidal compounds are being made and new formulations developed aimed to improve effectiveness while lowering their environmental impact. The degradation of the world's stone cultural heritage by biodeterioration is something that probably will required an ongoing study as microorganisms develop resistance to biocide and adapt to changed environments. New preventive and control methods for biodeterioration of stone are being developed in order to prolong the lifespan of stone monuments and buildings.

Chapter 5. Sandstone Characterization

5.1 Geology

The sediments for the sandstone that was eventually used for the Fisher Fine Arts Library, were deposited 299 to 307 million years ago during the late Carboniferous Period, also known as the Pennsylvanian Period, of the Paleozoic Era. Generally, the sandstone is referred to as Pictou Sandstone, after the geological grouping into which it falls, corresponding to the final stage of sediment deposited in the Cumberland Basin that covers most of northern Nova Scotia. This particular sandstone is part of the Balfron formation which comprises of red-brown subarkosic sandstone, mudrock, minor pebbly sandstone, calcareous mud-chip conglomerate, minor grey beds, and rare, thin, discontinuous limestone beds.[70]

The sandstone was chosen by Furness for its rich dark red color. Most likely it came from the Amherst Redstone Quarry, Amherst, Nova Scotia, Canada. The quarry opened in the mid 1800's and produced red sandstone until it closed in the 1930's, and remains abandoned. Currently it is surrounded by farm land and housing subdivisions. There is still a large quantity of excellent stone in the quarry, representing one of the best sandstone developments in Nova Scotia. The stone was used locally in buildings in Amherst and Halifax, Nova Scotia, as well as in Toronto, Hamilton and Stratford, Ontario.[71]

[70] R. J. Ryan, and R. C. Boehner. Geology of the Cumberland Basin, Cumberland, Colchester and Pictou Counties, Nova Scotia. (Halifax, N.S.: Dept. of Natural Resources, 1994). 36.
[71] G. B. Dickie, *Building Stone in Nova Scotia, Economic Geology Series*. (Halifax, N.S.: Nova Scotia, Dept. of Natural Resources, Mines and Energy Branches, 1996). 82, 84.

5.2 Previous Analyses

Past analysis carried out on the sandstone from the Amherst Red Stone Quarry were carried out by the Nova Scotia Department of Natural Resources Mineral Resources Branch as part of Nova Scotia's Building Stone Project and by the Canadian Department of Mines, reporting on the mineralogical and physical properties of the sandstone. According to the report of the Building Stone Project, the sandstone from the Amherst Quarry is made up of quartz and feldspar grains with a thin film of iron oxide. The feldspars are badly decomposed and the cement is composed of clay and iron oxide.[72] Physical properties of the stone (Table 5.1) for the project were taken from W. A. Parks' 1914 publication.[73]

Table 5.1: Physical Properties (W. A. Parks)

Physical Properties	
Specific Gravity	2.7
Weight per cubic foot	142.93 lbs
Pore Space	15.20%
Ratio of Absorption	6.89%
Coefficient of Saturation:	
One hour	0.47
Two hours	0.59
Crushing Strength	11,122 lbs/in2
Crushing Strength, wet	6938 lbs/in2
Crushing Strength, wet after freezing	4000 lbs/in2
Loss on treatment with carbonic acid and oxygen	0.00454 g/in2
Transverse Strength	551 lbs/in2
Chiseling Factor	4.8 g
Drilling Factor	19.5 mm
Ferrous oxide	1.80%
Ferric oxide	3.71%

Prior to the restoration of the Fisher Fine Arts Library in the late 1980's, Dr. Seymour Lewin analyzed samples of the sandstone taken from the building to determine the characteristics of the stone to aid in its restoration. The sandstone was analyzed using X-ray diffraction, thin section petrography, and scanning electron microscopy as part of the restoration of the library. The results obtained from the analyses of three samples taken from the sandstone on the west side of the were:

[72] Ibid. 82.

[73] Wm A. Parks, *Report on the Building and Ornamental Stones of Canada*. Vol. 2. (Ottawa: Governement Printing Bureau, 1914). 67-68

- Sample 1: 90% quartz, 6% muscovite., 2% kaolinite, 2% albite
- Sample 2: 92% quartz, 4% muscovite., 2% kaolinite, 2% anorthite
- Sample 3: 88% quartz, 8% muscovite., 4% kaolinite

Lewin classified the sandstone as moderately hard, undifferentiated, quartzose stone with quartz grans having rounded edges and 95% lie in the size range of 0.02-0.06 mm.

5.3 Present Analyses

For the present study, small samples that were flaking off the building were collected to carry out complementary analyses. These ranged from a simple crushing and sieving, testing for the presence of expansive clays using methylene blue test[74], to X-Ray powder diffraction (XRD), and petrographic thin section analyses, to confirm previous mineralogical analyses as well as Scanning Electron Microscopy (SEM), was used to view the micromorphology of a fracture sufrace of the stone as well as to examine the surface biocolonization.

5.3.1 Hydrostatic Weighing

Hydrostatic weighing was done on an irregularly shaped piece of stone that detached from the library to determine the porosity of the stone. Table 5.2 contains all of the data collected and the calculated values that were used to determine the samples porosity which was found to be 13.62%.

Table 5.2: Hydrostatic weighing

Hydrostatic Weighing								wire (g)	3.99
M_1 (g)	M_2 (g)	M_3 (g)	V_p (cm^3)	V_a (cm^3)	V_r (cm^3)	ρ_r (kg/m^3)	ρ_a (kg/m^3)	ε	%ε
97.4	55.8	103.9	6.6	48.1	41.6	2343	2024	0.1362	13.62

[74] E. E. Stapel and P.N.W. Verhoef. "The Use of the Methylene Blue Adsorption Test in Assessing the Quality of Basaltic Tuff Rock Aggregate." *Engineering Geology* 26 (1989): 244-45.

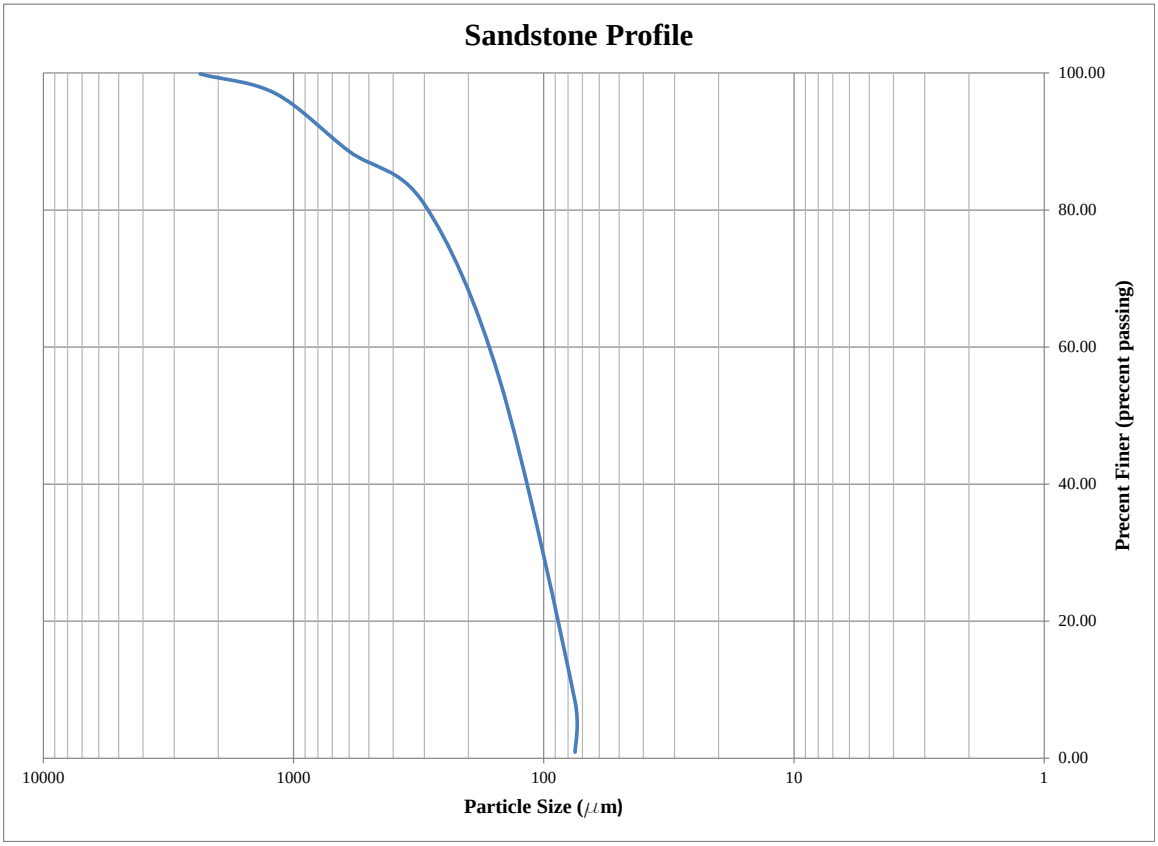
5.3.2 Crushing, Sieving and Methylene Blue Testing

A sample taken from a flaking sandstone block was used for this test. The sample, weighing 48.32 g, was crushed in a porcelain mortar and sieved through standard ASTM sieves to determine the overall particle size distribution of the stone and to concentrate the clays in the finer section. The amount of sample retained on each sieve was recorded and from this the percent passing through each sieve was calculated. All data and calculations are reported in Table 5.3 and the corresponding graph is shown in Figure 5.1. Approximately 75% of the grains were between 150 and 75 microns in size, i.e., approximately 0.15 to 0.08mm, indicating that they are mostly uniform in size, confirming Lewin's analysis, although he reported a lower size. The grains were examined under the microscope to determine their overall appearance and can be classified as being sub-angular (Figure 5.2), differing from the previous evaluation which listed them as rounded.

Table 5.3: Sieve Analysis

Sieve Number	**Screen Size** (μm)	**M_c** (g)	**M_2** (sample + container) (g)	**M_r** ($M_2 - M_c$) (g)	**$\%M_r$** (M_r/M_s) *100%	**$\%M_{rt}$** Σ $\%M_r$ (on or above)	**$\%M_{pt}$** 100% - M_{rt}%
8	*2360*	2.92	2.99	0.07	0.14	0.14	99.86
16	*1180*	3.02	4.40	1.38	2.86	3.00	97.00
30	*600*	3.00	7.07	4.07	8.42	11.42	88.58
50	*300*	2.74	6.48	3.74	7.74	19.16	80.84
100	*150*	2.62	14.99	12.37	25.60	44.76	55.24
200	*75*	2.93	25.61	22.68	46.94	91.70	8.30
Pan	*<75*	2.90	6.48	3.58	7.41	99.11	0.89

Figure 5.1: Particle size distrbution.



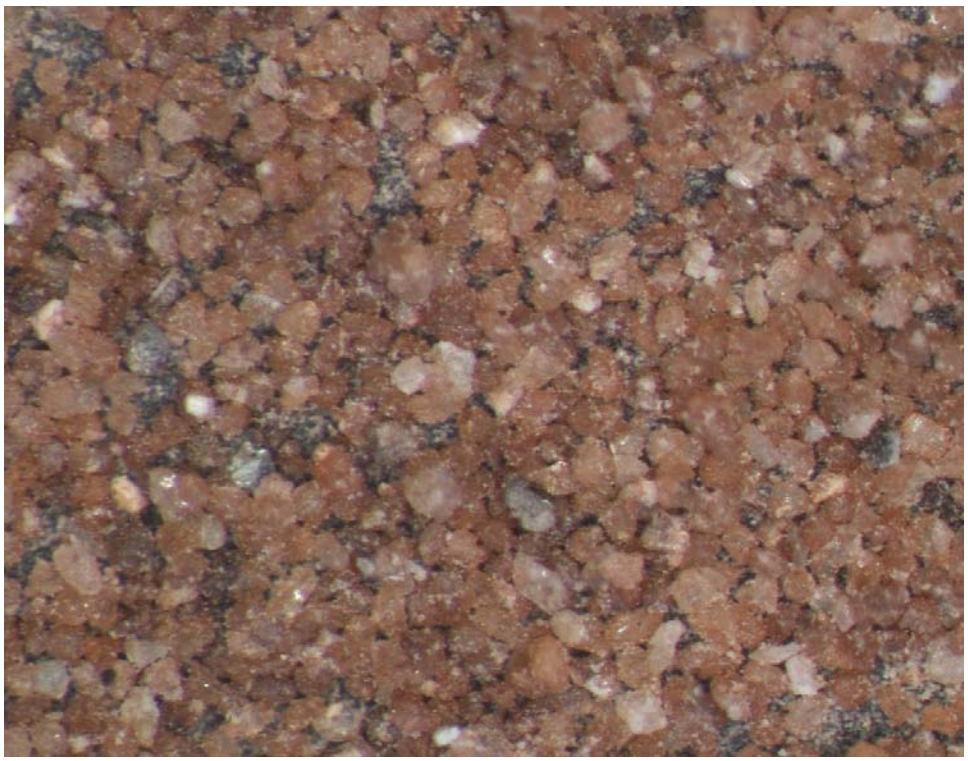


Figure 5.2: Particles retained on the 150 mm sieve magnified to 4.0x.

The Methylene Blue test[75] was applied to all particle sizes retained on 1180, 600, 300, 150, and 75 micron screens. Since a halo was formed around all test spots, as shown in Figure 5.3, this indicates that none or very few expansive clays are found in this stone.

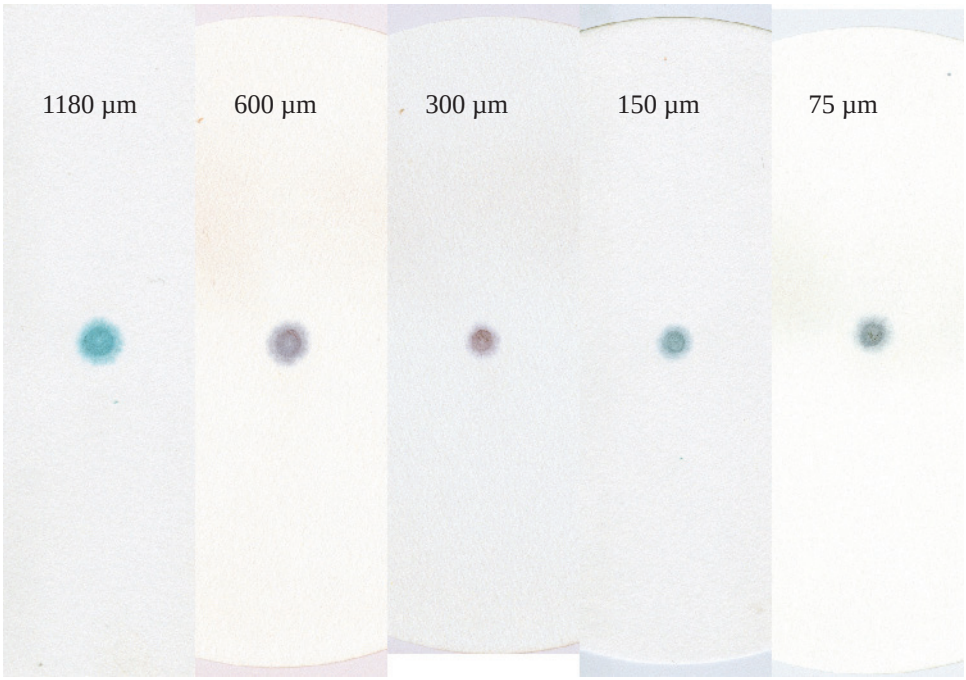


Figure 5.3: Methylene Blue test results.

5.3.3 X-Ray Powder Diffraction

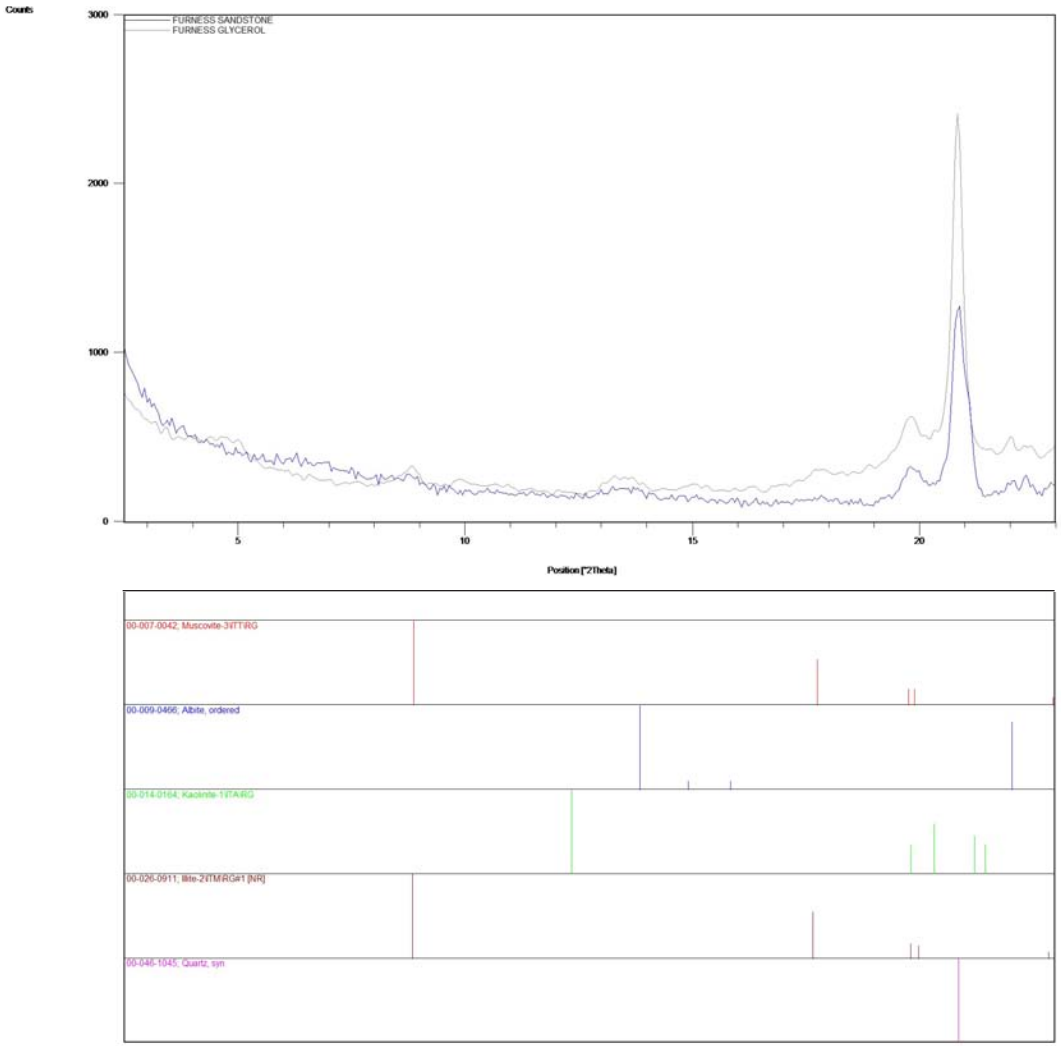
XRD was carried out only on the particles that passed through the 75 µm screen, and in order to better visualize any clay peaks present in the sandstone, the sample was analyzed only up to 23 degrees 2θ, to include the first peak of quartz. Two samples were run, one of the powdery fines (blue line) and the other one of a slurry of fines mixed with glycerol following the procedure described by Novich and Martin[76] (gray line) (Figure 5.4). The test was also run on an expansive Portland sandstone and showed that the preparation method used was not appropriate to determine the presence of expansive clays as no shifting of the clay peaks was observed. However, in the present instance,

[75] Ibid.

[76] Bruce E. Novich and R. Torrence Martin. "Solvation Methods for Expandable Layers." *Clays and Clay Minerals* 31.3 (1983): 235-38.

as indicated by the methylene blue test, no significant amount of expansive clays were found. Analysis showed that quartz, albite (a sodium based plagioclase feldspar), muscovite (a mica), illite, and kaolinite (clays), are present in the sandstone.

Figure 5.4: XRD analysis of the finer fraction of the sandstone from the Fisher Fine Arts Library. Quartz, albite, muscovite, illite and kaolinite were found in the sample.



5.3.4 Polarized Light Microscopy

A petrographic thin section was made from a small sample of the library sandstone which was covered with black biocolonization. The thin section was analyzed to further identify its mineralogical composition and texture, and to investigate any interaction of the biocolonization with the surface of the stone. Optical observations

were made by looking at the thin section in plane polarized light (ppl) and cross polarized light (xpl). The sandstone is clast-rich with fine grains that consist of quartz, argillite, i.e., lithified clay clusters (Figure 5.5), tablet shaped plagioclase feldspar (Figure 5.6), and mica (Figure 5.7). Argillite, also known as mudstone, is a fine-grained sedimentary rock composed predominantly of hard clay particles, basically lithified mud. Iron oxides, some mixed in with clays, e.g., ferruginous clays, coat the original grains, which gives the sandstone its red color, while new cement grows over the iron oxide. This indicates that the iron oxides are not a product of weathering but rather they were part of the original stone. The cement is composed of clays and minerals within the stone that have weathered.

Although the sample had a black biocolonization, when observing the biocolonized surface, patches of green biogrowth were apparent (Figure 5.8), with some of them growing deeper in weathered crevices (Figure 5.9), and its subsurface, approximately 60 µm deep into the stone (Figure 5.10).

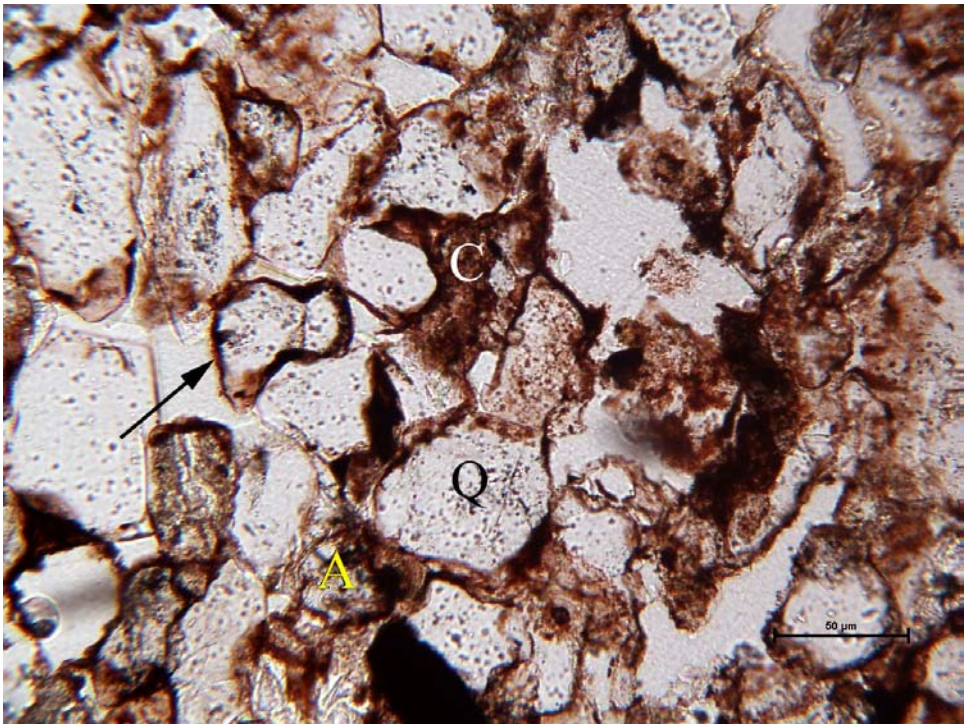


Figure 5.5: Thin section of sandstone from Fisher Fine Arts Library at 200x in ppl. Scale 50 µm.

A = argillite,

C = cement,

Q = quartz,

and the arrow is pointing to the iron oxide coating the grain.

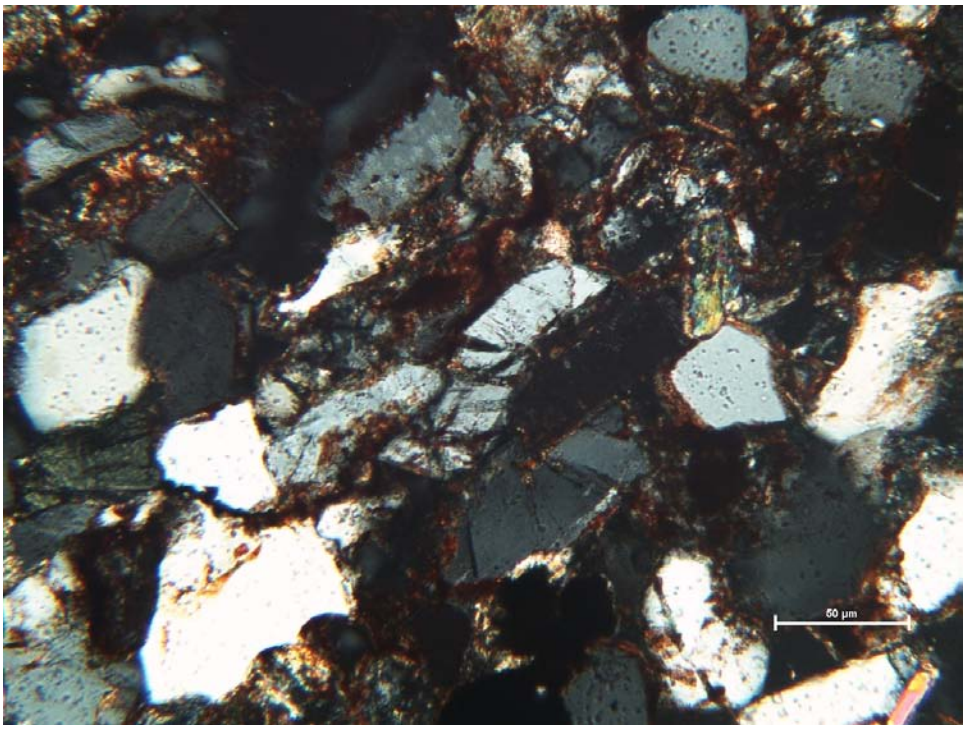


Figure 5.6: Thin section in xpl at 200x showing the tablet shaped plagioclase feldspar (center of image). Scale 50 µm.

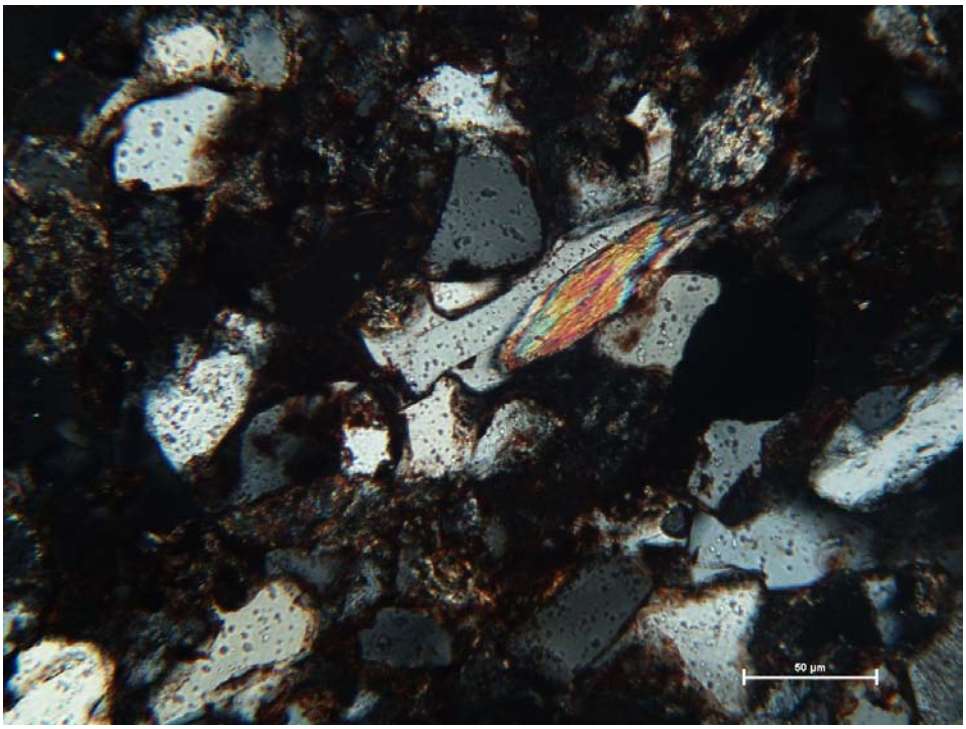


Figure 5.7: A booklet of mica can be seen in the center in xpl at 200x Scale 50µm.

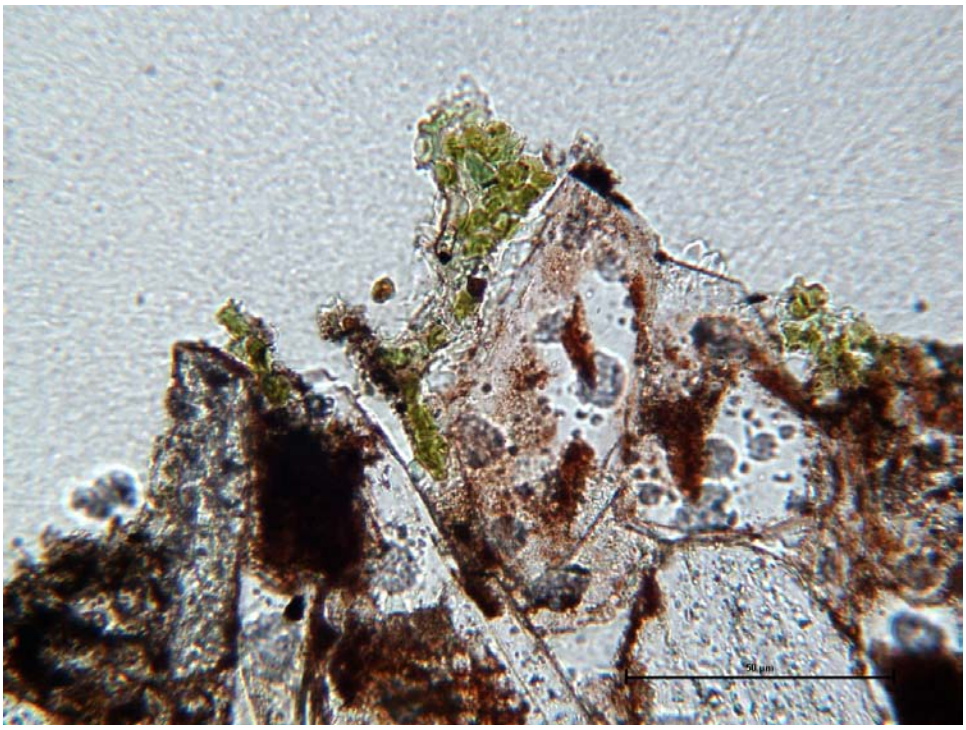


Figure 5.8: Biocolonization on the surface of the sand stone at 400x. Scale 50µm.

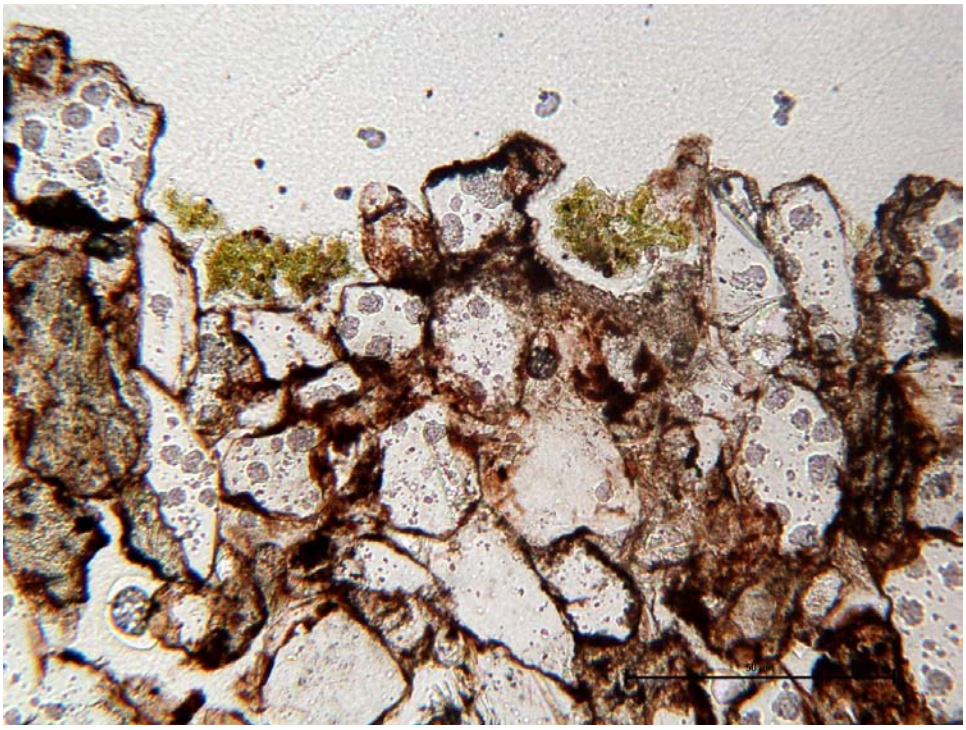


Figure 5.9: Biocolonization within the surface crevices of the sandstone at 200x. Scale 50µm.

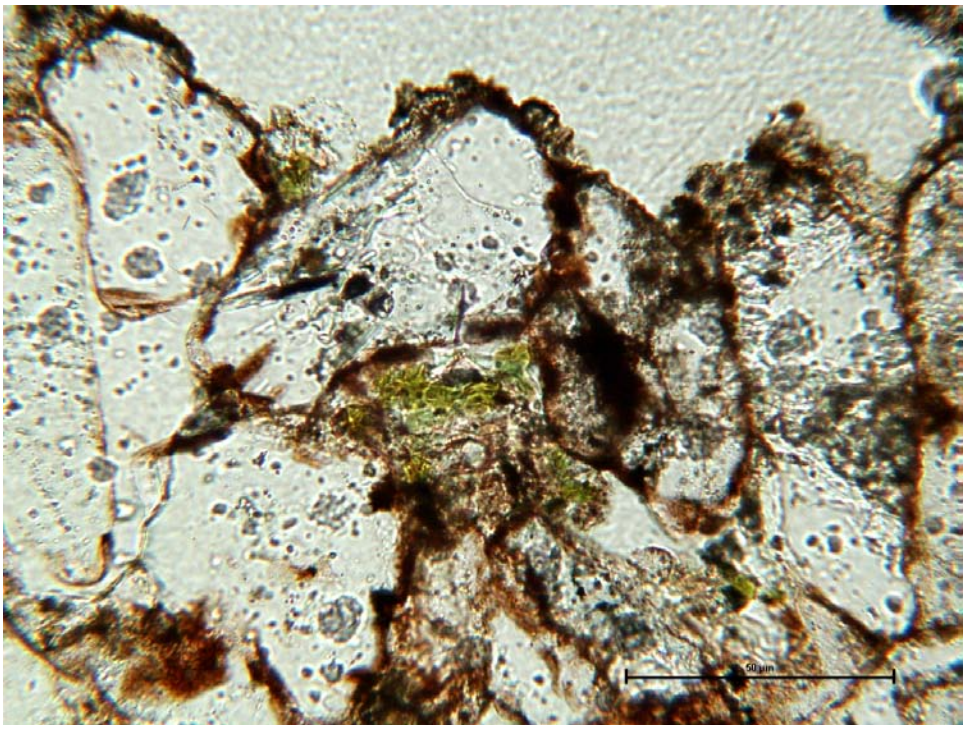


Figure 5.10: Biocolonization in the subsurface of the sandstone at 400x. Scale 50µm

5.3.5 Environmental Scanning Electron Microscopy

ESEM was carried out on three sandstone specimens from the library; a stone free of biocolonization that served as a control, one with green biocolonization, and the third one with black biocolonization. The images obtained with the ESEM provide three-dimensional images of the sandstone morphology as well as that of the surface biocolonization. These images also contribute information about the micromorphology and mineralogy of the stone. The control sample was a fracture surface and provided a clear look at a fracture surface, showing the individual quartz and plagioclase feldspar grains as wells as the overall texture (Figure 5.11). Figure 5.12 exemplifies the plate structure of the plagioclase feldspar grains that can be found in this sandstone. When looking at this sample, a honeycomb like coating was noticed on some of the grains (Figure 5.13). Using Energy Dispersive Spectrometry (EDS) it was determined that this coating corresponds to iron oxide and/or ferruginous clays.

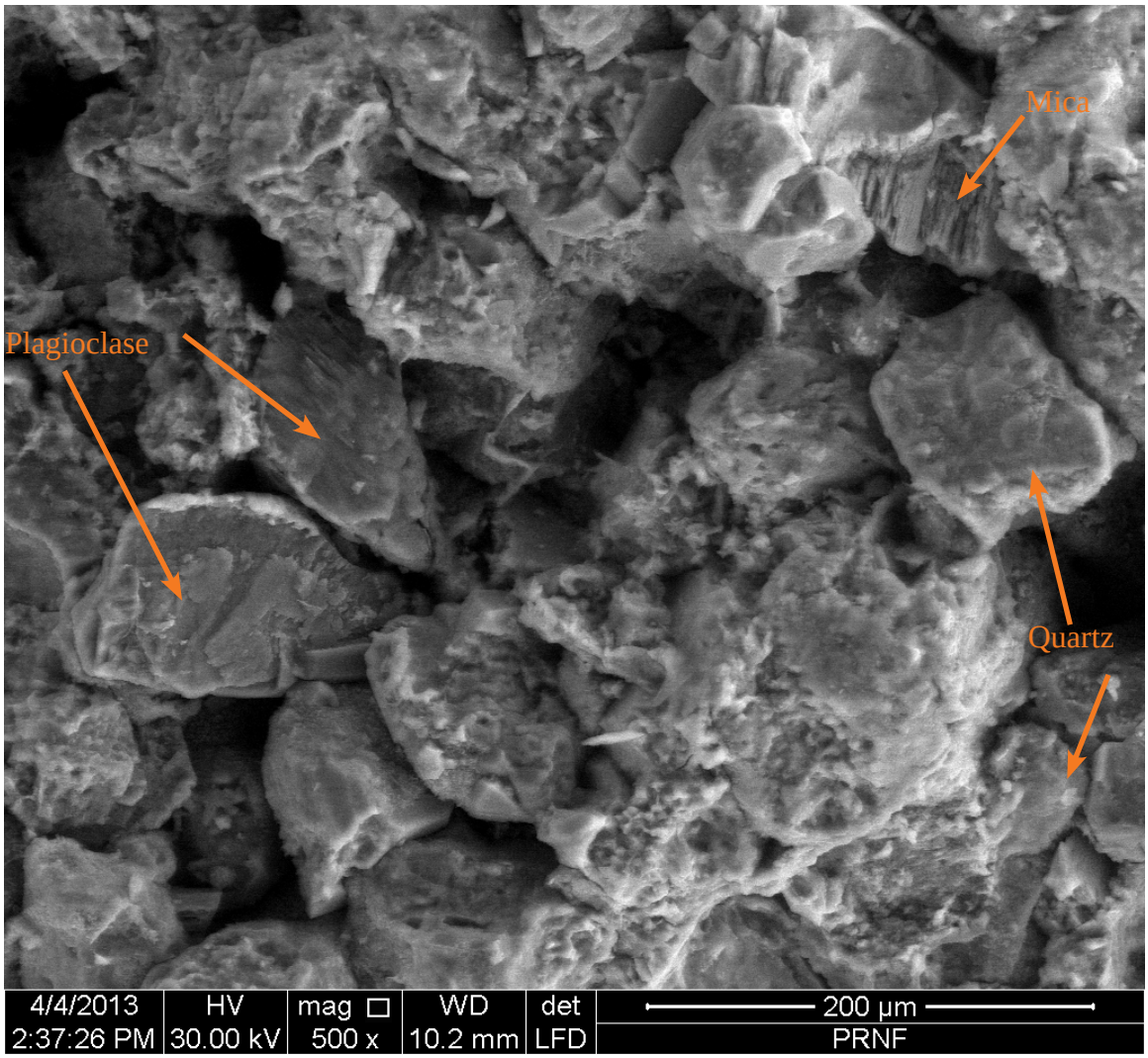


Figure 5.11: Quartz and plagioclase feldspar grains in the fracture surface of the control sample.

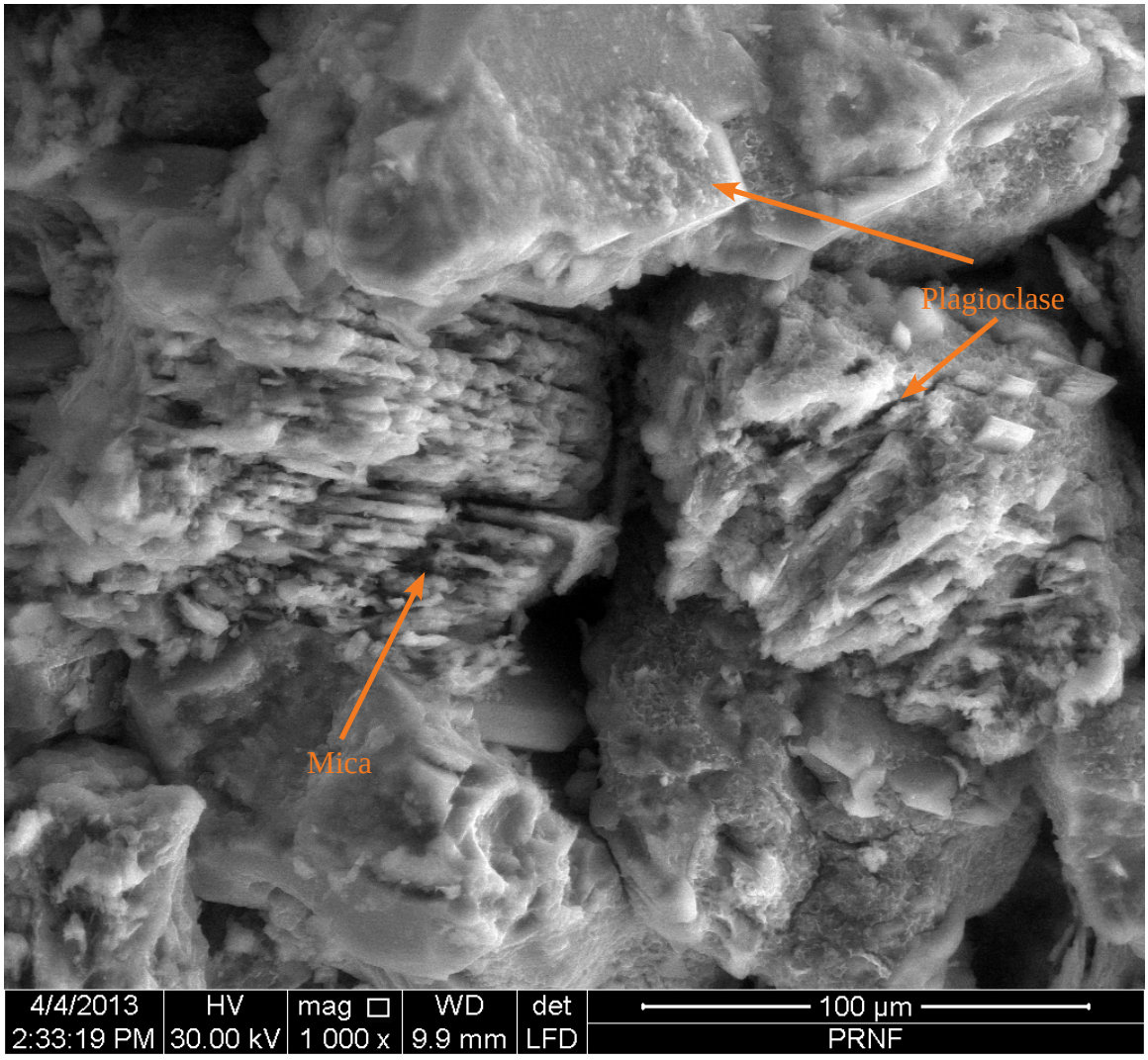


Figure 5.12: Grains of weathered plagioclase feldspar and detail of mica booklets from the above control sample.

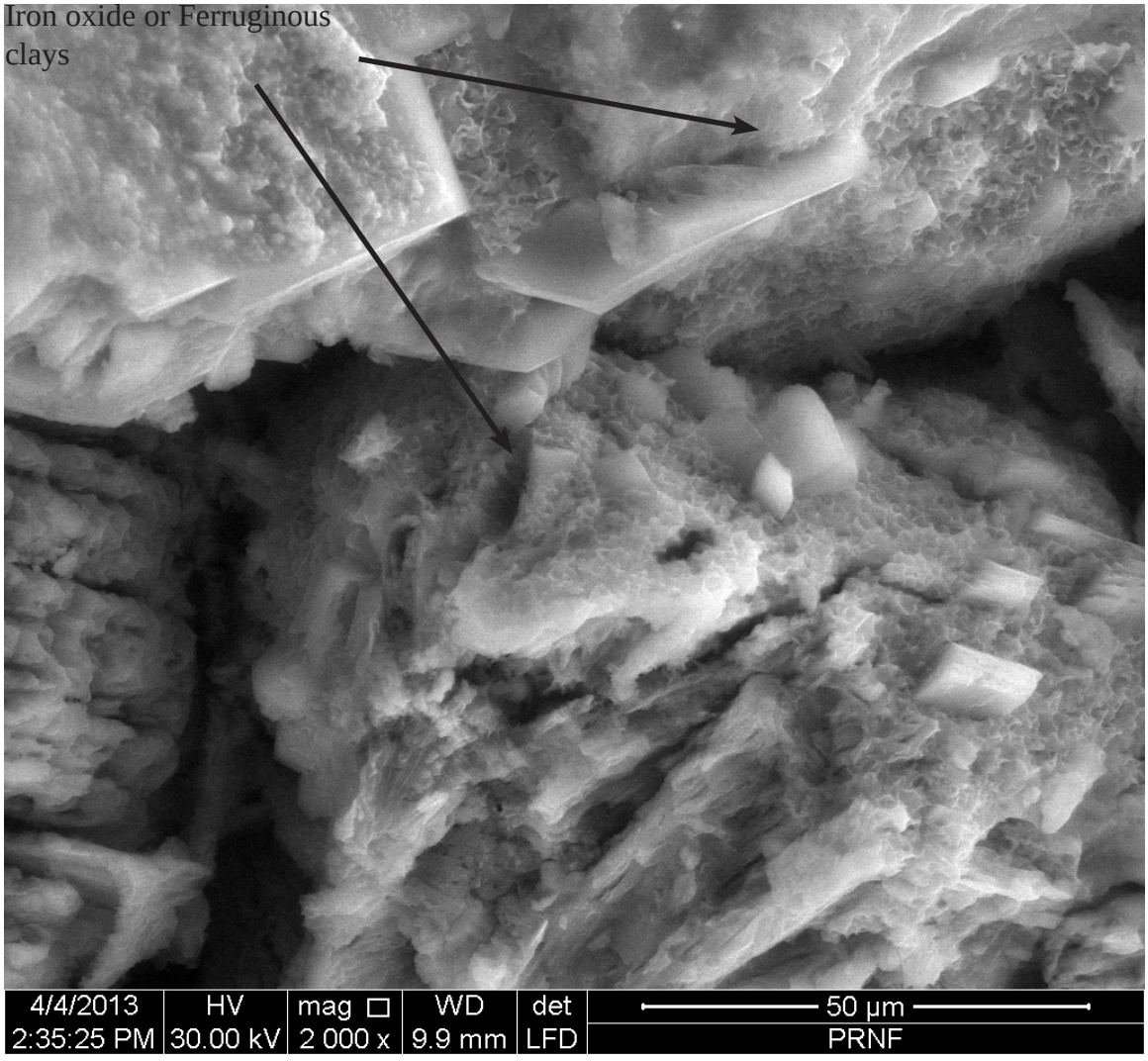


Figure 5.13: Iron oxide or ferruginous clay coating on the grains on the fracture surface of the control sample.

Two areas of the black biocolonized stone surface were observed. In the first area the biocolonization completely coated the surface of the mineral grains (Figure 5.14) and in the second area the biocolonization was thinner and more sporadic, which allowed the grains to be seen through the biocolonization (Figure 5.15). Under higher magnification it became evident that biocolonization had established itself between the grains of the stone (Figure 5.16).

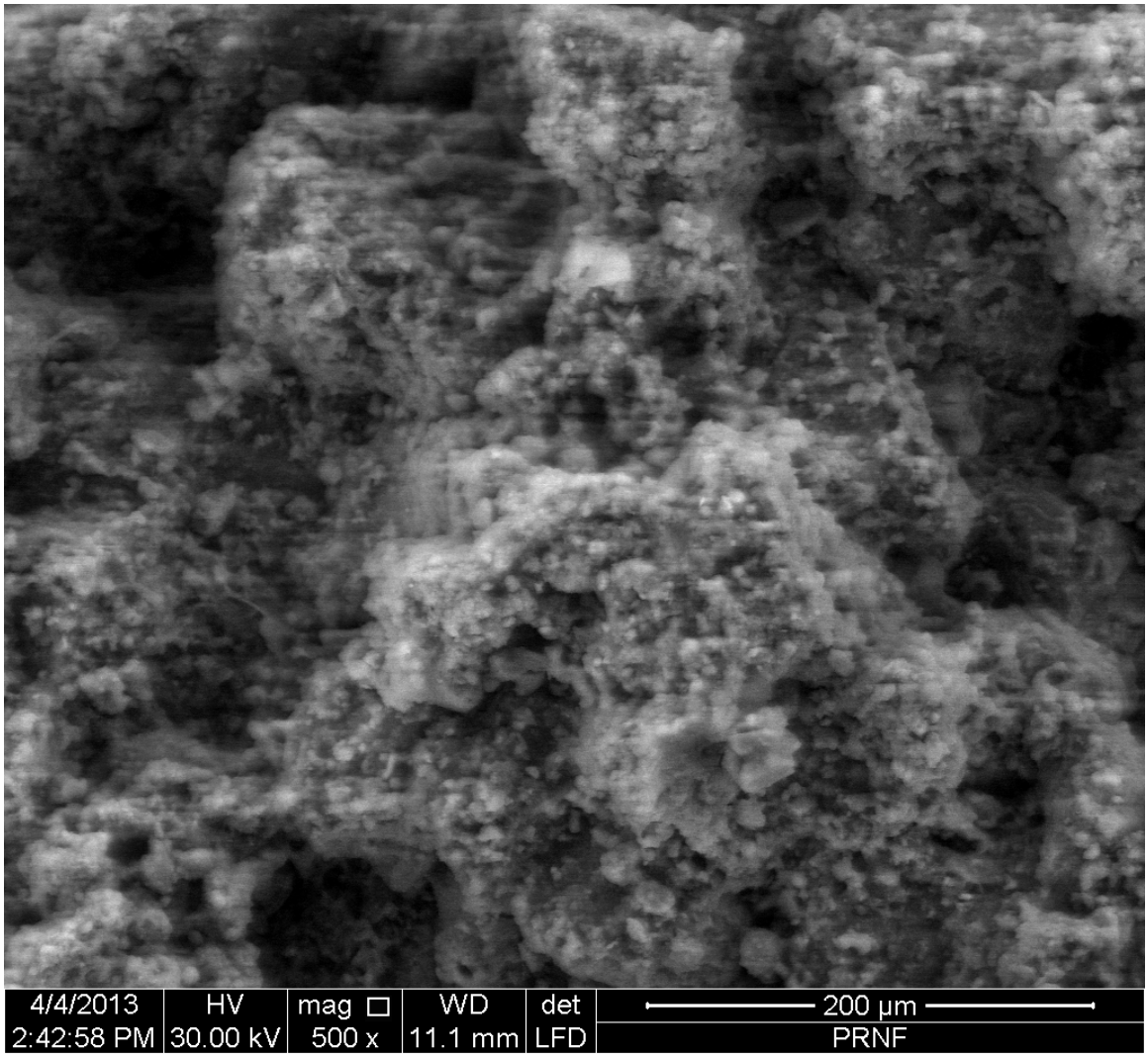


Figure 5.14: Appearance of the black biocolonization covering the surface of the stone.

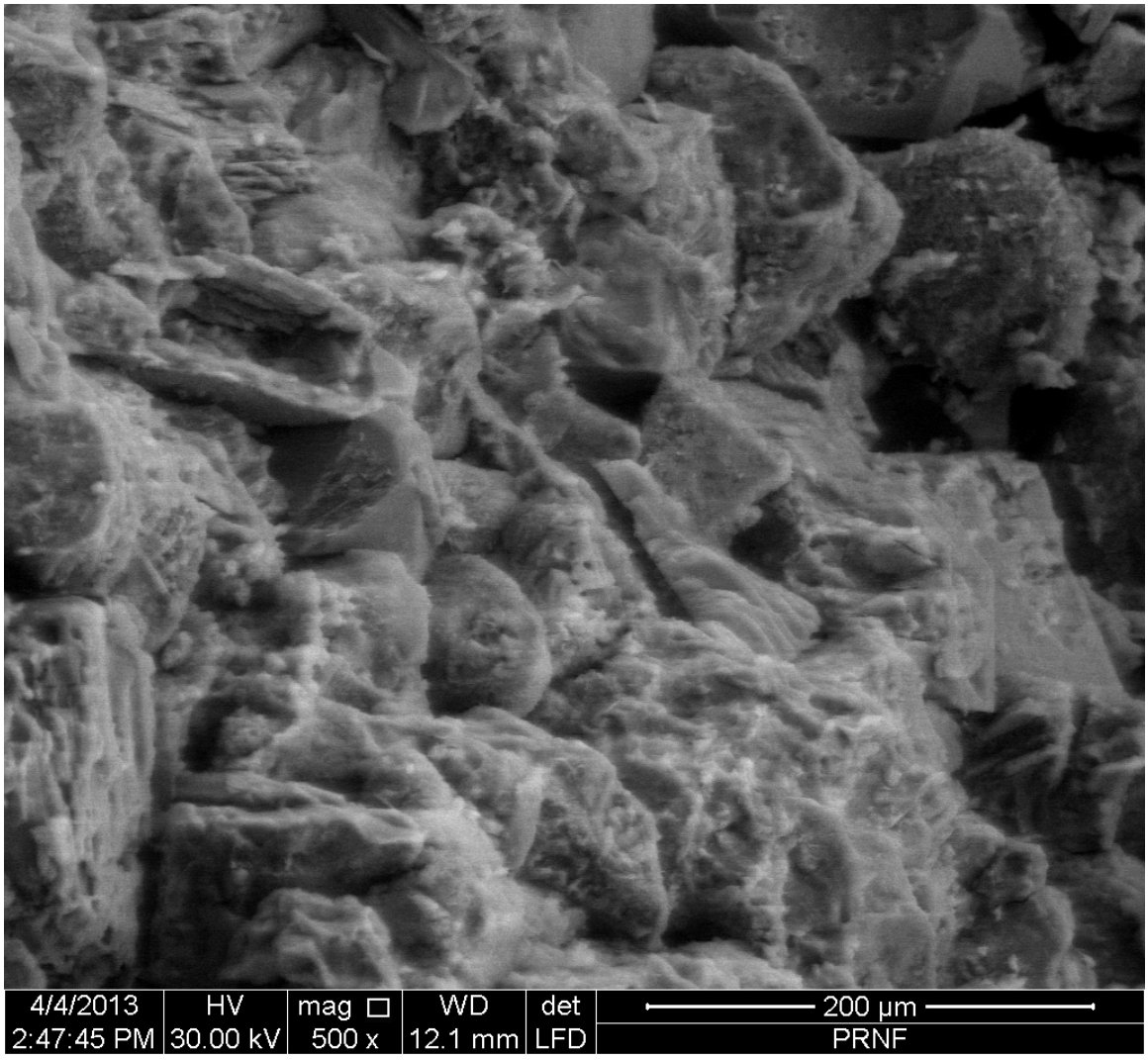


Figure 5.15: Black biocolonization in the subsurface of the stone.

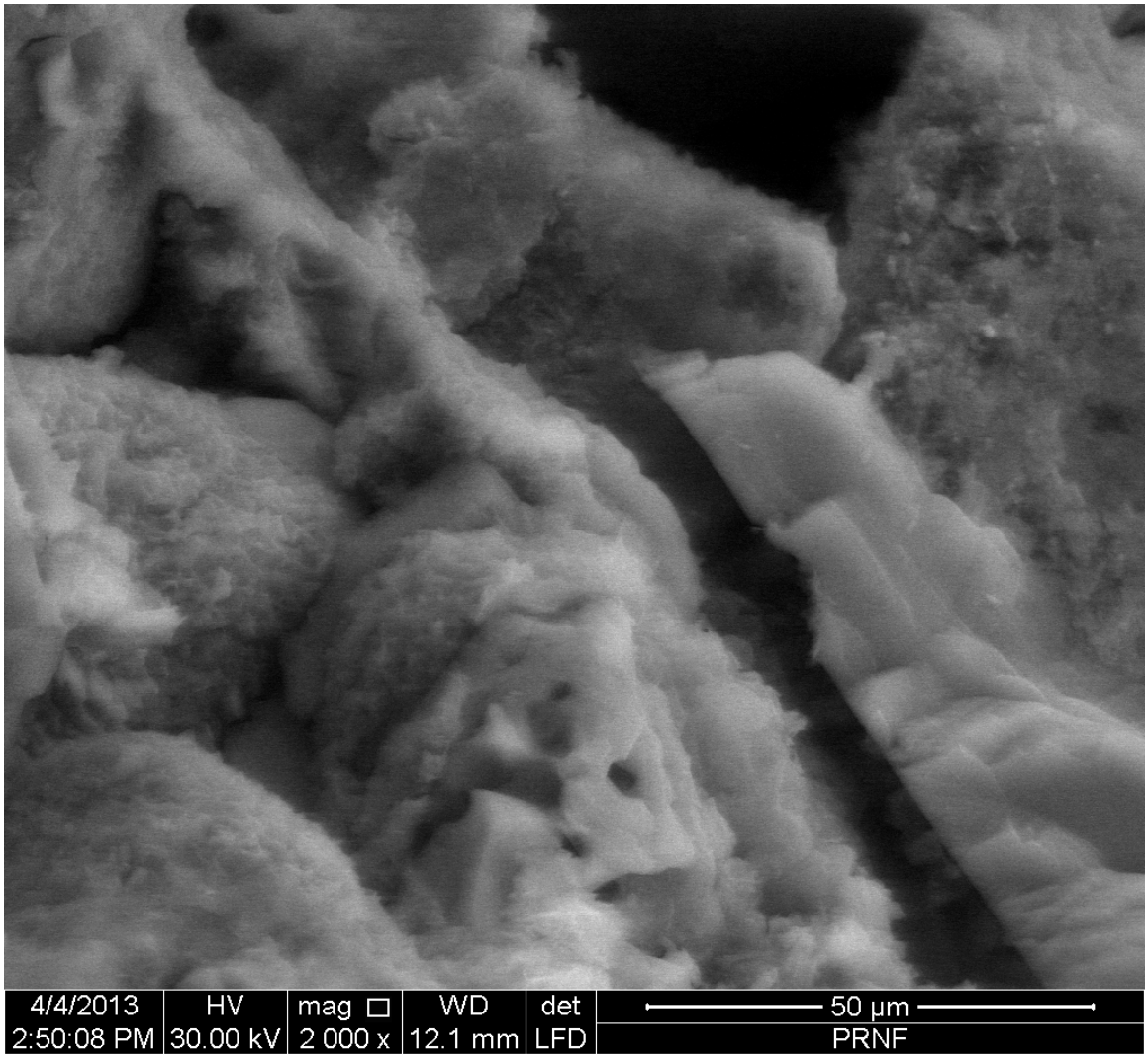


Figure 5.16: Detail of the black biocolonization from the previous figure.

The green biocolonization appears to be more evenly distributed on the surface of the sample. Two areas were examined and on both the mineral grains could be observed under the green biofilm (Figure 5.17 and 5.18). At higher magnification the individual cells of the microorganisms present in the biofilm could be distinguished, as seen in Figure 5.19. While individual species of microorganisms could not be explicitly identified, it was suggested that the spherical cells seen in figure 5.19 might be algae.

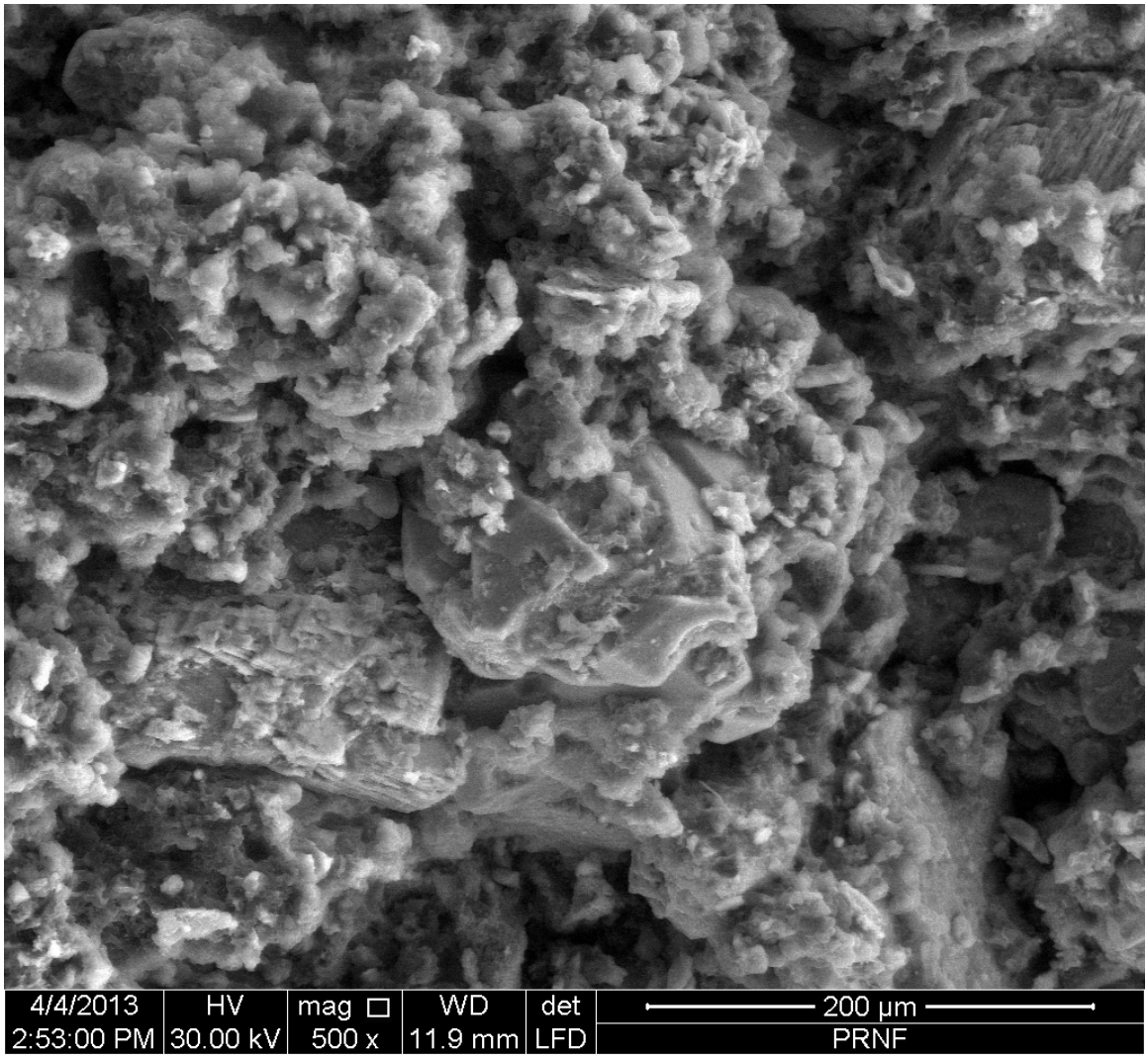


Figure 5.17: Green biocolonization covering the surface of the stone.

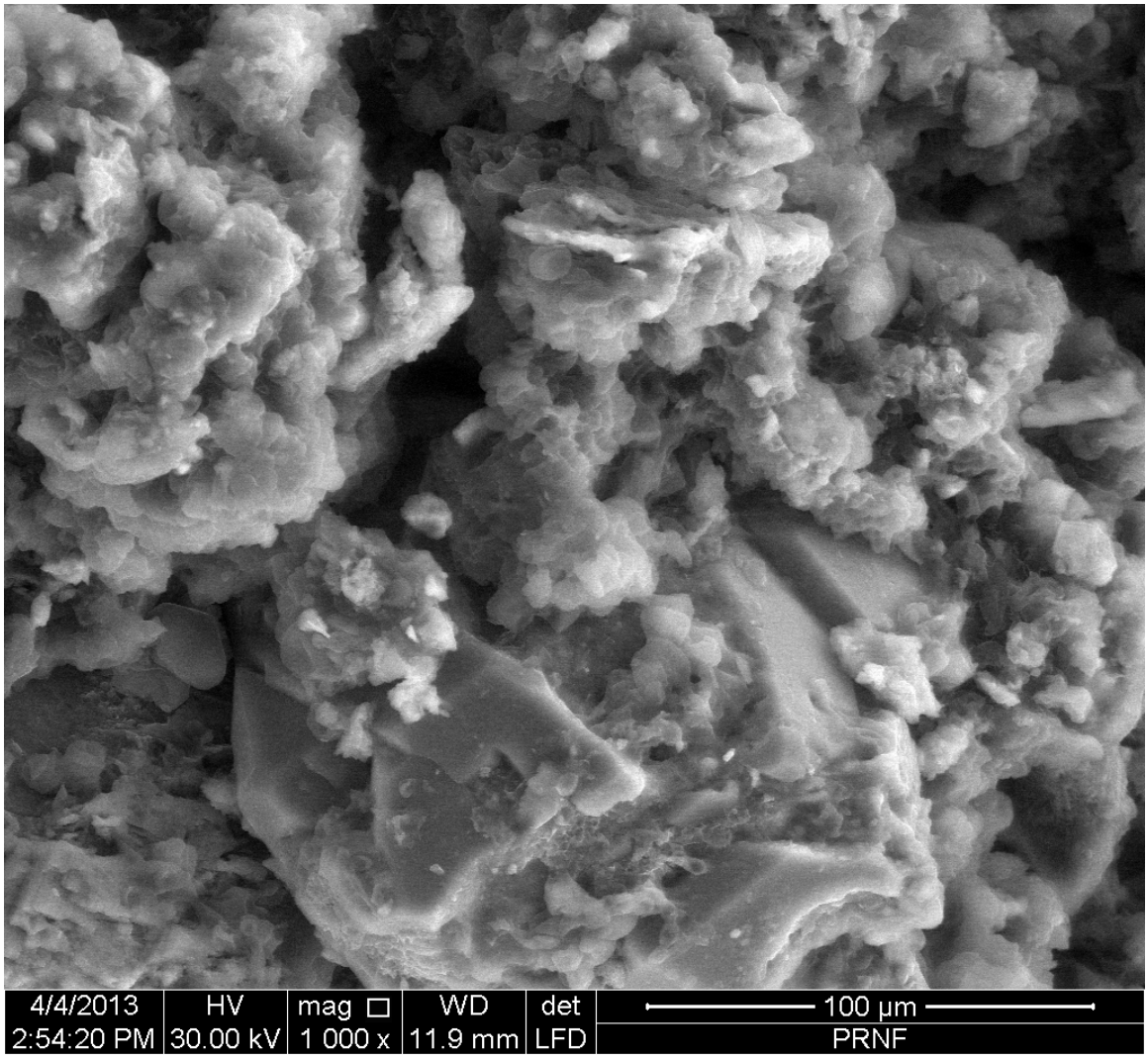


Figure 5.18: Green biocolonization partly covering a quartz grain.

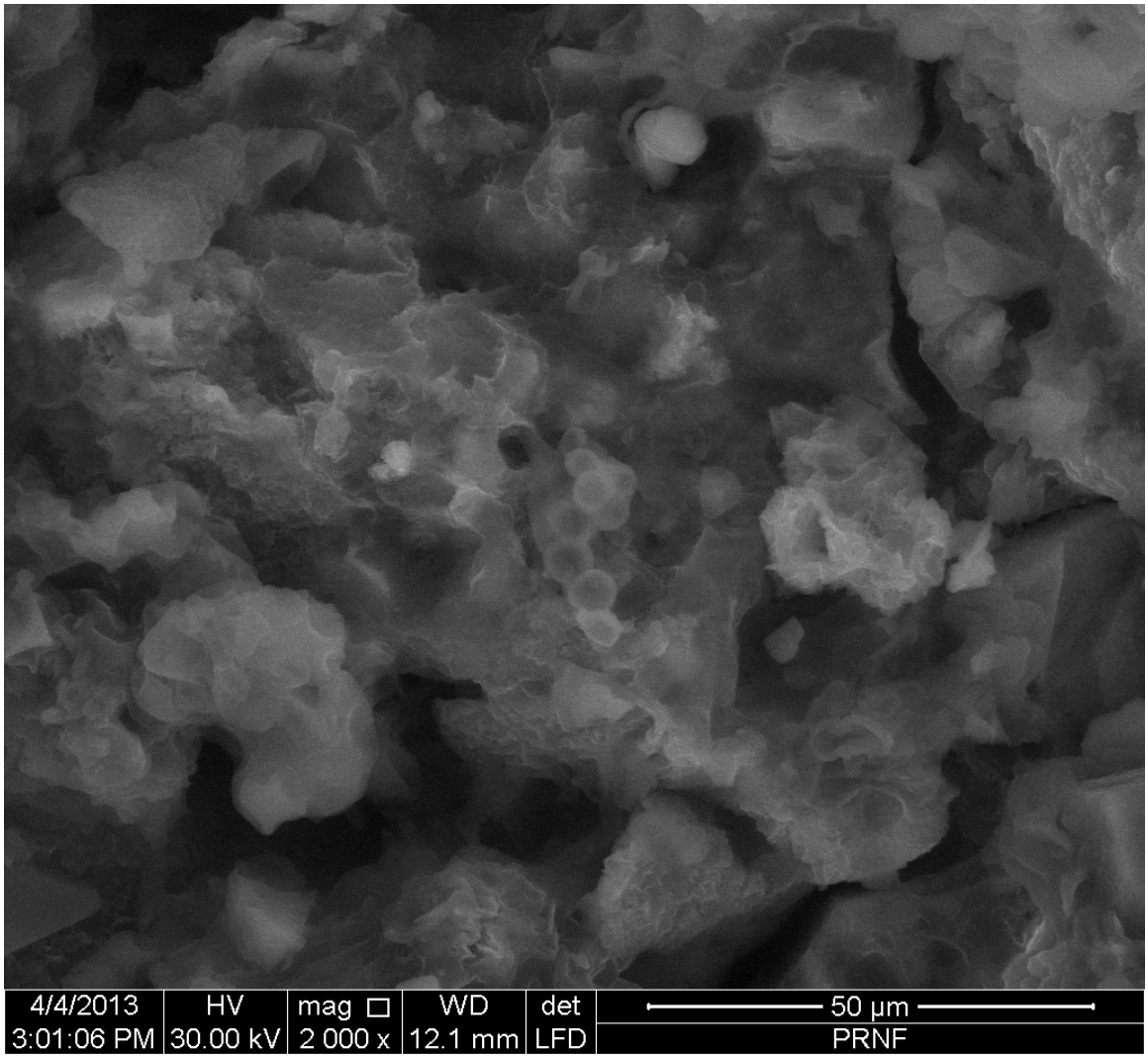


Figure 5.19: Some organisms of green biocolonization are visible in the center of the photo.

5.3.6 Optical Microscopy

A detached flake of the sandstone with green biocolonization was observed under a microscope to try to visualize the appearance of the microorganisms that are growing on the sandstone. Identification of these possible microorganisms is based on their appearance and would require a microbiologist to identify them. The different looking microorganisms are shown in Figures 5.20 through 5.23.

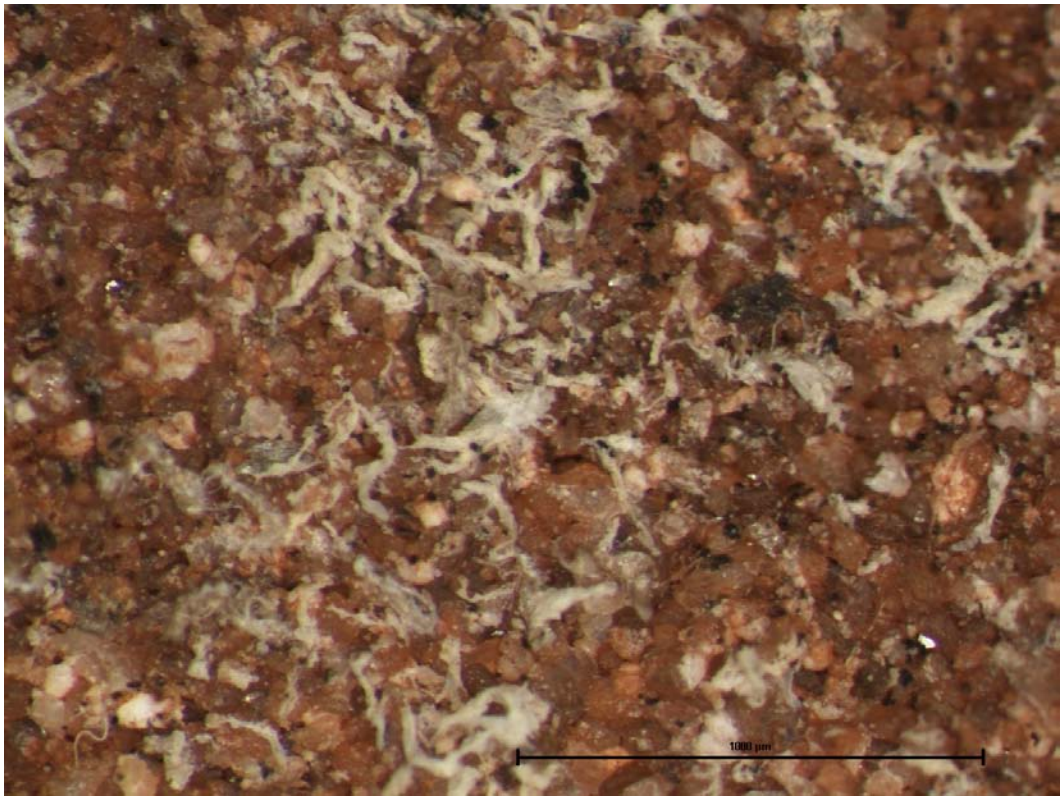


Figure 5.20: Underside of green biocolonized sample. The white threads appear to be fungal hyphae. Magnification 4x. Scale bar 1000µm.

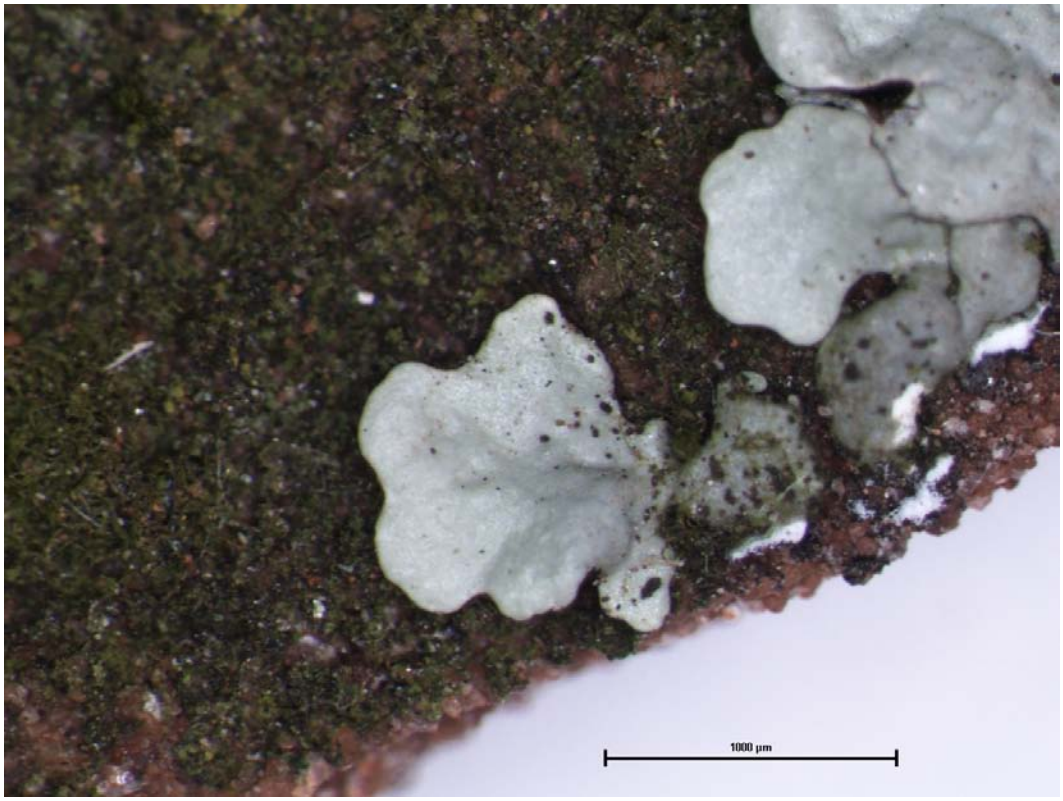


Figure 5.21: White lichen attached to the sandstone. Magnification 2.5x. Scale bar 1000µm.

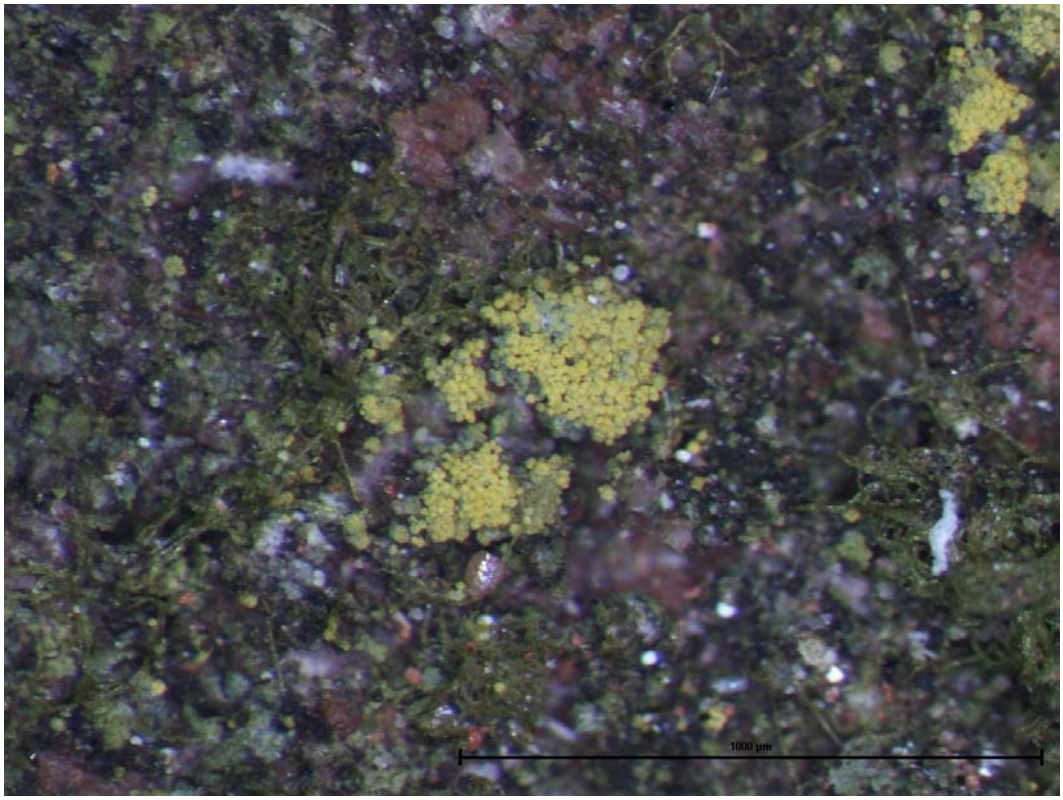


Figure 5.22: Lichen attached to the sandstone. Magnification 5x. Scale bar 1000µm.

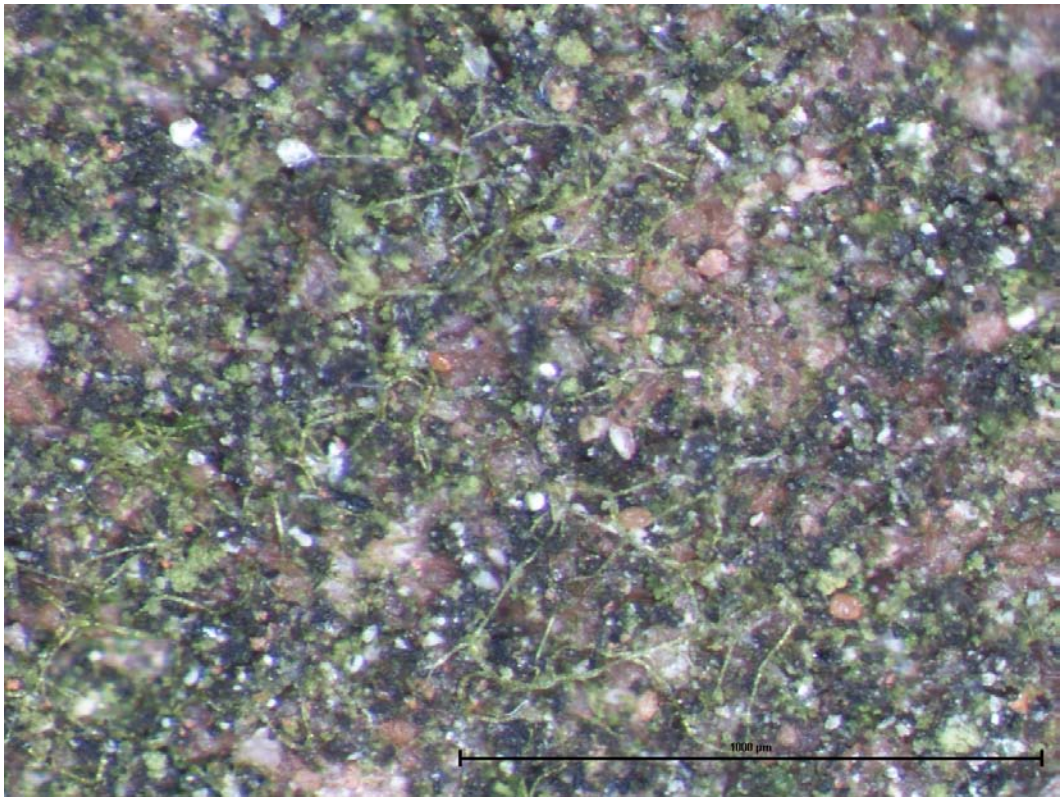


Figure 5.23: The green organisms on the surface of the flake are presubably algae, while the strands would correspond to fungal hyphae.. Magnification 5x. Scale bar 1000µm.

Chapter 6. Methodology

6.1 Biocides Used for Testing

The two biocides tested in this thesis were, D/2 Biological Solutions and Enviro Klean BioWash, both based on quaternary ammonium salts. D/2 is manufactured by D/2 Biological Solutions, Inc., distributed by LimeWorks and marketed as "a biodegradable, easy to use liquid that removes stains from mold, algae, mildew, lichens and air pollutants."[77] This product does not contain bleach, acids, or inorganic salts. While the product's MSDS sheet lists the ingredients as surfactants, wetting agents, and buffers, all of which are proprietary, the active ingredients are known to contain octyl decyl dimethyl ammonium chloride, dioctyl dimethyl ammonium chloride, didecyl dimethyl ammonium chloride, and alkyl (C14, 50%, C12, 40%, C16, 10%) dimethyl benzyl ammonium chloride.[78] BioWash is manufactured and distributed by PROSOCO, Inc. as a "biological soiling remover for monuments and gravestones" that "removes mold and mildew staining and atmospheric staining that disfigures and degrades many types of construction materials."[79] The active ingredients in BioWash are di-(C8-10)-alkyl dimethyl ammonium chlorides, alkyldimethyl benzyl ammonium chloride (C12-16), and nonyl phenol ethoxylate, a non-ionic surfactant (See Appendix C).

6.2 Infrared Thermography

Infrared thermography has been used in the building industry since the 1980s, mostly for detecting heat loss in building envelopes. However, over the past two decades the technology has developed significantly and has become a vital tool for determining performance characteristics of buildings. In the field of conservation

[77] "Product Data Sheet, D/2 Biological Solution." (D/2 Biological Solutions, Inc. 2012), 1.
[78] Michael Trinkley, "Conservation Talk." *AGS Quarterly* 36 3(Fall 2012): 23.
[79] "Product Data Sheet, BioWash." (PROSOCO, Inc.,2011), 1.

infrared thermography is used as a nondestructive tool to detect the presence of moisture in masonry walls by means of changes in heat transfer brought on by conductance of water and phase change heat loss or gain.[80] It is also used to gain information about wall construction, locate subsurface conditions, voids, and infilled doors and windows. Infrared thermography camera, or thermal imager, produces a color image mapping the difference in surface temperature known as temperature maps.

Thermal imaging measures surface temperature, not water content. However, it is possible to map moisture distribution within a wall due to the absorption of energy during evaporation. Each gram of evaporating water absorbs 2,500 J of energy, cooling the surface very effectively, resulting in moist areas being colder than dry ones, assuming the same atmospheric boundary conditions exist across the surface.[81] This naturally occurring phenomenon depends on the air temperature, relative humidity levels, air movement, and direct sun exposure. When at equilibrium the moist material supplies the water flux, which is mainly related to the porosity of the material and its soluble salts content.[82] Phase change of moisture from liquid to gas requires energy and is considered an endothermic reaction. The energy from the phase change is absorbed from the building materials holding this moisture. Porous materials show greater variable temperature effects as a result of moisture accumulation. The amount of surface cooling is directly proportional to the rate of evaporation and the amount of moisture within the wall.[83]

All objects on earth radiate infrared energy and the amount of energy radiated is based on two primary factors: surface temperature of the object and the emissivity of

[80] Antonio Colantonio, "Detection of Moisture and Water Intrusion Within Building Envelopes By Means of Infrared Thermographic Inspections." *Journal of Building Enclosure Design* (Summer/Fall 2008) 47.

[81] Elisabetta Rosina and Jonathan Spodek. "Using Infrared Thermography to Detect Moisture in Historic Masonry: A Case Study in Indiana." *APT Bulletin* 34.1 (2003): 12.

[82] Ibid.

[83] Colantonio. 54.

the object's surface. Emissivity of a material is the ratio of the radiant energy emitted by a surface to that emitted by a blackbody at the same temperature. Thermal imagers detect infrared energy from an object and use this information to estimate the object's temperature. When the thermal imager is set to the proper emissivity value the imager automatically calculates a corrected surface temperature providing an accurate surface temperature reading.[84] Emissivity values for common materials can be found in reference tables. The reported emissivity value for red sandstone is between 0.60-0.83. For testing in this thesis the emissivity values was set at 0.67, the reported values for sandstone.[85]

The Fluke Ti32 Industrial-Commercial Thermal Imager was used for this thesis. It takes images in both visible and infrared light, automatically aligning the two images to produce a single superimposed image. The visible light camera has a minimum focus distance of 46 cm and takes images that are 2.0 megapixel in size. The infrared lens has a minimum focus distance of 15 cm. The thermal imager is a handheld device with no zoom capabilities, the area that the imager captures is dependent on the proximity of the imager to the object. Each pixel in the image contains temperature data, that can be viewed using SmartView, the software that accompanies the thermal image.

6.3 Preliminary Testing

Preliminary tests were carried out using the Fluke Ti32 to determine if a distinguishable difference in temperature could be found during the cooling resulting from wetting dry stones with various levels of biocolonization. These tests appeared to indicate that a difference in the temperature change response could be observed between wetting of clean stone and biocolonized stones, either with green or black biogrowth, thus

[84] *Fluke Ti32, TiR32, Ti29, TiR29, Ti27, TiR27 Thermal Imagers Users Manual*. (Everett (WA): Fluke Corporation, 2011). 25-26.
[85] Jon S. Wilson, *Sensor Technology Handbook*. (Amsterdam: Elsevier, 2005). 627.

leading to the experimental procedure that was used for this thesis.

During the first test using the thermal imager, images were taken of each type of stone, uncolonized, with green, and black biogrowth, when the stone was dry to determine the base temerature for each of them. Water was then applied to each stone until they were thoroughly wet. Thermal images were then taken of the wet stones. The images were then analysed using SmartView to determine the average temperture of the stone under each condition.

The test was started with the uncolonized stone, henceforth referred to as clean stone. As expected there was a decrease in the temperature upon wetting it, the water having been collected the previous day and kept in an unheated room. Since more water was required to continue the test with the green and the black colonized stones, tap water was collected which was presumably at a higher temperature than the one used for the clean stone. Furthermore, both colonized stones were cooler than the clean stone, with the green one warmer than the black one, and there was an overall increase in temperature. The results are reported in Table 6.1. This served to highlight the importance of recording the water temperature used as well as the environmental conditions.

Table 6.1: Preliminary testing data 1.

Average Temperature (°C)			
	Clean Stone	**Green Biocolonization**	**Black Biocolonization**
Dry	13.48	10.52	8.59
Wet	9.79	12.37	13.2

Further preliminary testing was carried out to determine the most appropriate size of the area to be tested (Appendix A) and it was determined that a complete block was possibly the best approach to obtain a response from a more representative area. Subsequent testing developed the protocol that was later adapted into the experimental procedure as well as determining what size area should be used and the time intervals at

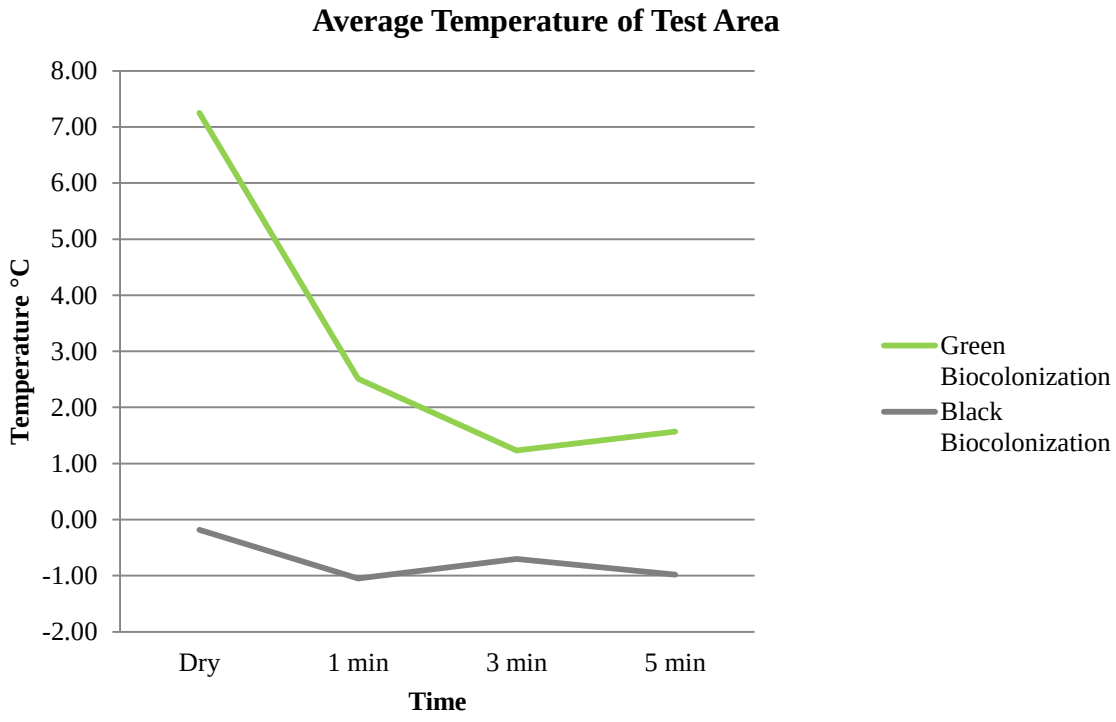
which images should be taken after wetting. Table 6.2 provides data for a single stone block when it is dry and then at one, three, and five minutes after wetting with water at 23°C. Figure 6.1 illustrates how the temperature changed as the water evaporated from a green and black biocolonized stone.

The protocol developed consisted in measuring the temperature change of the stone every minute for five minutes after water was applied to the surface of the stone. This protocol appeared initially to be useful to evaluate the performance of the biocides, as the changes induced by the dying off of the biogrowth would be reflected in the amount of water absorbed by the stone.

Table 6.2: Single stone temperature readings for the two bicolonizations after wetting with water at 23°C.

Average Temperature (°C) of Test Area		
	Green Biocolonization	**Black Biocolonization**
Dry	7.25	-0.18
1 min	2.51	-1.05
3 min	1.23	-0.70
5 min	1.57	-0.98

Figure 6.1: Change in the average temperature of a single stone by biocolonication type.



6.4 Experimental Procedure Developed

Test areas on the Fisher Fine Arts building were selected based on the extent of biocolonization covering the stone and at an easily accessible height. There are test areas for the two main types of biogrowth present on the building, i.e., green and black, including a control area for each of them, and an apparently uncolonized, i.e., clean, stone was chosen as a "blank" for monitoring. The test areas for the green and black biocolonization are at the same height from the ground to avoid introducing other variables that might be present due to the test area's proximity to the ground. However, the green colonized area is on the north wall of the building while the black is on the west side of the apse. Each test area was divided into three sections, one section for each biocide, D/2 and BioWash, and an untreated control section between the two.

The test areas located on the fifth course of stone from the ground and consist of three stones each, one for each of the two biocides and the third, located between the tow, an untreated stone serving as a control. Having the untreated stone in the middle provides a buffer between the two biocides. Due to the fact that different types of biocolonization appear on the building in different locations, the green biocolonized test area is on a north facing wall (Figure 6.2) so as to have it at the same height as the black biocolonized test area on a west facing wall (Figure 6.3).

The biocides were applied to the test areas by brushing them onto the surface of the stones with a natural bristle paint brush and no mechanical cleaning of the stones was attempted so that the effectiveness of biocide action could be studied over the course of four months. The changes in temperature induced on the stone by the applied biocides were measured following the protocol described above, while using water for the control areas. This set of data was considered the starting point for the evaluation.

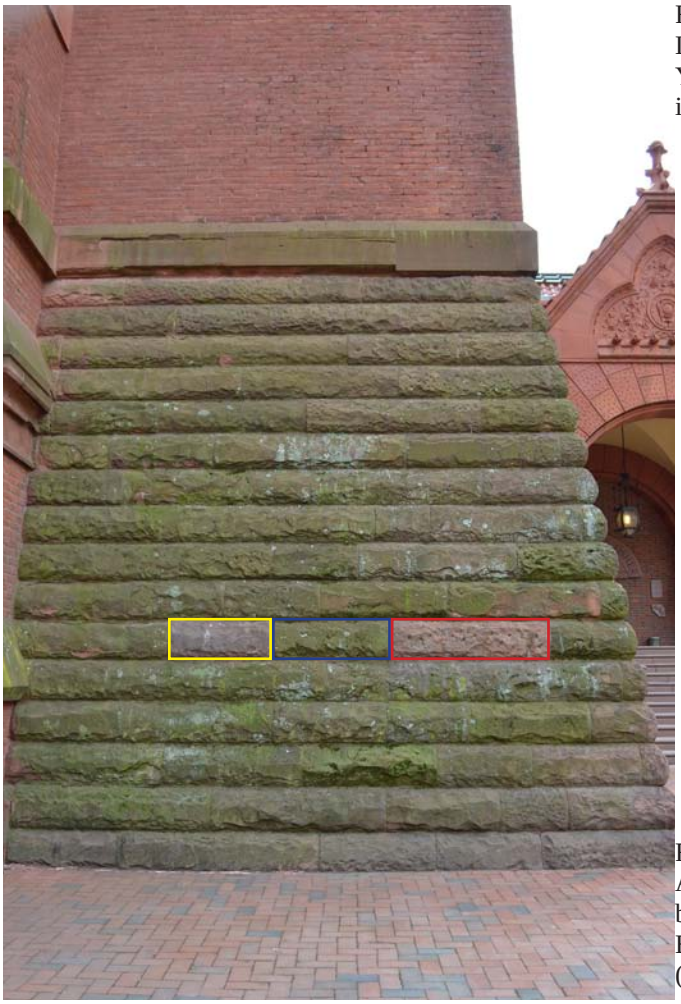
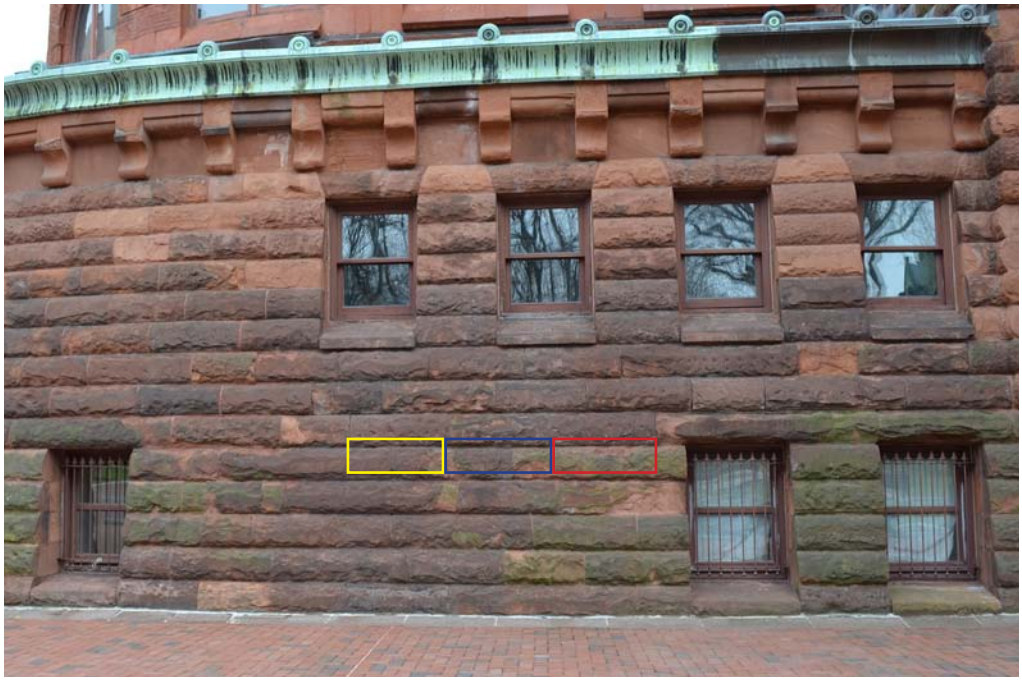


Figure 6.2: North wall of Fisher Fine Arts Library. Test area for green biocolonization. Yellow is D/2 test area. Blue is control. Red is BioWash test area. (Right)

Figure 6.3: West side of the Fisher Fine Arts Library apse. Test areas for black biocolonization. Yellow is D/2 test area. Blue is control. Red is BioWash test area. (Bottom)



The test areas were monitored once a week for the first two weeks and then once every month using infrared thermography. During each measurement thermal images of the test areas were taken and the effectiveness of the biocide was evaluated from the data that these images provided. The temperature data that was collected was plotted for each test area based on the average temperature of the stone for each time interval.

Since the thermal imager measures changes in temperature, the temperature of the stone decreases after it is wetted because of the evaporation of the water. On a stone covered with biogrowth, the biocolonization will change the amount of moisture absorbed and that evaporated, therefore in principle, the change in temperature will be different than that of a clean stone. For each measurement that was taken the weather conditions were noted, including ambient temperature, relative humidity, amount of shade of the test area, the presence and intensity of wind, and the cloud cover of the sky.

Upon the completion of the experiment the visible light photographs from before treatment and after treatments were compared to determine to what extent the biocolonization has been eliminated. The thermal image and temperature data that was collected for each test was compared for each biocide and each type of biocolonization to determine if it could be correlated to the effectiveness of the two biocides used. Water absorption test using a RILEM tube was also carried out to compare between the biocolonized control stone and the one(s) treated with the biocides, to determine any difference in water absorption between them.

Chapter 7. Results and Evaluation

7.1 Photographic Evaluation

Photographs of the apse and portions of the Fisher Fine Arts Library that is clad in sandstone were taken in May 2012 to capture the extent of biocolonization on the building. As shown in Fig. 7.1 the apse is colonized in the lower courses by green bioorganisms, such as green algae, lichens and protonema, and then the colonization takes on a black appearance, mostly due to cyanobacteria, while the top courses do not show any obvious biocolonization. Differences in colonization can be attributed to the amount of moisture that is available to the various courses. The lower, green colonized courses receive more water (rising damp, backsplash from the rain, residues from deicing salts, and washing of the court) than the middle courses. These, receiving less water and having a higher exposure to sunshine, can only be colonized by organisms that develop black pigments that protect them from UV radiation and of surviving dry spells. To be noted is that black colonization starts at a point where water falls down on them from the roof. The uncolonized top courses are protected by the overhanging copper roof eaves.

Figure 7.1 View of the Fisher Fine Arts Library apse showing the two areas with green and black biocolonization. Blue arrow show where overflowing rain from the roof hits the apse, 2012. (J. Focht)

Prior to the application of the two biocides photographs were taken of the chosen test blocks. The same images were then taken after treatment to visually compare the effectiveness of the biocides. Comparison of photographs before and after a treatment application is fundamental in documenting any action taken on a historic building. The photographs below (Figure 7.2) shows the area on the building where green biocolonization has developed, located on the north wall adjacent to the apse, before and after treatment. Note that this wall does not receive much sunlight which explains why green biocolonization has reached higher courses than on the apse.

Figure 7.2: North wall of the Fisher Fine Arts Library where the treatment was applied to remove green biocolonization. Left, in May 2012 . Right, March 2013 four months after biocide application. Note that the stones to which the biocide(s) was applied can be easily identified.

Close up photographs of the blocks show the results better, but it has to be considered that the initial photographs were taken in the fall, when biocolonization was in decline. Figure 7.3 depicts the control block for green biocolonization, before and four months after treatment. Note the increased biocolonization as spring begins.

Figure 7.3: Top. Control block for green biocolonization, November 2012. Bottom. The same block ,four months later, March 2013.

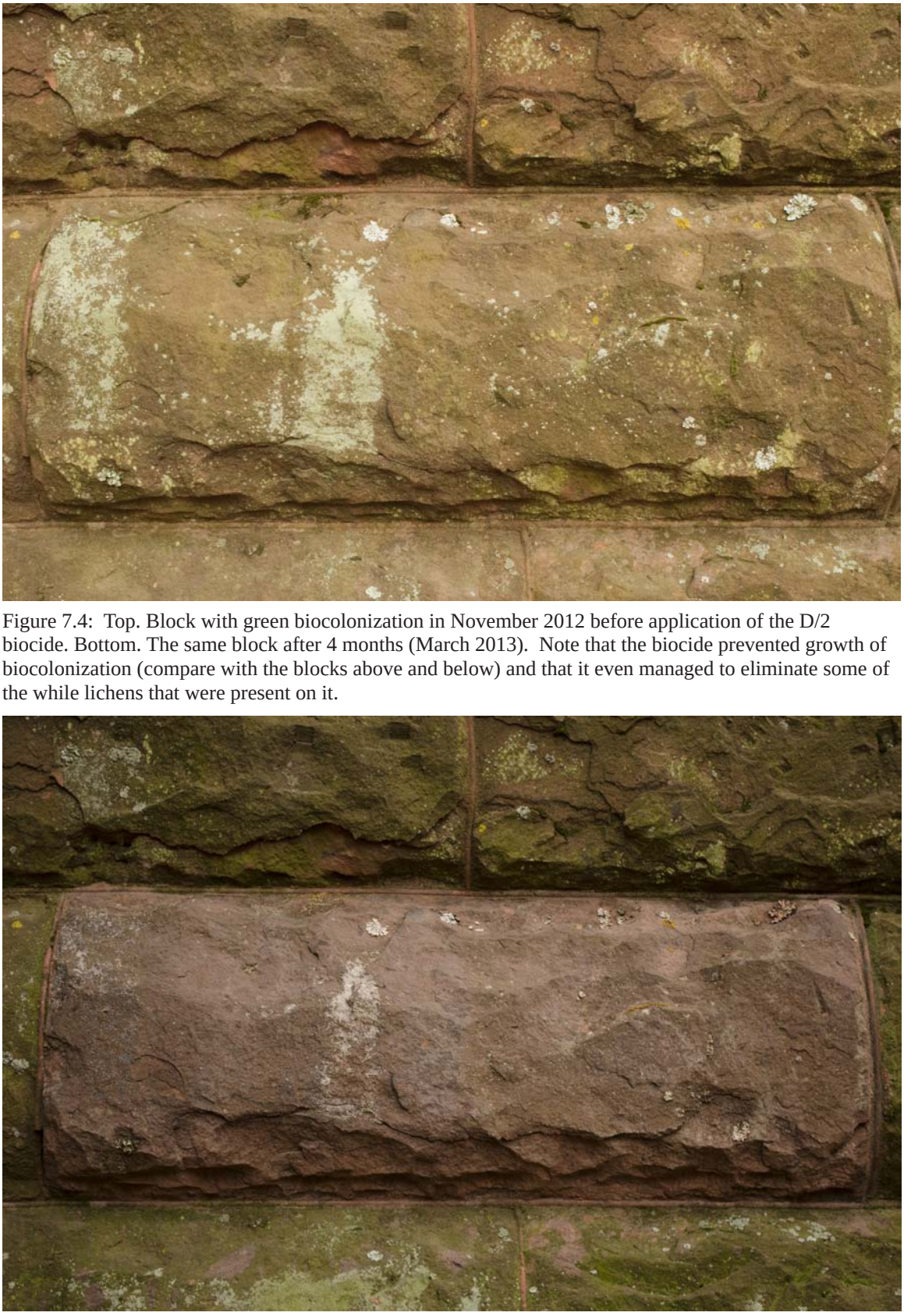


Figure 7.4: Top. Block with green biocolonization in November 2012 before application of the D/2 biocide. Bottom. The same block after 4 months (March 2013). Note that the biocide prevented growth of biocolonization (compare with the blocks above and below) and that it even managed to eliminate some of the while lichens that were present on it.

Figure 7.5: Top. Block with green biocolonization in November 2012, before application of the BioWash biocide. Bottom. The same block after 4 months, March 2013.

The green biocolonized block treated with D/2 is shown in Figure 7.4 and it can be seen that the biocide prevented biocolonization (compared with blocks above and below) and that it even managed to eliminate some of the white lichens that were present on it. As a result of the biocide application the microorganisms died, and the color of some of them changed. This can be seen with the lichen in the upper right hand corner of this block, which turned a reddish-brown color, almost the same color as the sandstone.

The block with green biocolonization that was treated with BioWash can be seen in Figure 7.5. Practically no biocolonization developed and some of the white lichens were removed as a result from the application of the biocide. The moss that was present on this stone, mostly in the dimpling, turned from a dark green to golden brown as a result from treatment. These mosses are now detached from the surface and fall off the stone with the slightest touch.

Testing on the black biocolonization was carried out on the apse, where it is predominantly present on the center courses. The test area is located on the west facing side of the apse. The photographs below (Figure 7.6) depict the area prior to treatment and four months after the biocides were applied. Figure 7.7 shows a close up of the control block with black biocolonization. Colonization has not changed significantly as in the case of the green biocolonization. The same trend occurs with the black biocolonized blocks that were treated with D/2 (Figure 7.8) and BioWash (Figure 7.9) where no significant change can be seen between the before and after treatment photographs. However, because of the rather wet spring, some traces of green biocolonization can be found on the control block and surrounding blocks of the biocide treated one, but none on the treated surfaces.

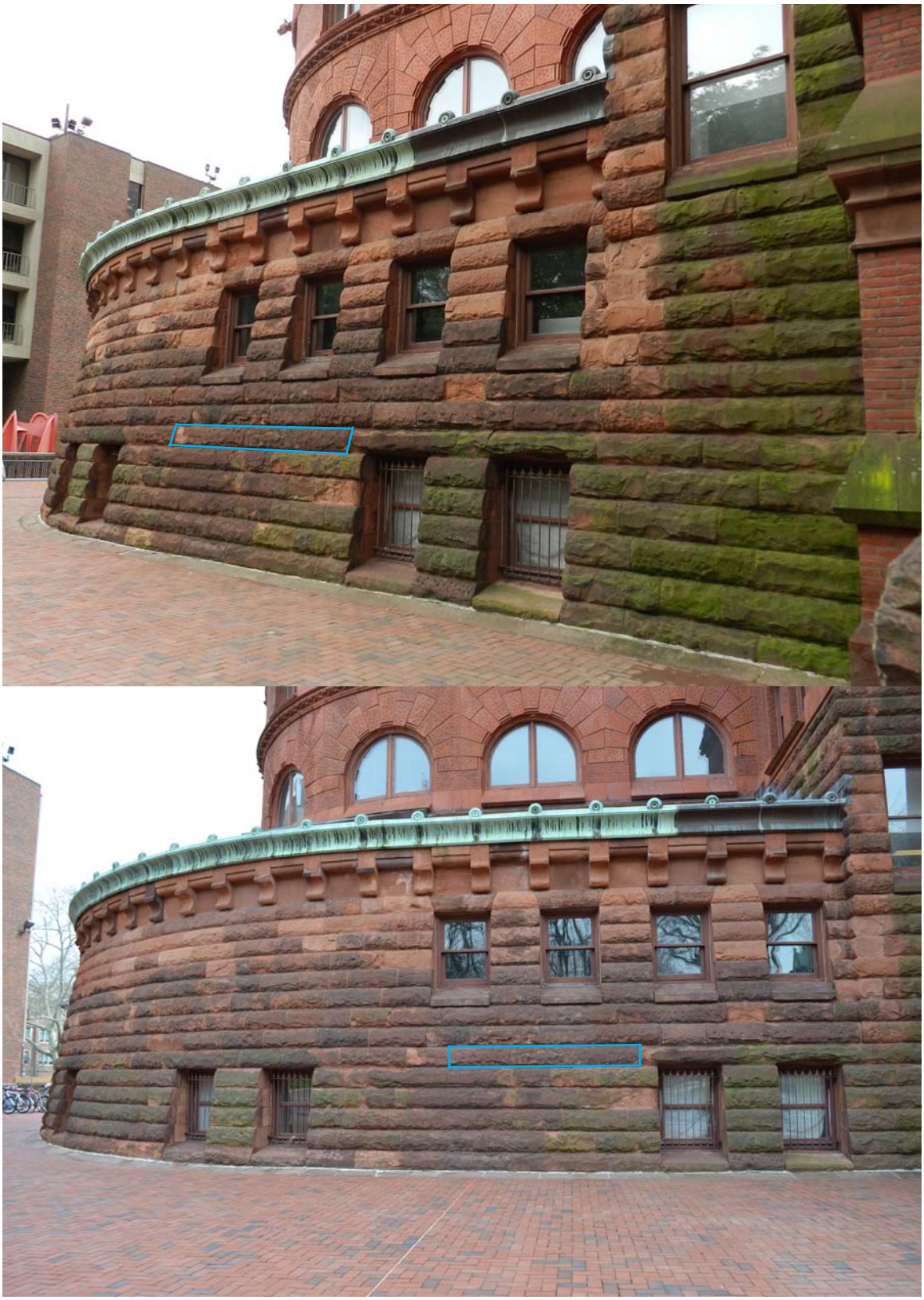


Figure 7.6: West wall of the Fisher Fine Arts Library apse where the treatment was applied to remove black biocolonization. Top, in May 2012 . Bottom, March 2013 four months after biocide application.

From the photographic evaluation of the applied biocides, it can be shown that both biocides are effective for the green biocolonization, mostly algae, lichens and protonema, i.e., threads of cell chains that will develop into moss, but appear not to affect the black biocolonization. However, since this colonization is more resistant than the green one, the effect of the biocide may require more time than for the green ones. Futhermore, it is known that sandstones containing iron, may develop a black patina on areas that are regularly wetted from the migration of iron oxides to the surface of the stone. The formation of black varnish on rain washed surfaces, caused by wetting and drying cycles is a result of the migration of solubilized iron (and manganese) oxides from within the stone, to the surface of the stone where they redeposit as a black oxide layer. This surface layer changes the porosity and consequently the water absorption characteristics of the stone.[86] The mechanism of this migration is possibly aided by microorganisms and it is still under study.[87]

[86] C. Thomachot-Schneider, M. Gommeaux, and G. Fronteau. "Modifications of the Porous Network of Sandstone Accompanying the Formation of Black Varnish." *Environ Geol* 56 (2008): 580.

[87] Caitlin O'Grady, "The Occurrence of Rock Varnish on Stone and Ceramic Artifacts." *Reviews in Conservation* 6 (2005): 33-34.

Figure7.7: Top: Control block with black biocolonization in November 2012. Bottom: The same block in March 2013. Note that some green biocolonization can also be found.

Figure 7.8: Top. Block with light black biocolonization before application of the D/2 biocide in November 2012. Bottom. The same block 4 months after the biocide was applied, March 2013

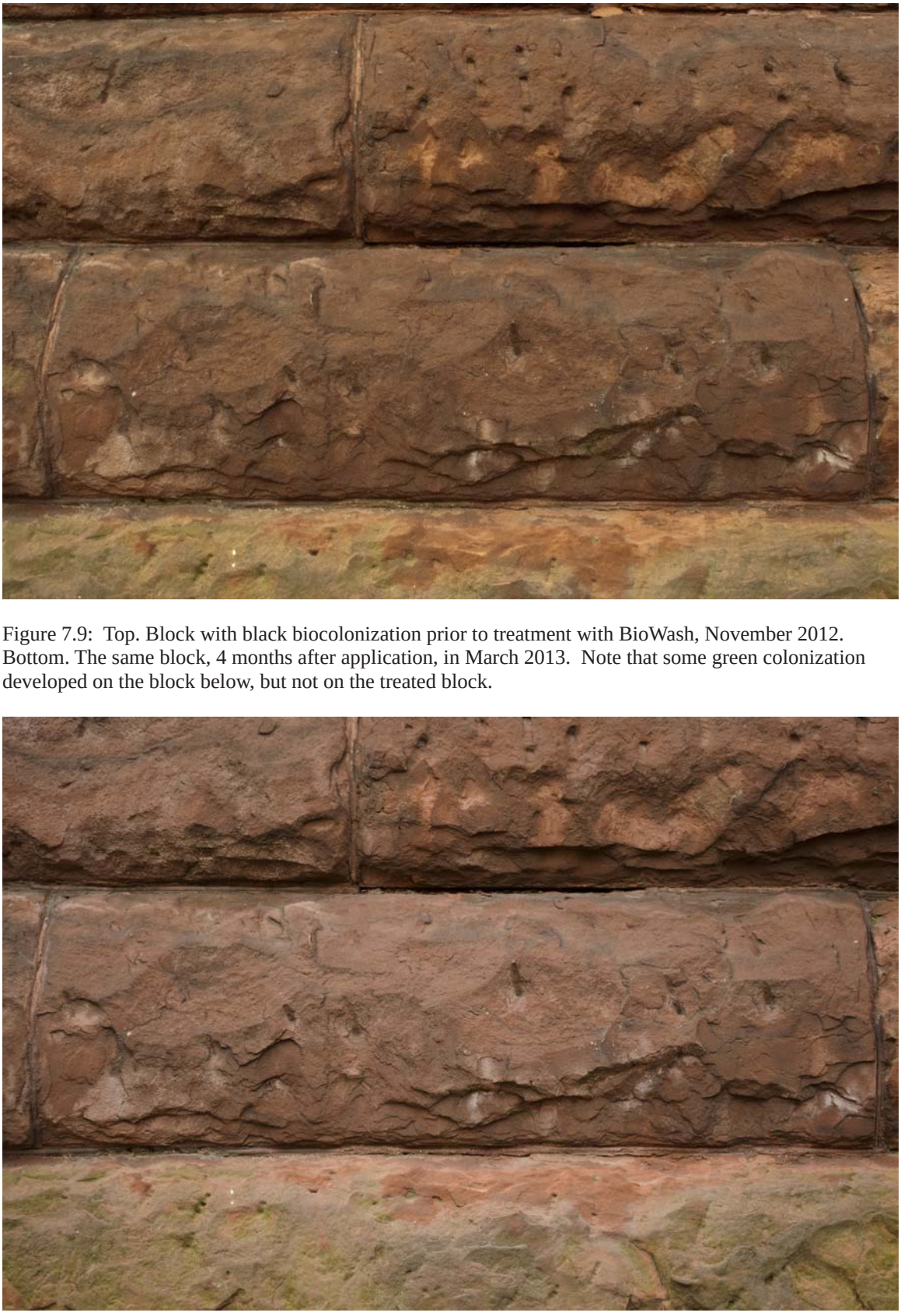


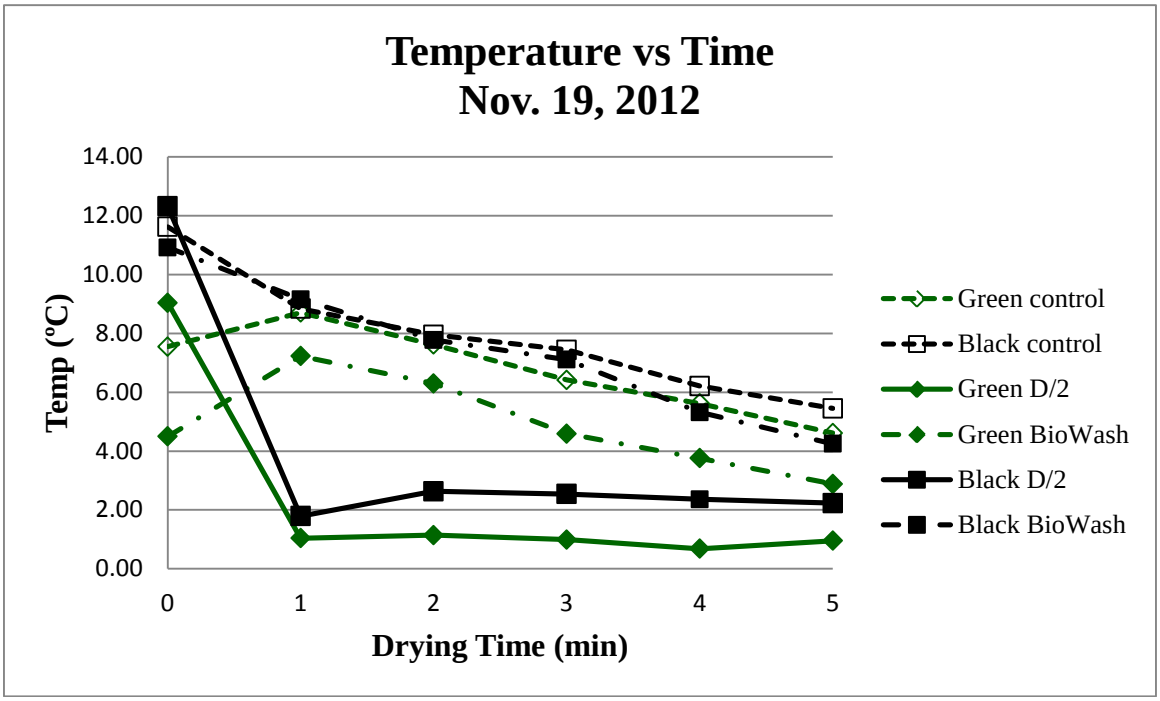
Figure 7.9: Top. Block with black biocolonization prior to treatment with BioWash, November 2012. Bottom. The same block, 4 months after application, in March 2013. Note that some green colonization developed on the block below, but not on the treated block.

7.2 Thermal Imaging Results

The changes in temperature that resulted from wetting an originally dry surface was followed for five minutes with the thermal imager camera. In total, seven areas were measured: three blocks with green biocolonization, three with black biocolonization, and a block that appears to be free of biocolonization, henceforth referred to as "clean". Of the three blocks of each biocolonization type, the central block served as a control and the other two were treated with a biocide, D/2 and BioWash, for the left and right block, respectively. The results obtained were plotted for easier comparison and the data obtained from the thermal images, plus all the climatic data are presented in Appendix B.

The first reading was obtained using the biocides brushed on, rather than spray wetting with water, except for both the control blocks, green and black, where water was used. Figure 7.10 shows the data corresponding to this first reading, November 2012, for all biocolonized stones. Apart from the differences in the initial temperature of the

Figure 7.10: Temperature change of blocks after application of biocides, November 2012.



blocks, due to their location and time of day when the treatment was applied, the most noticeable feature is the divergence in behavior for the blocks treated with D/2. The two control blocks and the black biocolonized block treated with BioWash all had similar temperature readings once they were wet.

Figures 7.11 through 7.14 show the graphs of temperature changes for the different colonized blocks corresponding to readings taken from December to March. The mild temperatures in December 2012 resulted in all the changes in temperature being fairly similar to each other (Figure 7.11).

Although the dry temperature of each stone was different, after water was applied the three stones with green biocolonization recorded almost exactly the same temperature change for all subsequent months, from January to March 2013 (Figures 7.12 to 7.14). The right (D/2) and center blocks always had the same temperature, while the left block (BioWash) was about 3°C colder, because of its location. The most significant difference in temperature of the dry stones was found in February 2013 with a difference of nearly 12°C between the black control and the green BioWash. While in January, differences were still observed for both green and black biocolonized stones treated with D/2, in the following three months, all the curves had similar slopes except for the one corresponding to the black biocolonization treated with D/2.

Figure 7.11: Temperature change of blocks, December 2012.

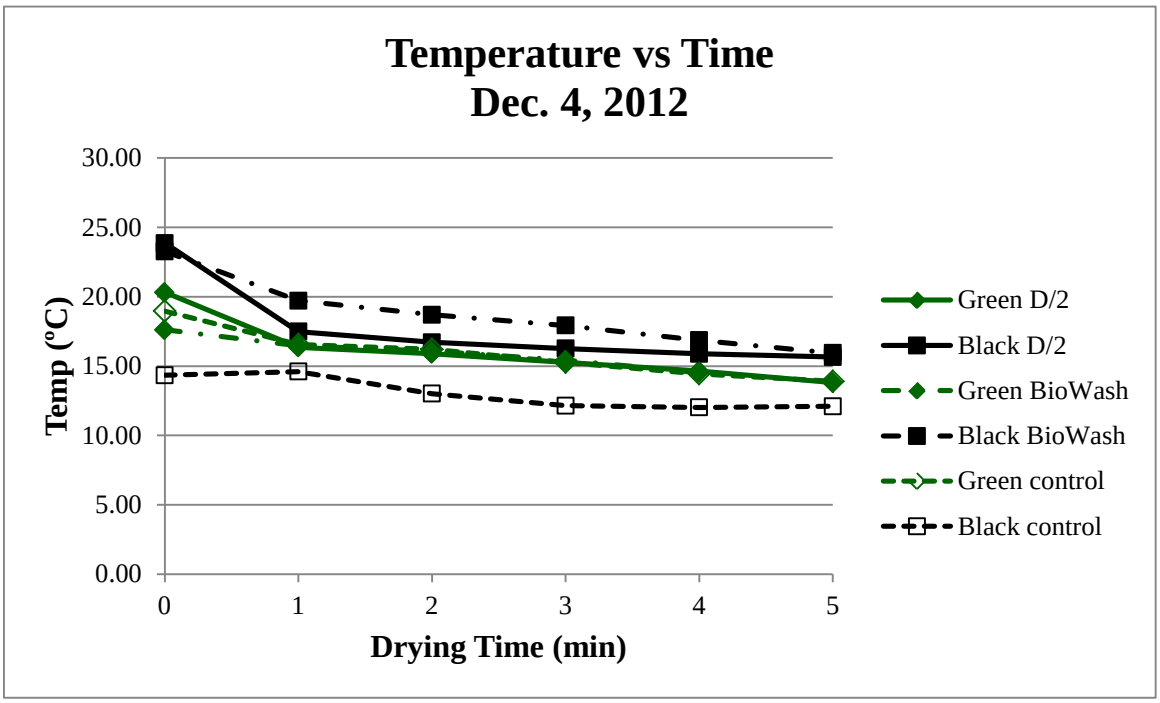


Figure 7.12: Temperature change of blocks, January 2013.

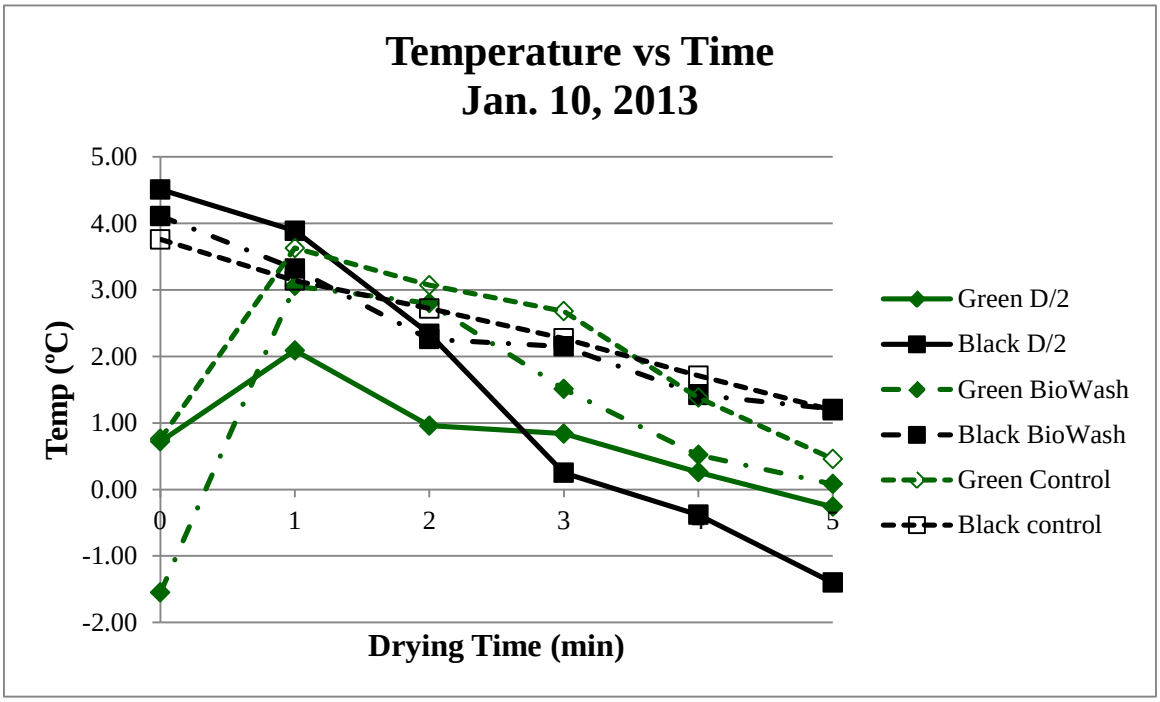


Figure 7.13: Temperature change of blocks, February 2013.

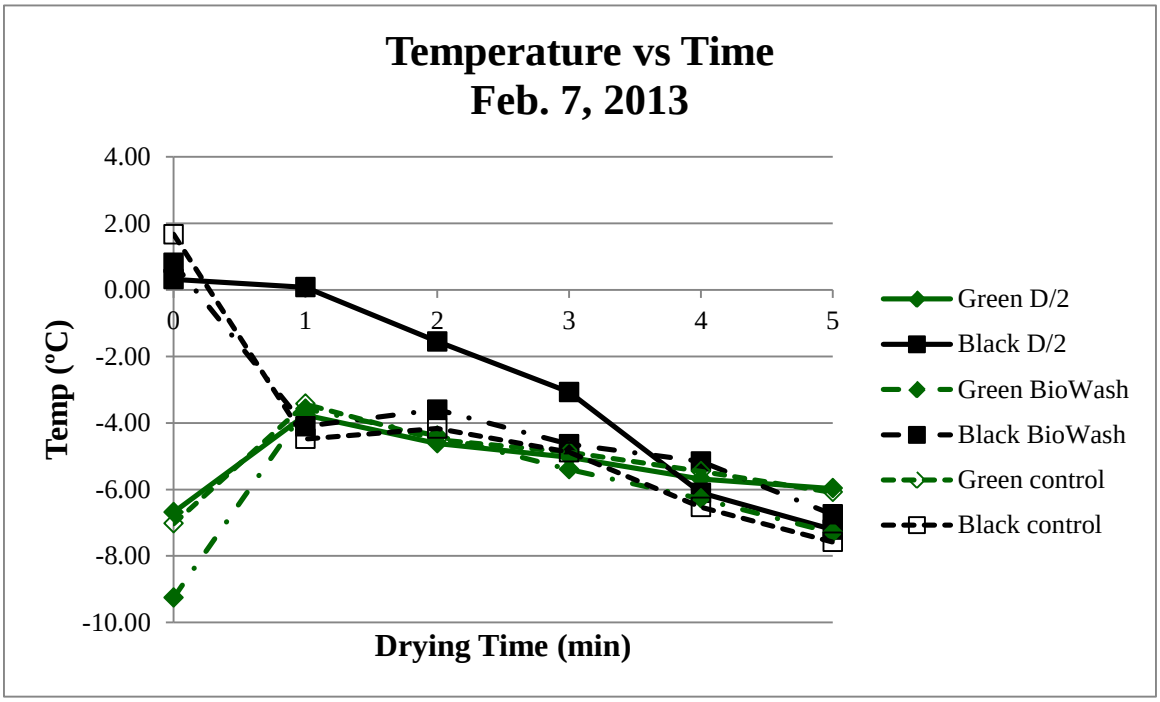
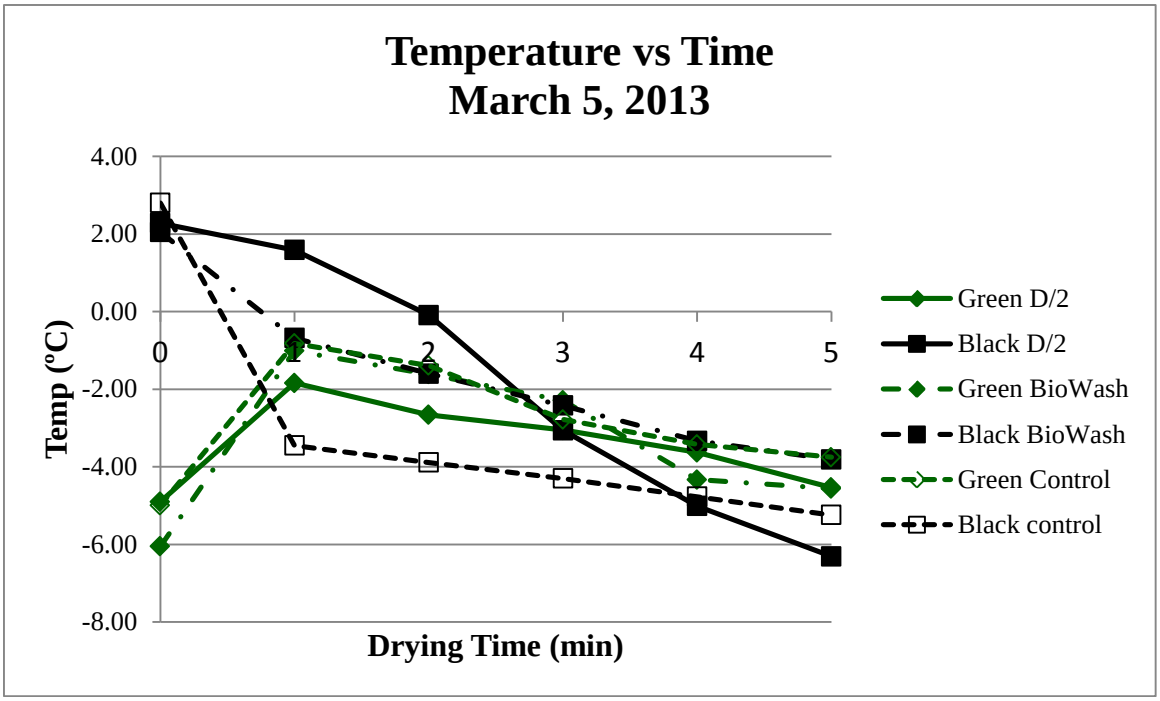


Figure 7.14: Temperature change of blocks, March 2013.



The data obtained were further analyzed and the temperature changes per unit time, what could be called instant slope, were calculated for all obtained readings. The formula used was:

Instant slope = (temp. @ time x – temp dry)/time x

Figures 7.15 through 7.19 show the resulting plots, one for each month, and include the values for the uncolonized, clean stone. The November data (Figure 7.15) corresponds to the cooling (and subsequent wariming in the case of the D/2) resulting from the application of the biocides for the treated stones and water for the controls. The D/2 curves are completely different from those of the BioWash and water and the spread of the initial data points is the largest, about 15°C. For December through March water was used for all measurements. All instant slopes tend to the same value, i.e., similar changes in temperature per minute, independently on whether the dry stone was warmer or colder than the water used to wet it. In December (Figure 7.16) and January (Figure 7.17) the initial spread of data is the smallest. Around 7°C, while it increased again for February

Figure 7.15: Instant slope graph, November 2012.

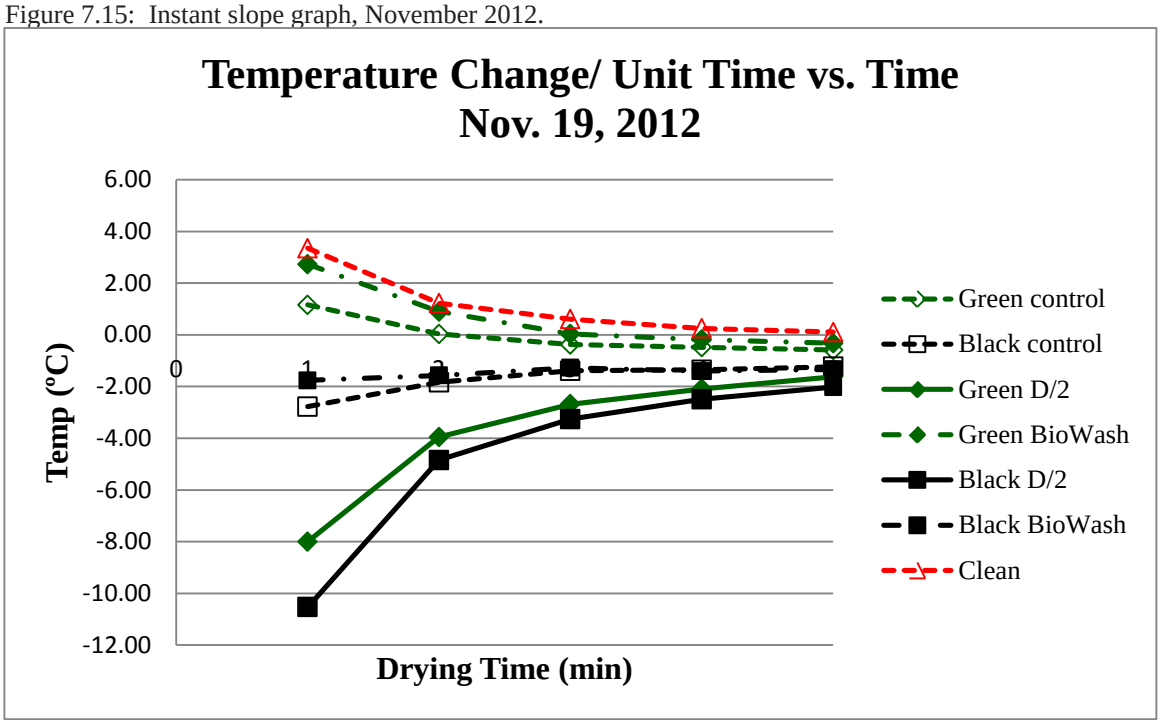


Figure 7.16: Instant slope graph, December 2012.

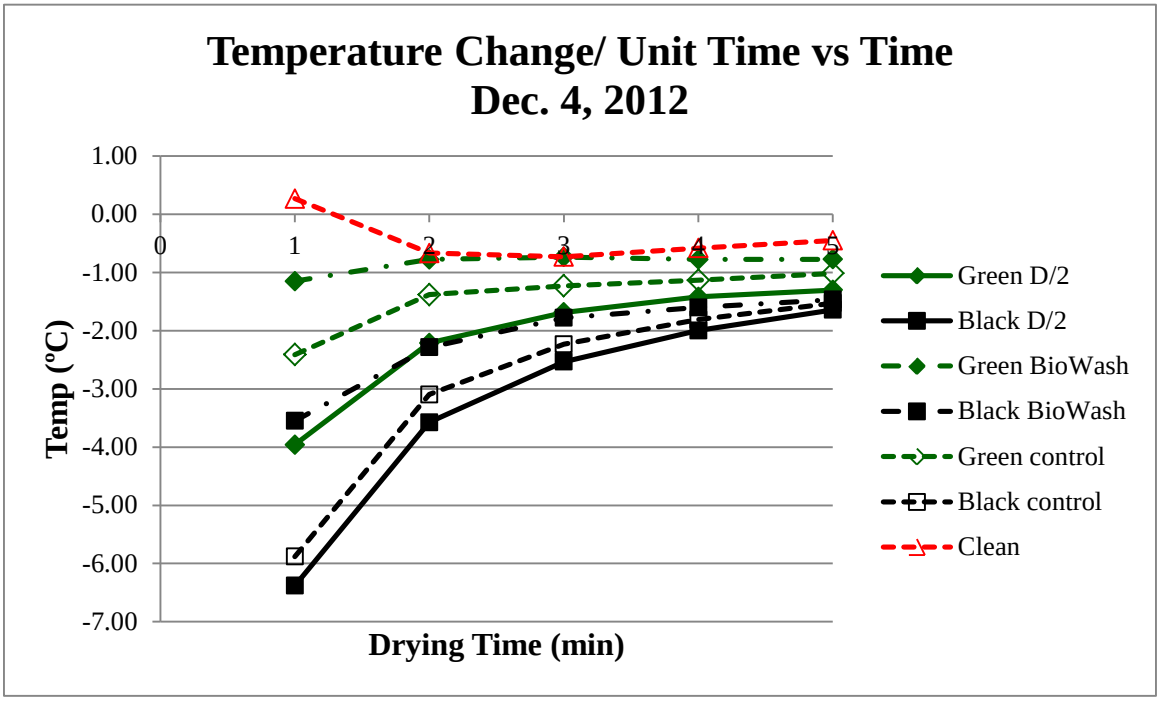


Figure 7.17: Instant slope graph, January 2013.

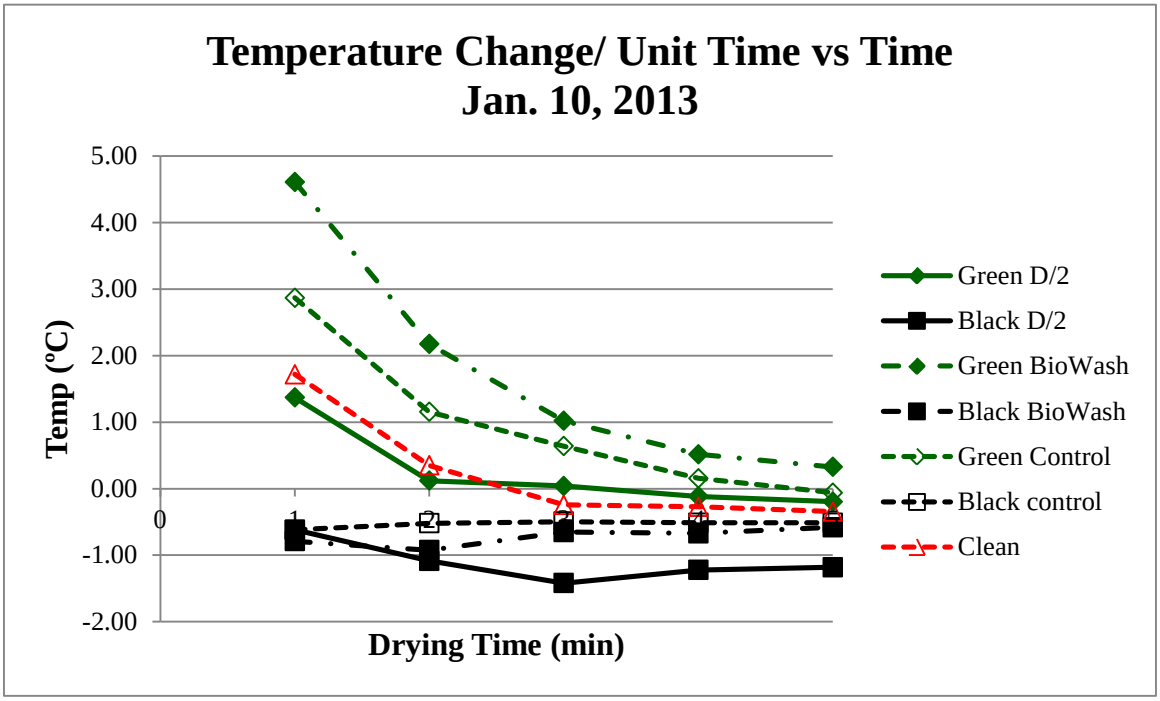


Figure 7.18: Instant slope graph, February 2013.

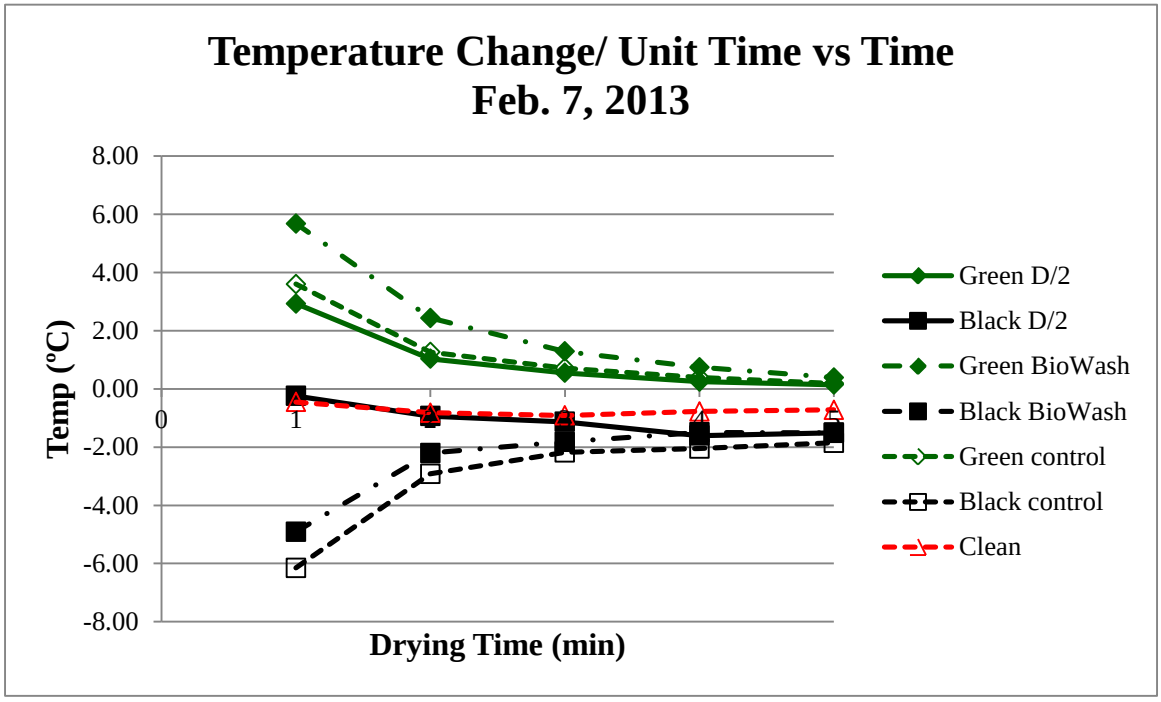
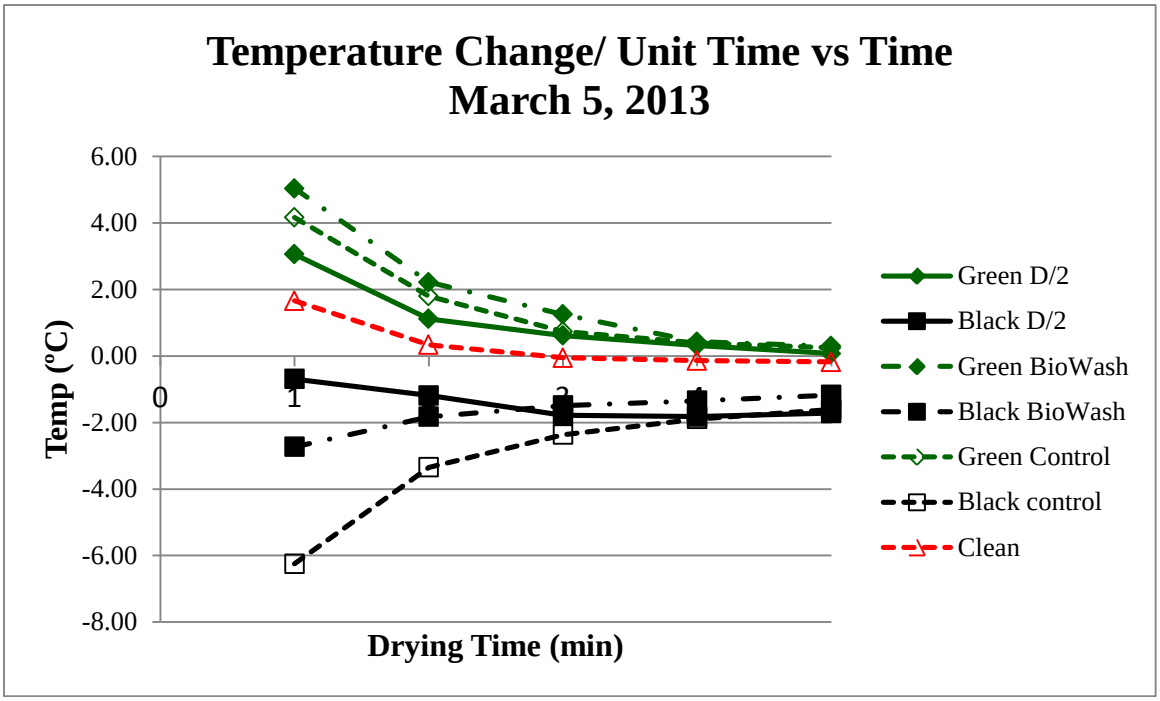


Figure 7.19: Instant slope graph, March 2013.



(Figure 7.18) and March 2013 (Figure 7.19). In these last months, the clean stone takes the center position between the green biocolonization above and the black one below and those treated with BioWash are the extreme ones. The fact that all the curves tend to attain similar changes in temperature in the last readings, be they from the uncolonized stone, the colonized ones and those treated with a biocide, appear to indicate that only the initial cooling data could provide information about the effect of the biocides. However, no clear attern could be detected between the colonized stone(s) and those treated with the biocide(s).

The overall slopes of the temperature change curves (Figures 7.11 to 7.14) were also calculated. The correlation factors for these slopes were mostly over 0.90, the great majority being over 0.97, with only two exceptions, those for the D/2 treated blocks in November. This can beattributed to the wetting with the biocide, that contain ingredients that will have a different cooling effect. The slopes correspond to the (wet-dry) temperature change rate and they are shown in Figures 7.20 and 7.21, plotted on the same scale to facilitate comparison. It is evident, that D/2, particularly when applied to black biocolonization has an unusual effect, very different from that of BioWash, and which can be attributed to differences in the formulation between these two products. On the other hand, BioWash applied to the black biocolonization shows a response close to that of the clean stone, as seen in the measurements from February and March.

For green biocolonization, BioWash shows initially no significant difference with the control (November through January) but this changes in the last two months where the slope increases indicating a higher cooling effect. In the case of D/2, the last three months show the most constant slope of all.

Figure 7.20: Temperature change rate of green biocolonization.

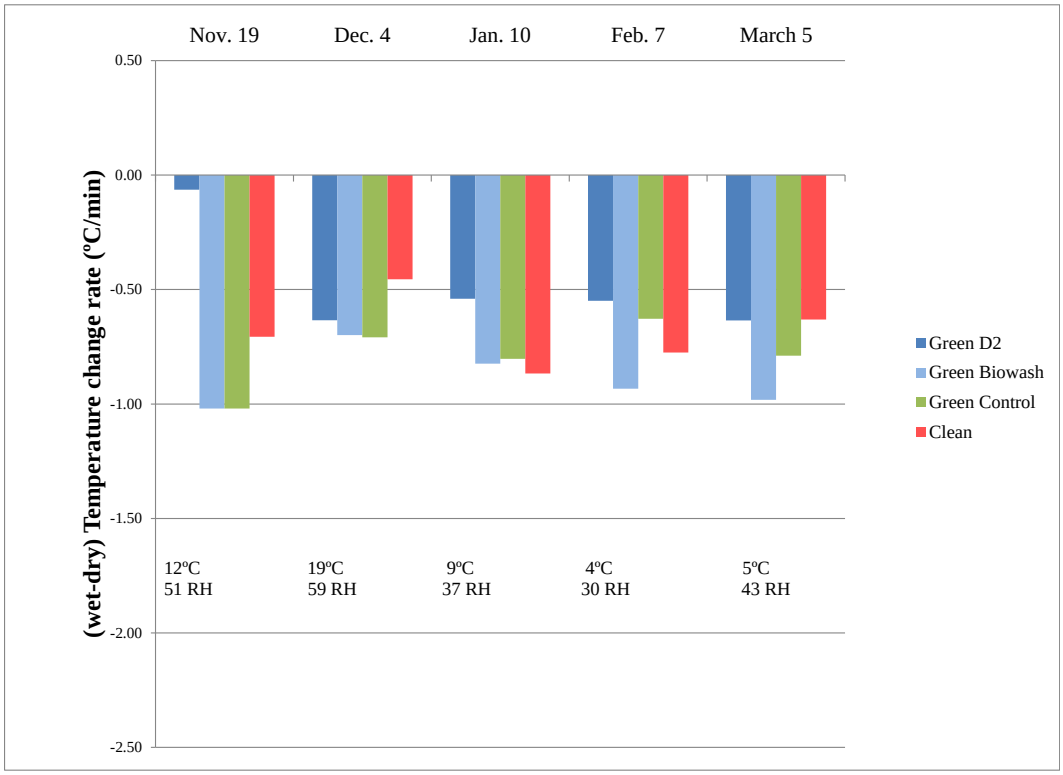
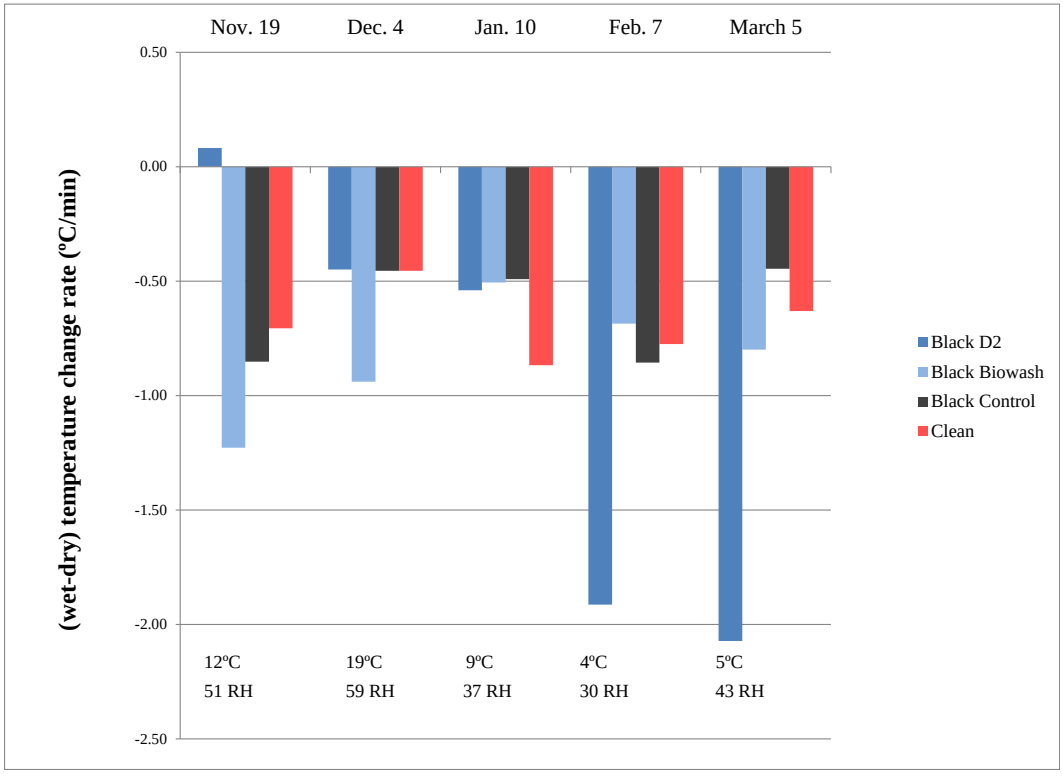


Figure 7.21: Temperature change rate of black biocolonization.



7.3 RILEM Tube Results

RILEM tube testing was carried out *in situ* on the three green and three black biocolonized stones and the clean stone to measure surface water permeability. Testing was also done on a second clean stone that is face bedded (the original one being naturally bedded), similar to the biocolonized stones (Figure 7.22). The tubes were applied with putty and left on the stones for a duration of two hours and the amount of water absorbed was recorded at 5, 10, 15, 20, 30, 60, and 120 minutes. The data collected for each stone was used to plot water absorption vs. time to determine rate of water absorption or water permeability. The data is presented in Table 7.1.

Figure 7.22: Top. Clean stone naturally bedded. Bottom. Clean stone face bedded.

After two hours the naturally bedded and face bedded "clean" stones absorbed almost the same amount of water, 1.5 mL and 1.4 mL respectively. However, it took the face bedded stone longer to start absorbing water compared to the naturally bedded stone. This indicates that the way the stone is bedded does not affect the way it absorbs water after prolonged exposure to moisture. Data collected from the clean stones were compared to the water absorption data for the green and black biocolonized stones as shown in Figure 7.23.

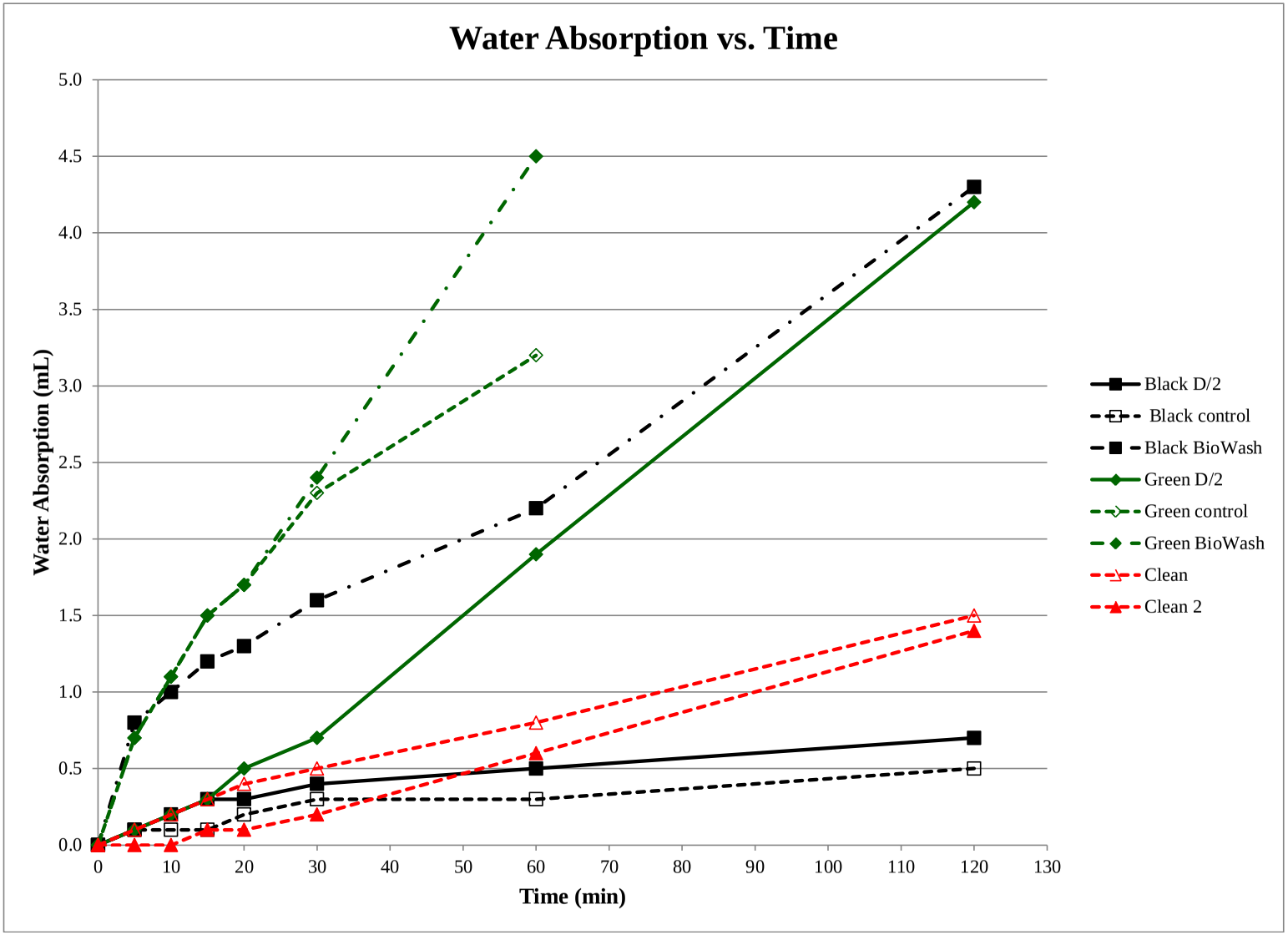
Table 7.1: RILEM tube Water absorption data for all stones.

Sample	Green Biocolonization			Black Biocolonization				
	D/2	Control	BioWash	D2	Control	BioWash	Clean	Clean 2
Bedding	face	face	face	face	face	face	natural	face
Temp (C°)	19.0	19.0	19.0	18.0	18.0	18.0	19.0	19.0
RH (%)	37	37	37	33	33	33	37	37
TIME (min)	Amount of water absorbed (mL)							
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.1	0.7	0.7	0.1	0.1	0.8	0.1	0.0
10	0.2	1.1	1.1	0.2	0.1	1.0	0.2	0.0
15	0.3	1.5	1.5	0.3	0.1	1.2	0.3	0.1
20	0.5	1.7	1.7	0.3	0.2	1.3	0.4	0.1
30	0.7	2.3	2.4	0.4	0.3	1.6	0.5	0.2
60	1.9	3.2	4.5	0.5	0.3	2.2	0.8	0.6
120	4.2	-	-	0.7	0.5	4.3	1.5	1.4

During the first half hour of testing the stone with green biocolonization treated with D/2 absorbed water at a similar rate to that of the clean stone. For the same time period the green colonized control stone and BioWash treated stone behaved very similar to each other, but absorbed water at a faster rate than the green D/2 stone. Between one and two hours both the green control and BioWash stones absorbed the remaining water in the tube suggesting that in the case of the control, the biocolonization was absorbing moisture, while the block treated with BioWash might still have subsurface colonization or the significant surface pitting could explain it. A reading could be obtained for two hours D/2 and the clean stones, since they absorbed water at a slower rate. The difference in water absorption of the stone treated with D/2 with respect to the clean one could be explained by the presence of remaining surfactants within the stone.

As the green D/2 and BioWash stone absorbed water, water could be seen being absorbed into the stone surrounding the tube suggesting a high pore interconnectivity.

Figure 7.23: Water apsorption graph for all biocolonization



This was not observed for the control stone, possibly because the biofilm was absorbing the water as mentioned above. The fact that all stones with green biocolonization, whether removed or not, had a higher water absorption suggests that the biogrowth can contribute significantly to the deterioration of the stone.

The stones with black biocolonization absorbed water at a very different rate than those which have green biocolonization. The stone treated with BioWash showed again the highest water absorption. Except for the black biocolonized stone treated with BioWash the other two stones, the black control and that treated with D/2 both showed a similar behavior to the clean stones. Actually, after one hour the clean stones absorbed more water than the other two. These results are partly at odds with those of the study by Warscheid and Leisen, where measurements carried out on a sandstone monument at Angkor Wat indicated that a cyanobacterial biofilm treated with an algal wash lowered the water absorption of the stone compared to an untreated cyanobacterial biofilm and even to one with no visible biocolonization.[88] In the present case, both the control and the D/2 treated are below the uncolonized stone. This could be explained if the black coloration is due to the presence of an iron oxide patina.

7.4 Conclusions

From all the data obtained it is evident that both biocides were effective in removing the green biocolonization. This is not so clear in the case of black biocolonization, since part of the black appearance might be the development of a black surface deposit of iron oxides, similar to the so-called “desert varnish”.

[88] Thomas Warscheid and Hans Leisen, “Microbiological Studies on Stone Deterioration and Development of Conservation Measures at Angkor Wat.” in *Biocolonization of Stone: Control and Preventive Methods*, ed. A. Elena Charola et al. (Washington D.C.: Smithsonian Institution Scholarly Press, 2011), 14.

The thermal imaging technique appears promising, however, interpretation of the obtained data is not easy. While photographic documentation confirmed that the green biodeterioration was affected by the application of the biocides, this is harder to correlate with either the obtained thermal data or the water absorption tests with the RILEM tube. The increased water absorption of the BioWash on both the green and the black colonized areas could be a result of residual compounds left in the stone and this could be correlated to the higher instant slope results in the last months (Figures 7.18 and 7.19) obtained with the thermal imager. However, this would have to be confirmed with a more thorough evaluation of the water absorption properties of this sandstone and further analysis to determine if organic residues from the biocides remain in the stone. On the other hand, the lower water absorption of both the green and black biocolonization areas treated with D/2 could be correlated to the lower changes in the instant slope results obtained.

Chapter 8: Final Conclusions and Recommendations

8.1 Final Conclusions

Both biocides tested, D/2 and BioWash, were effective in eliminating the green biocolonization that is present on the Fisher Fine Arts Library, including that found in some areas of the black colonized blocks (see Figures 5.8-5.10, 7.7) but do not appear to have made a difference on the black biocolonization. This might be due to the more resistant black colonization or the formation of a black iron oxide patina on the surface of the stones. The presence of biogrowth on the black colored areas was confirmed by SEM examination (see Figures 5.14-5.16). The water absorption of the two D/2 treated areas was lower than that corresponding to the BioWash. This difference could perhaps be attributed to variances in the formulation of the two biocides. The higher slopes of the cooling curves observed during the last two readings for the D/2 applied to the black biocolonization (Figure 7.21) appears to confirm that more evaporation took place, while for the case of this biocide applied to the green biocolonization (Figure 7.20) the slope remained practically constant for the last three readings, being lower than that for any of the other samples, a point that requires further evaluation.

The surface migration of iron (and manganese) oxides present in the stone may be aided by microorganisms. No samples were analyzed from the biocide treated areas because these were not spalling and sampling for this study was only carried out in areas where actual detachment was occurring, more frequent in the lower courses as a result of the higher moisture content that will increase freeze-thaw damage.

8.2 Future Investigation

One of the points that needs further consideration is the fact that if the metal gutter around the roof is made out of copper, as indicated in the Exterior Condition Assessment, Anne & Jerome Fisher Fine Arts Library, by John Milner Architects, Inc., then presumably copper ions should be washed down to the black colonized area. In this case, why did biocolonization appear? Could all of the black areas of biocolonization be the result of iron oxide surface migration? Samples from stones in the different black stained courses should be taken to determine whether apart from iron, and perhaps manganese, also copper is found. For this purpose, Scanning Electron Microscope Energy Dispersive X-ray Spectrometry could be used to carry out the required elemental analysis. Furthermore, a microbiological study would be needed to identify the presence of the different colonizers, particularly in the black stained area.

Further research and testing is needed to determine whether the thermal imager is effective as a monitoring tool for the evaluation of biocides. For this purpose, the correct emissivity of the biocolonized sandstones should be determined. The emissivity value used for testing was that of sandstone, however, sandstone covered with green or black biocolonization will alter its emissivity value. Is this difference significant? The exact emissivity should be determined to be able to obtain accurate measurements with this instrumentation.

It would be important to identify the quarry from which the red sandstone was quarried because it has not been identified as of yet. All that is known is that it is a red Pictou sandstone from Nova Scotia and that the Amherst Red Stone Quarry is one of the possible quarries while the other could have been the River John Quarry. This could be useful for obtaining replacement stones for future interventions and for a better characterization of the stone itself.

The presence of the higher moisture in the lower courses might be the result of deicing salts used over the years. A salt analysis campaign should be carried out to determine the presence of chlorides. If found, they could have migrated from the sodium chloride (NaCl) or calcium chloride ($CaCl_2$) used in the deicing products. Currently, more environmentally friendly deicing compounds are available.

8.3 General Recommendation for Maintenance

The following recommendations can be made for the maintenance of the apse:

- Monitoring of the biocide treated black colonized areas over the next six to eight months to determine whether any significant change can be observed as compared to the control.
- Application of D/2 to remove the green colored biocolonization.
- Only use environmentally friendly deicing salts.
- Ensure that there are no open joints in the structure, especially at the base of the apse, and in the surrounding pavement.

The D/2 biocide is recommended because it resulted in reducing the water absorption of the stone. The application of the biocide can be done by simple brushing and waiting for it to act, or by actively scrubbing the surface to eliminate the growth, as generally recommended by the manufacturers. This approach, however, is rather drastic for this building, considering the active flaking problem it presents. Therefore, the recommendation would be simply to brush it on and let the biocide slowly eliminate the colonization that will eventually fall off by itself.

The cleaning of the building with the mixture containing hydrofluoric acid in 1987, eliminated the surface layer of the stone through the reaction:

$$4\ HF + SiO_2 \rightarrow SiF_4 + 2\ H_2O$$

The resulting silicon fluoride (SiF_4) is volatile and eliminated. Any deposit on that surface will then be loose and eliminated. While it is an effective method for cleaning insoluble black deposits of any nature, it is a drastic approach as the acid will not only attack the surface but penetrate into the subsurface pore structure opening it by attrition of the pore walls.

Should it be proven that the black coloration is due to iron oxide surface migration, a natural process for this type of sandstone, the issue of its removal becomes a more complex decision. The iron oxides deposited on the surface are stable and so far no report has been found as to them inducing deterioration. Therefore, their removal can only be justified from an aesthetic point of view. However, their removal can only be achieved by eliminating part of the actual stone and its history, a procedure that is not acceptable within the ethical framework established by the Venice Charter.

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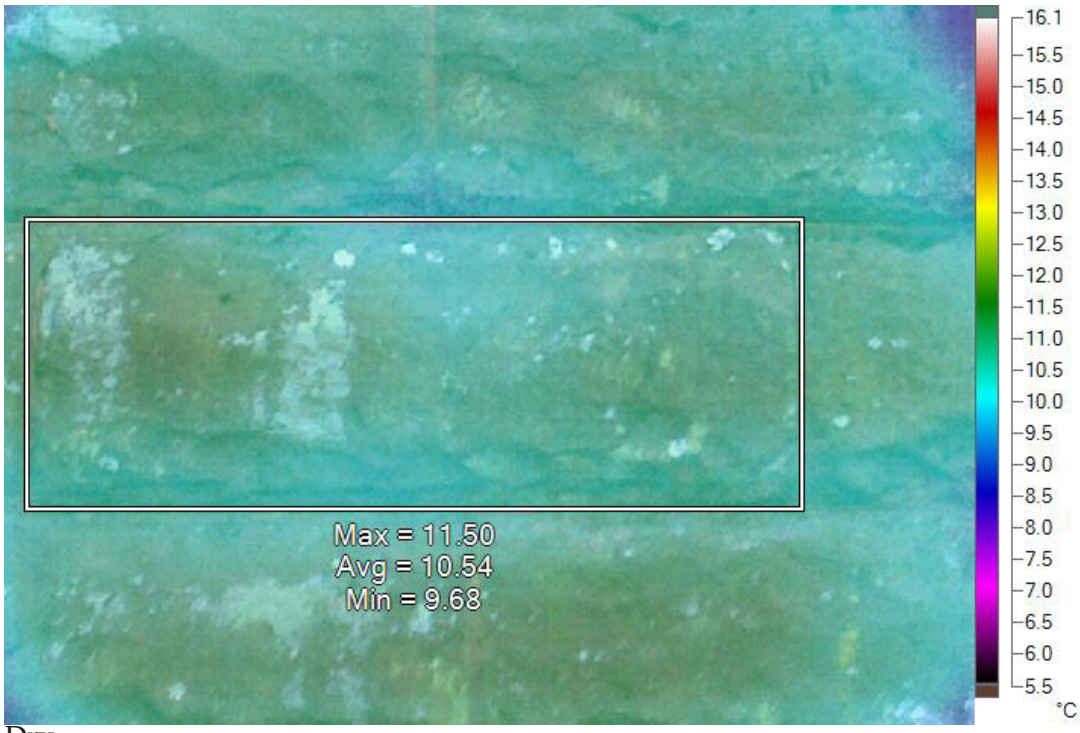
Appendix A. Preliminary Testing Data

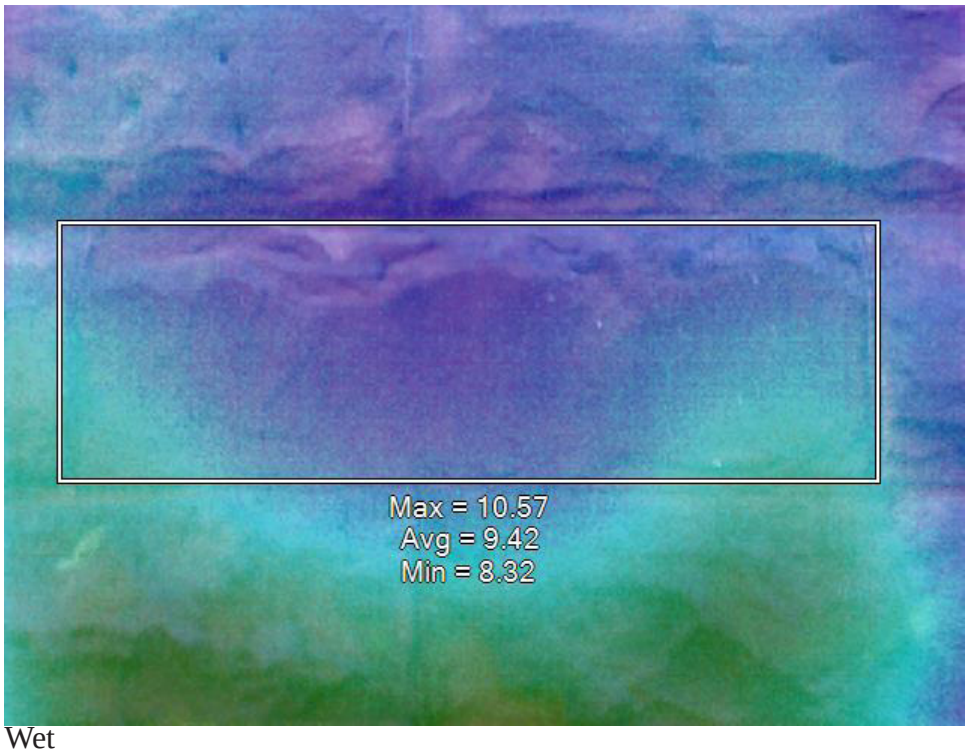
A.1 Single Stone Wet-Dry Test

October 12,2012

Green biocolonization

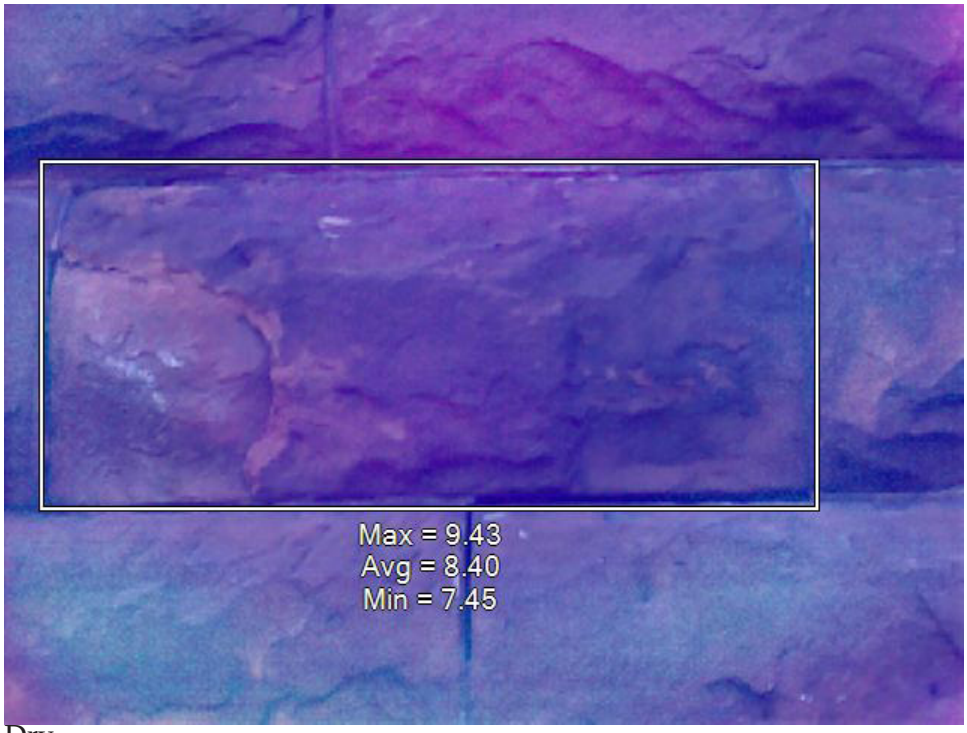


Dry

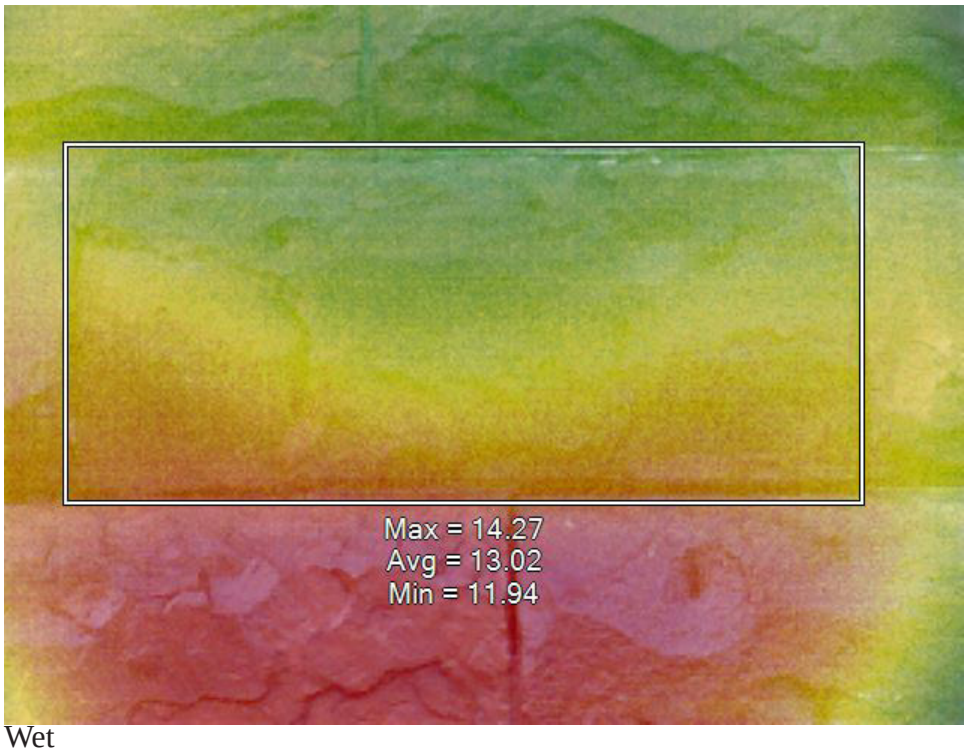


Wet

Black biocolonization

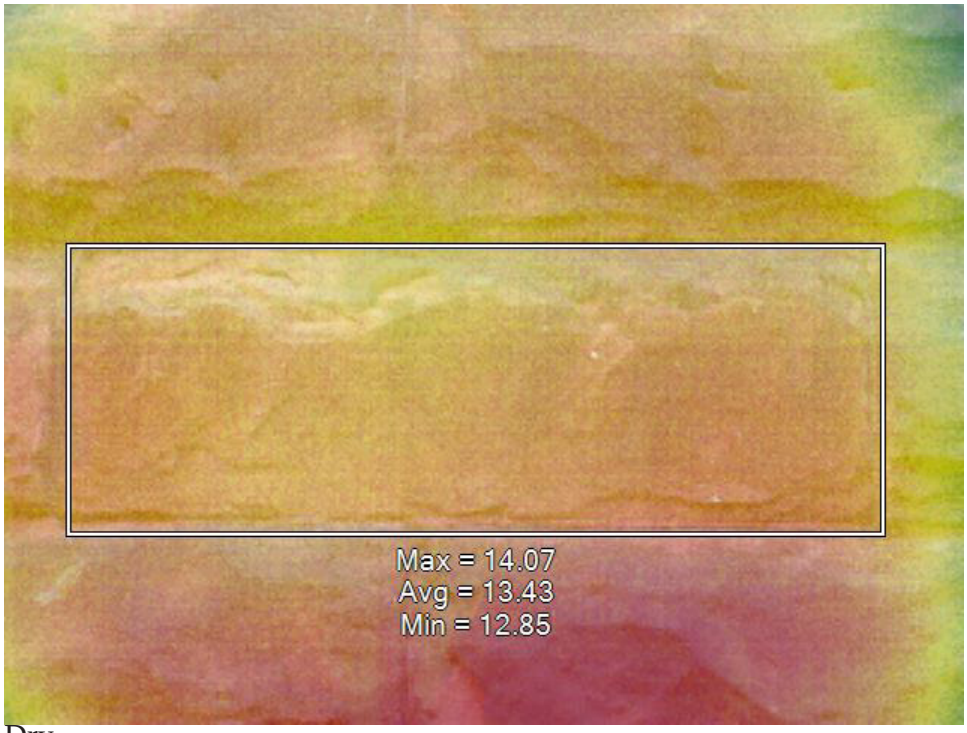


Dry

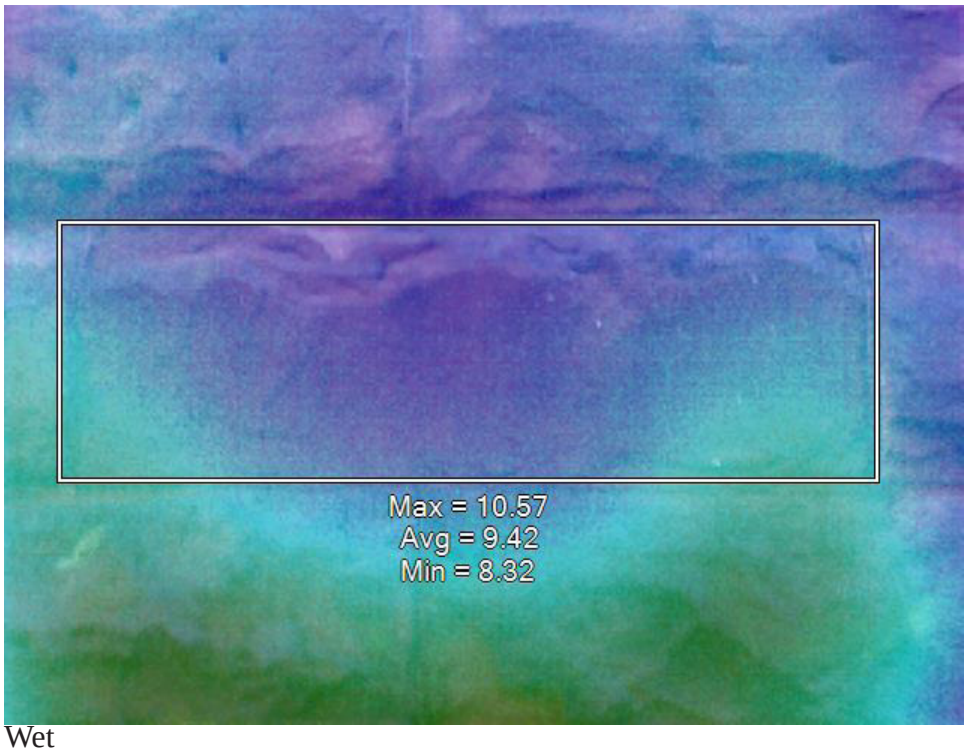


Wet

Clean stone



Dry

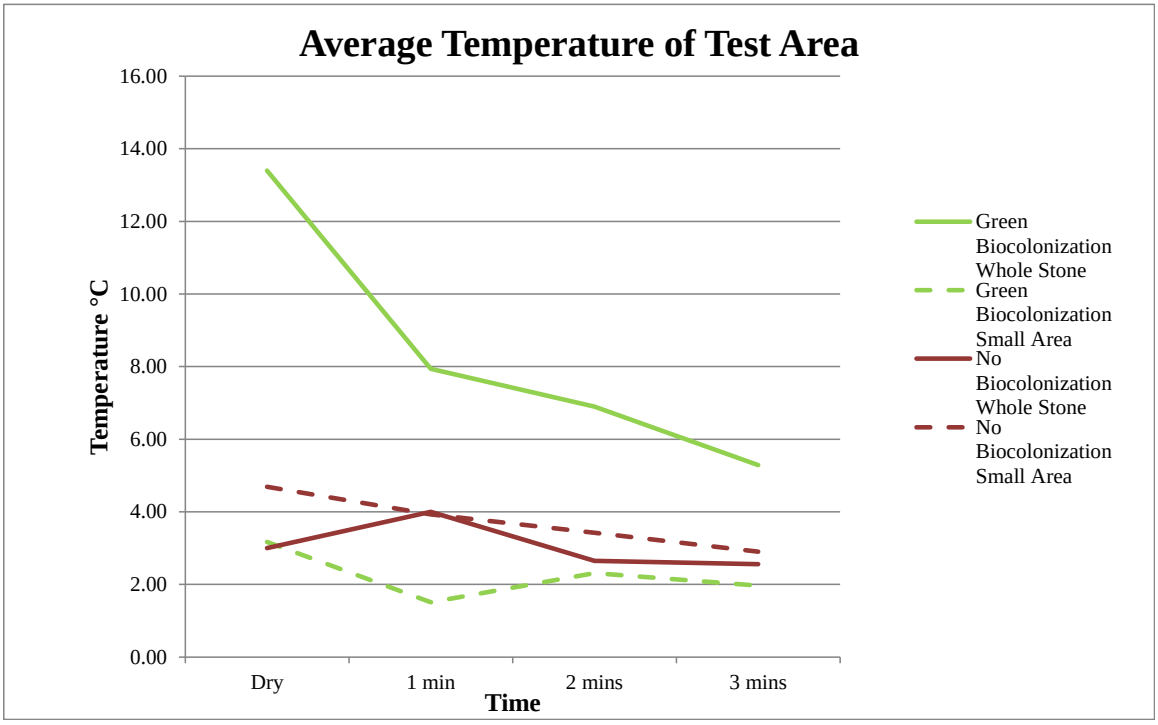


Wet

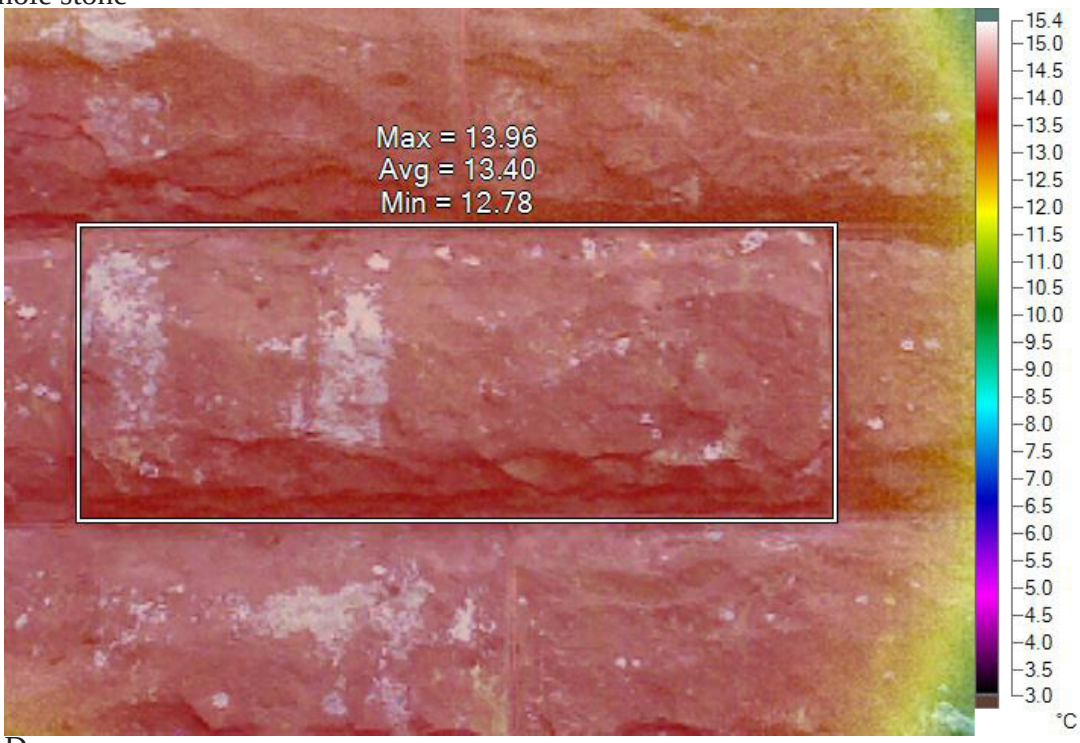
A.2 Single Stone vs. Small Area Test

November 2, 2012

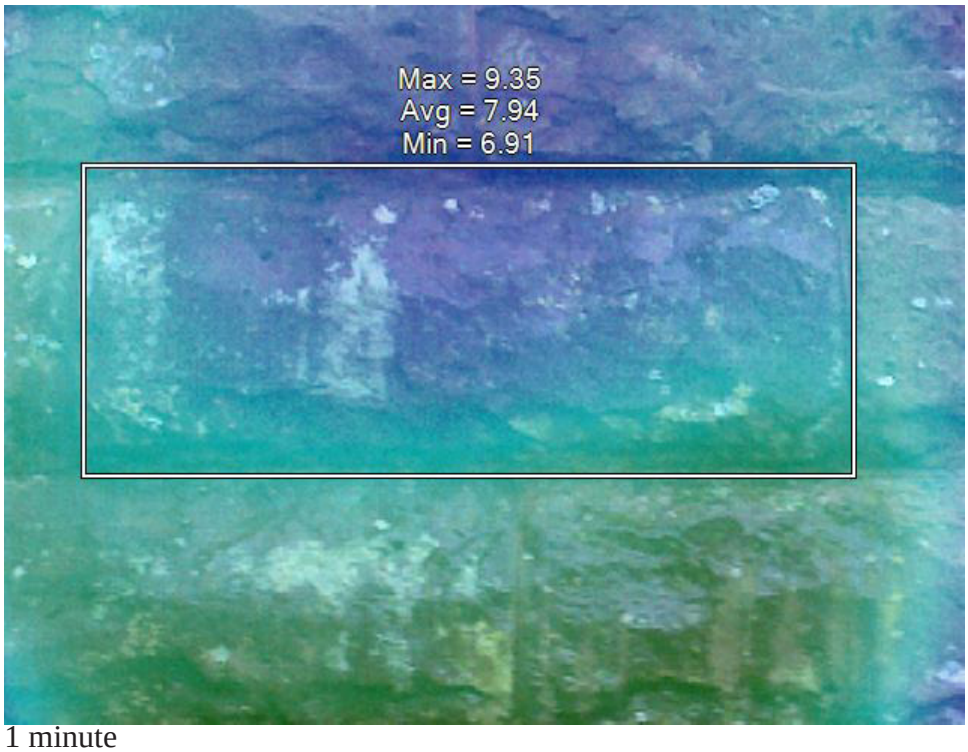
Average Temperature °C				
	Whole Stone		Small Area of Stone	
	Clean Stone	Green Biocolonization	Clean Stone	Green Biocolonization
Dry	3.00	13.40	4.69	3.17
1 min	4.00	7.94	3.93	1.51
2 mins	2.65	6.90	3.42	2.31
3 mins	2.56	5.29	2.90	1.97



Green biocolonization
Whole stone

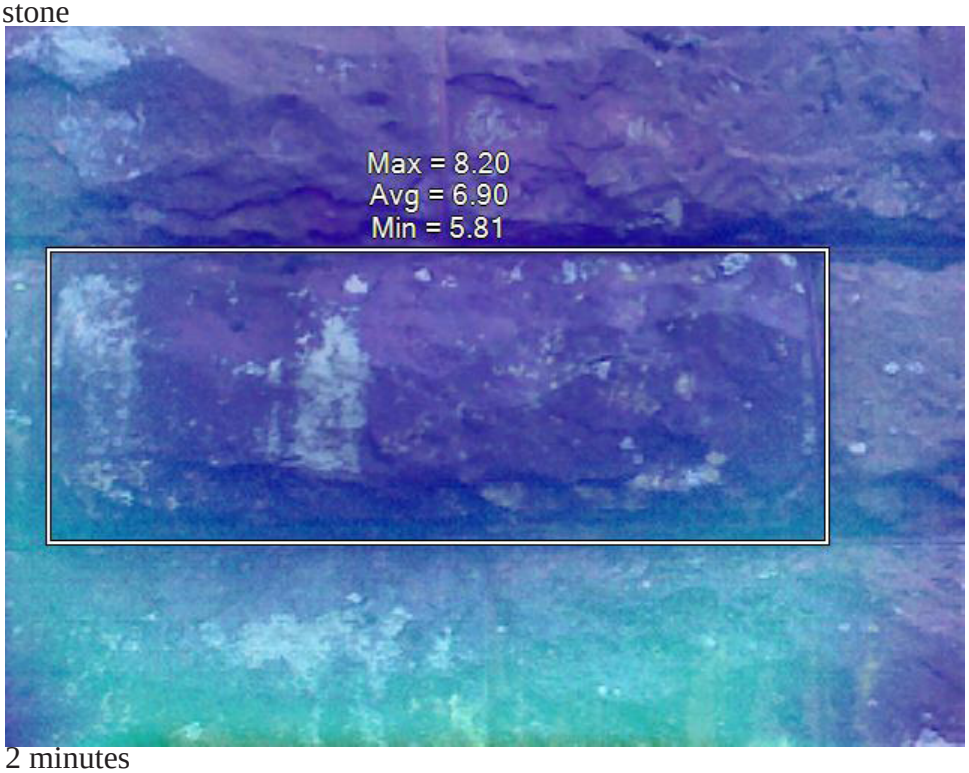


Dry

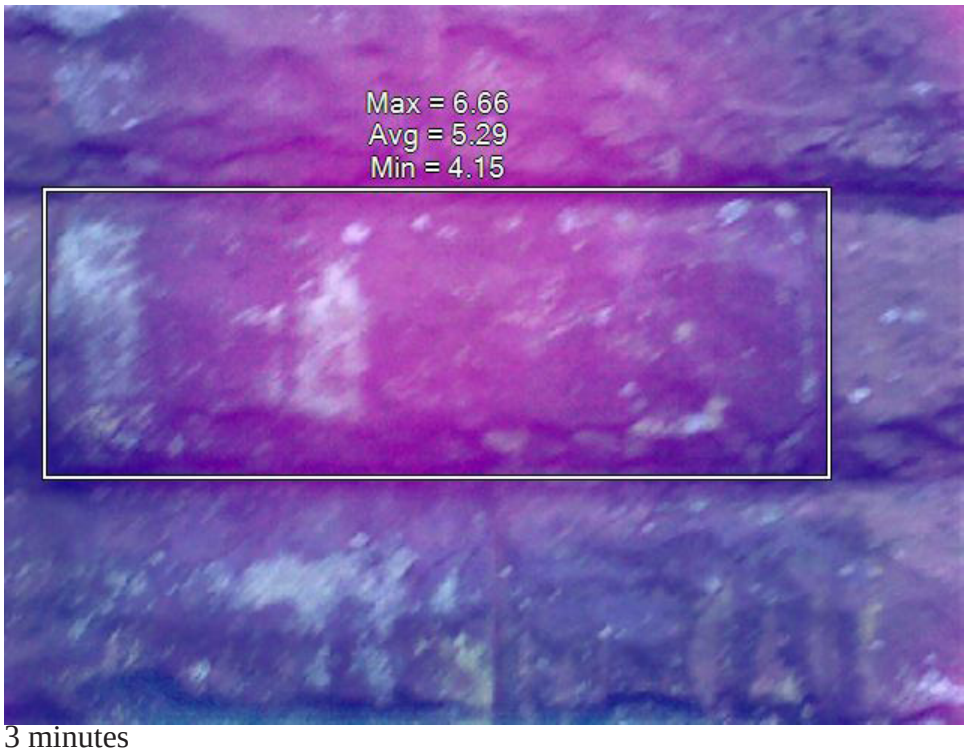


1 minute

Green biocolonization
Whole stone

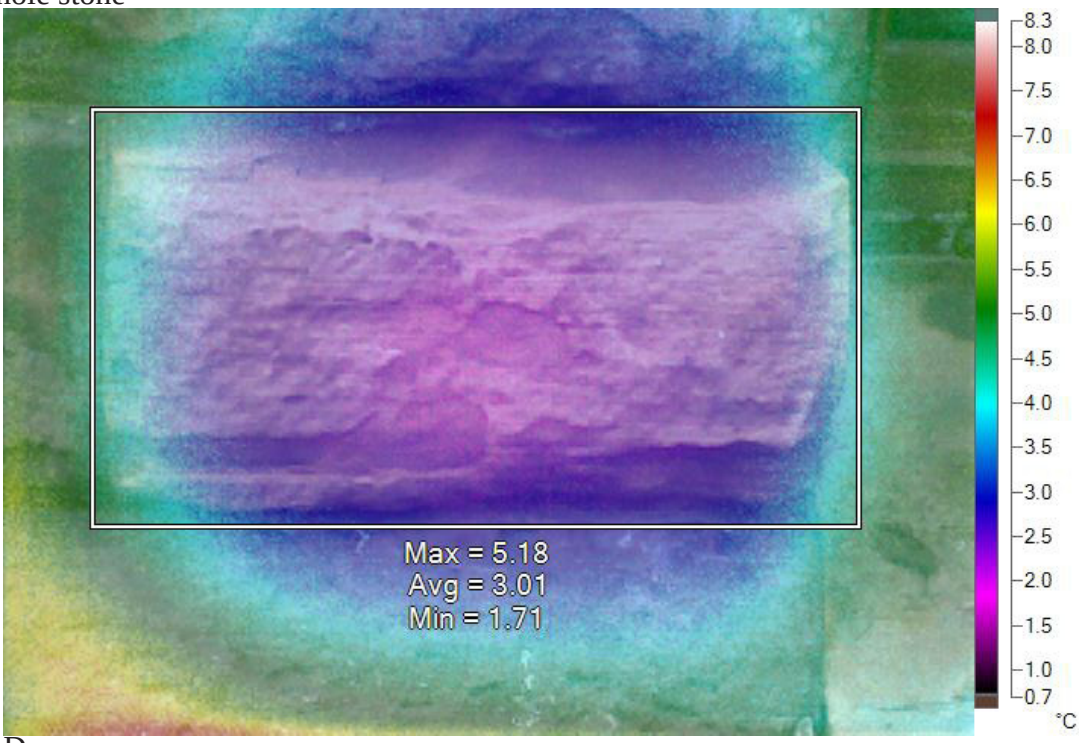


2 minutes



3 minutes

Clean stone
Whole stone



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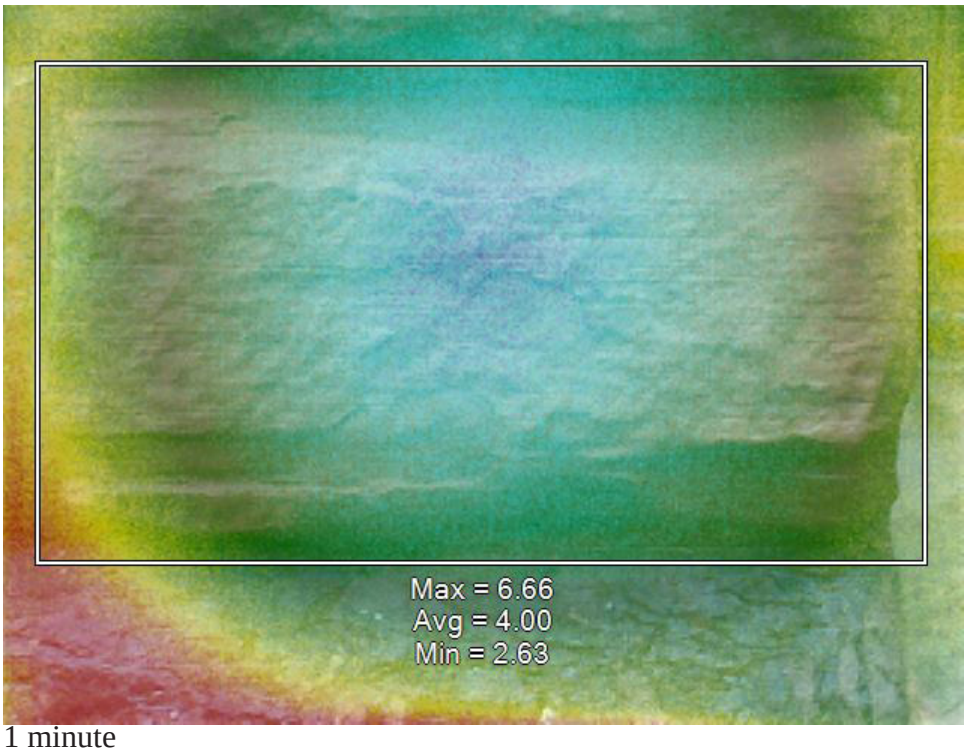
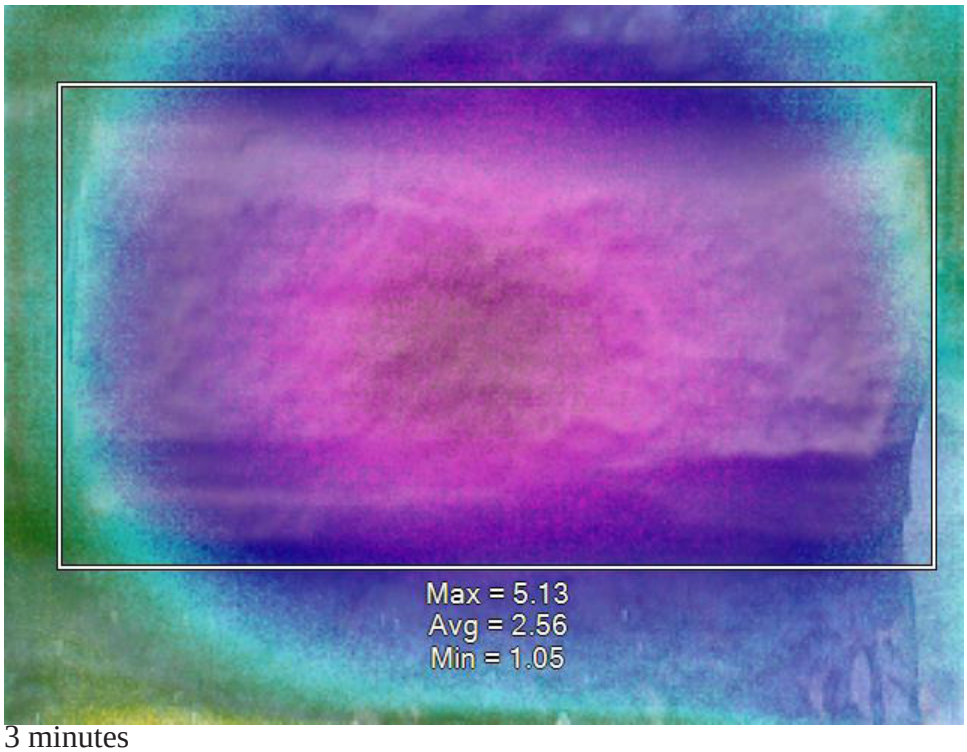


1 minute

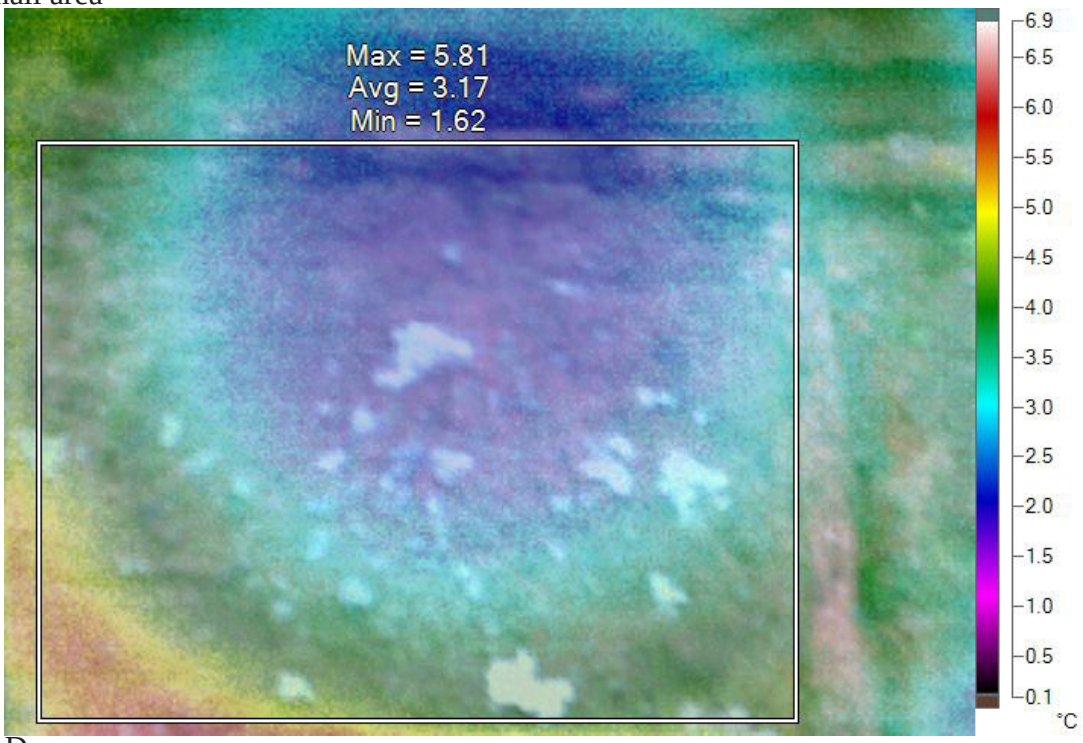
Clean stone
Whole stone

2 minutes



3 minutes

Green biocolonization
Small area



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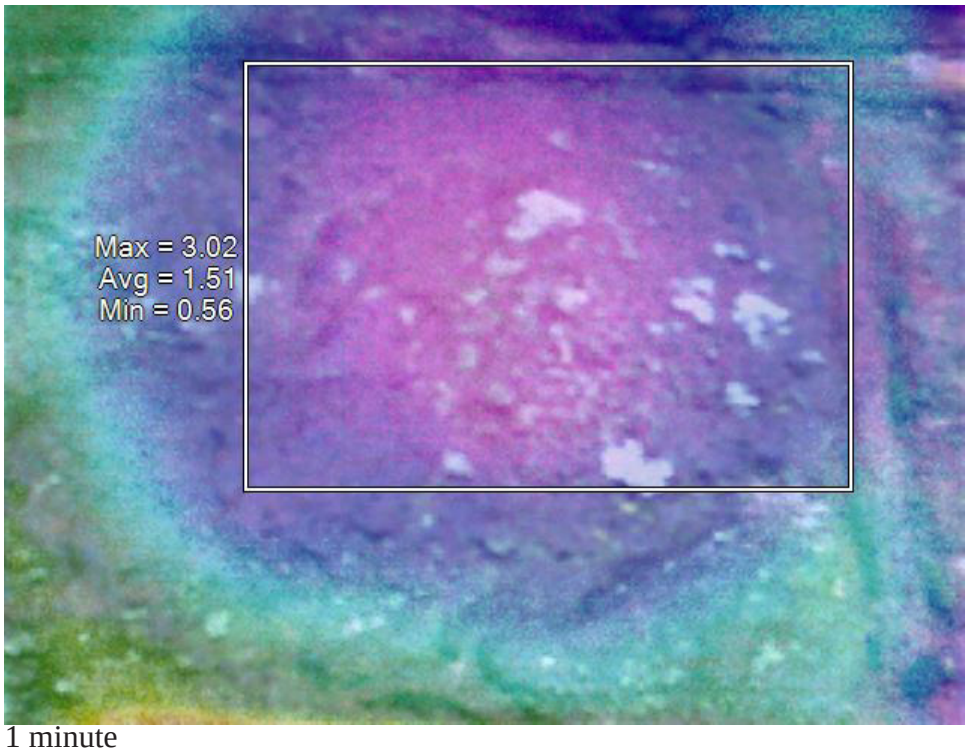
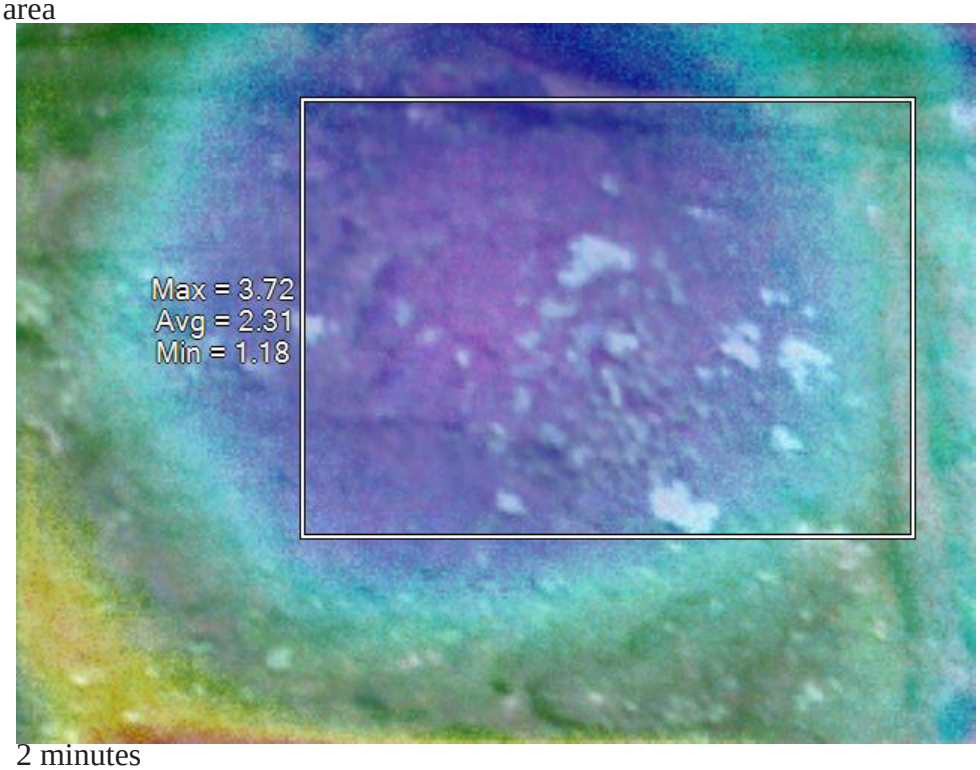
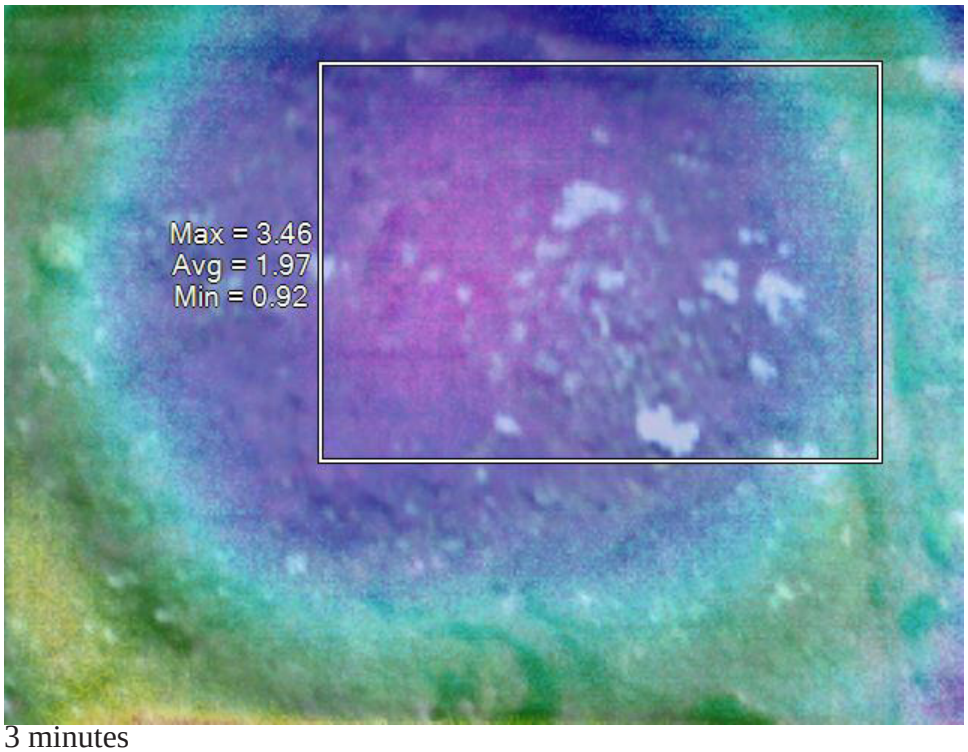


1 minute

Green biocolonization
Small area



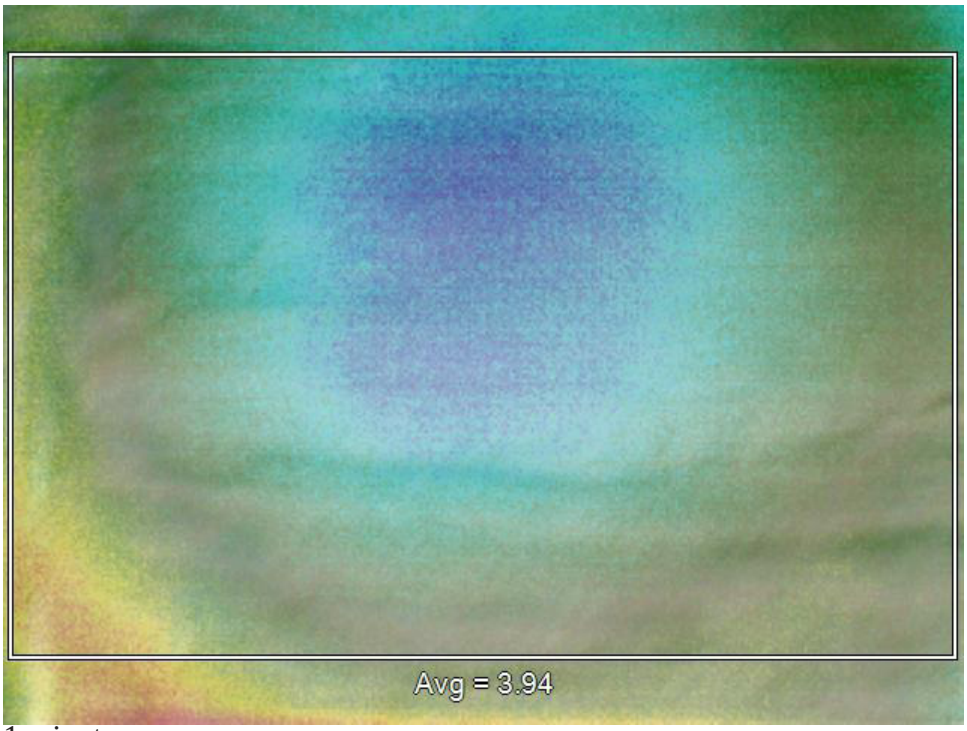
2 minutes



3 minutes

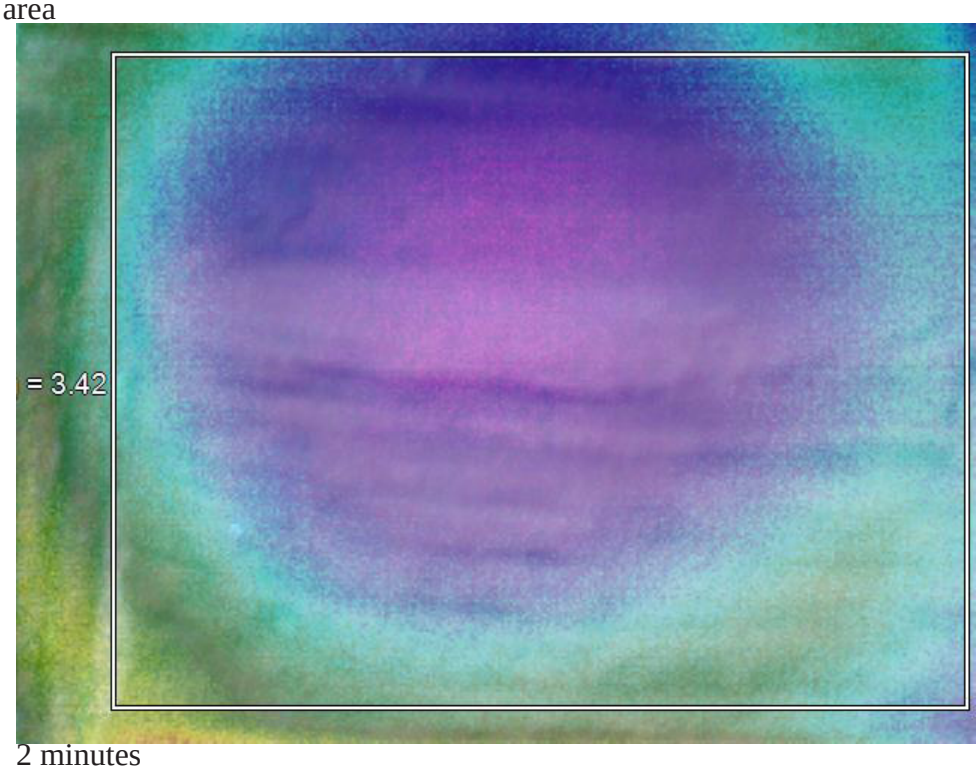
Clean stone
Small area

Dry



1 minute

Clean stone
Small area



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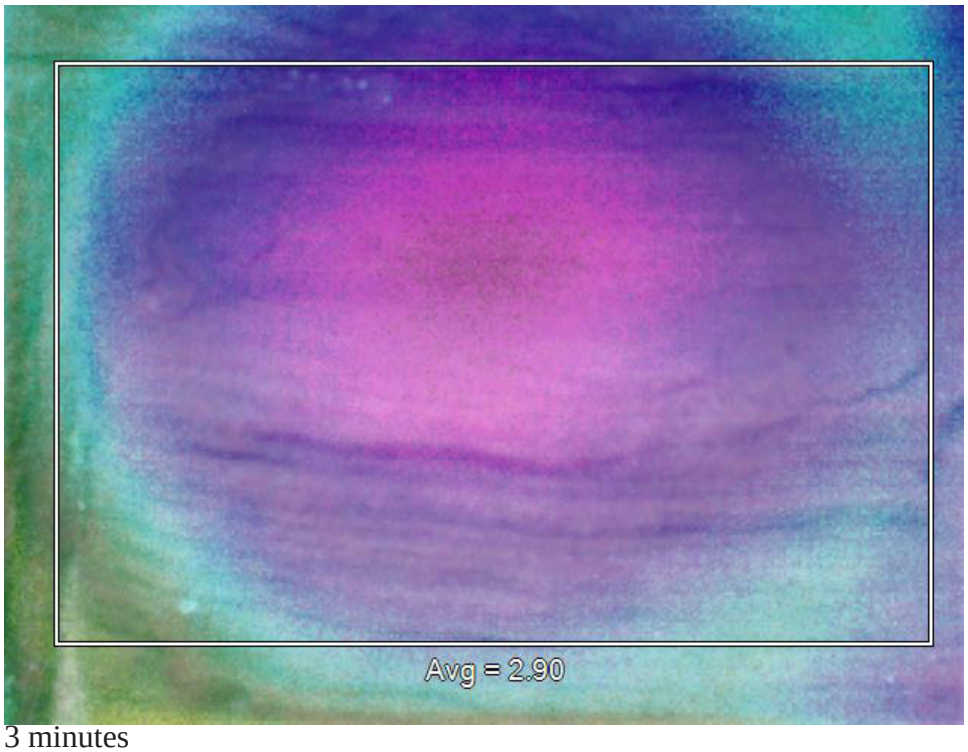
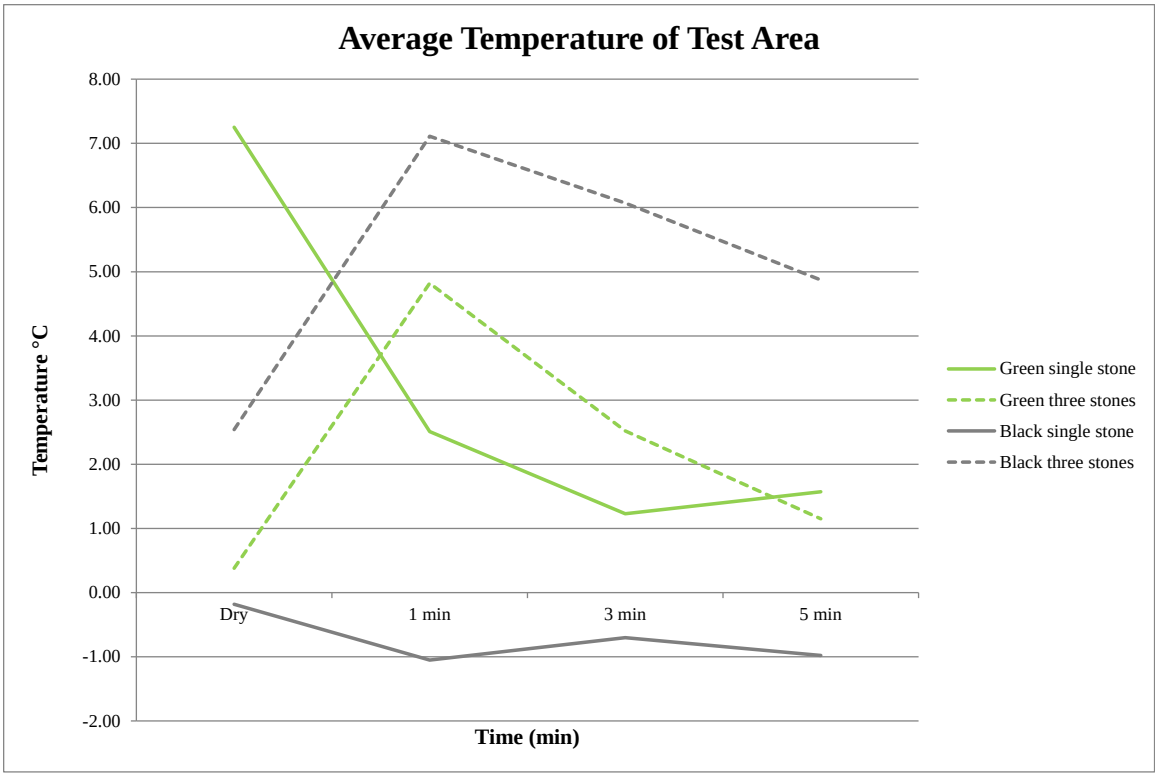


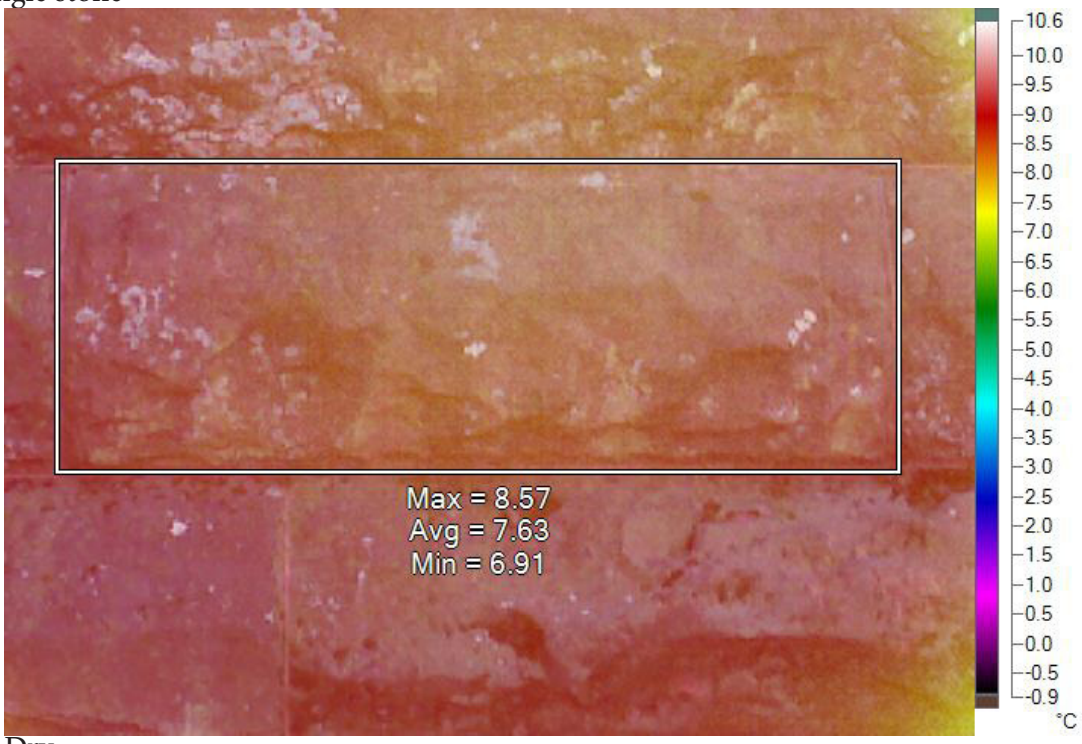
3 minutes

A.3 Single Stone vs. Three Stone Test Area
November 8, 2012

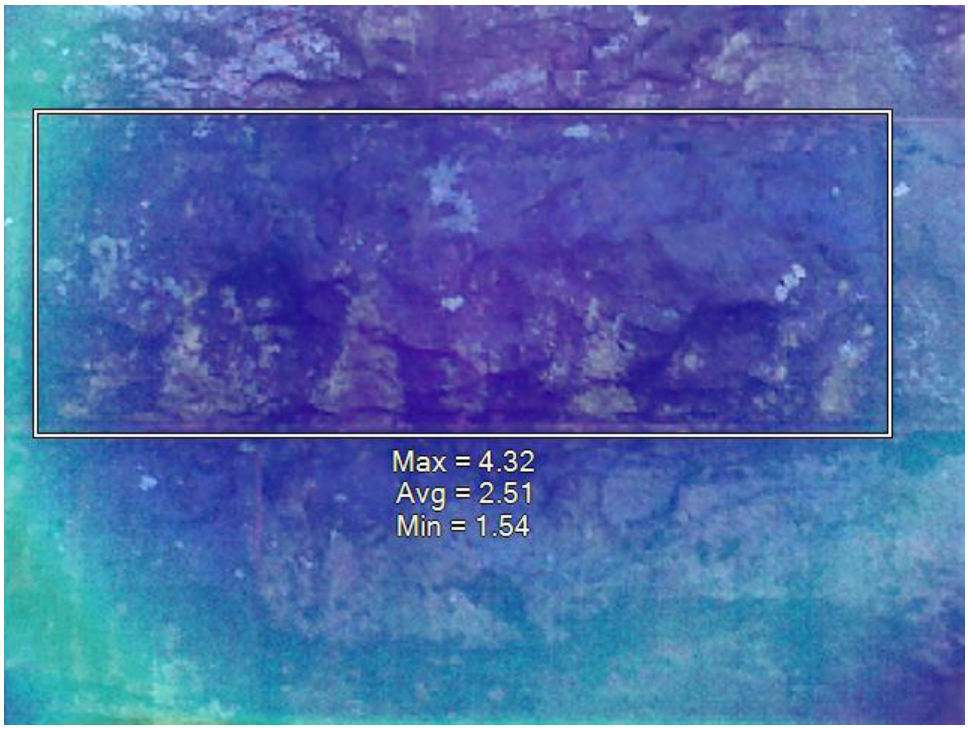
Average Temperature (°C) of Test Area				
	Green Biocolonization		Black Biocolonization	
	single stone	three stones	single stone	three stones
Dry	7.25	0.38	-0.18	2.54
1 min	2.51	4.82	-1.05	7.11
3 min	1.23	2.52	-0.70	6.07
5 min	1.57	1.15	-0.98	4.87



Green biocolonization
Single stone

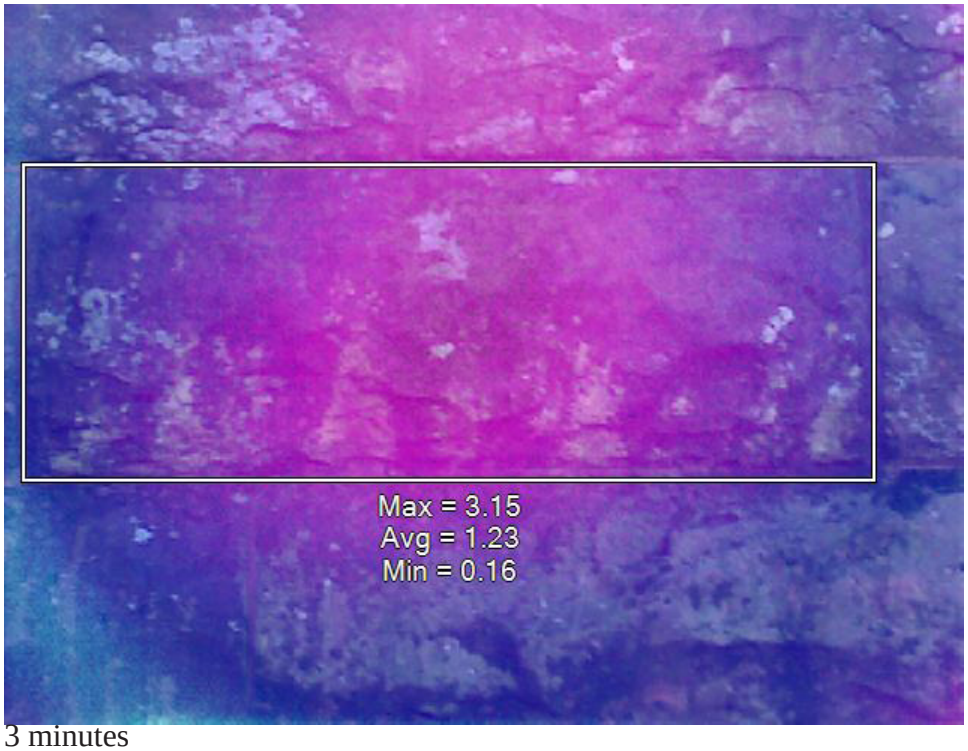


Dry

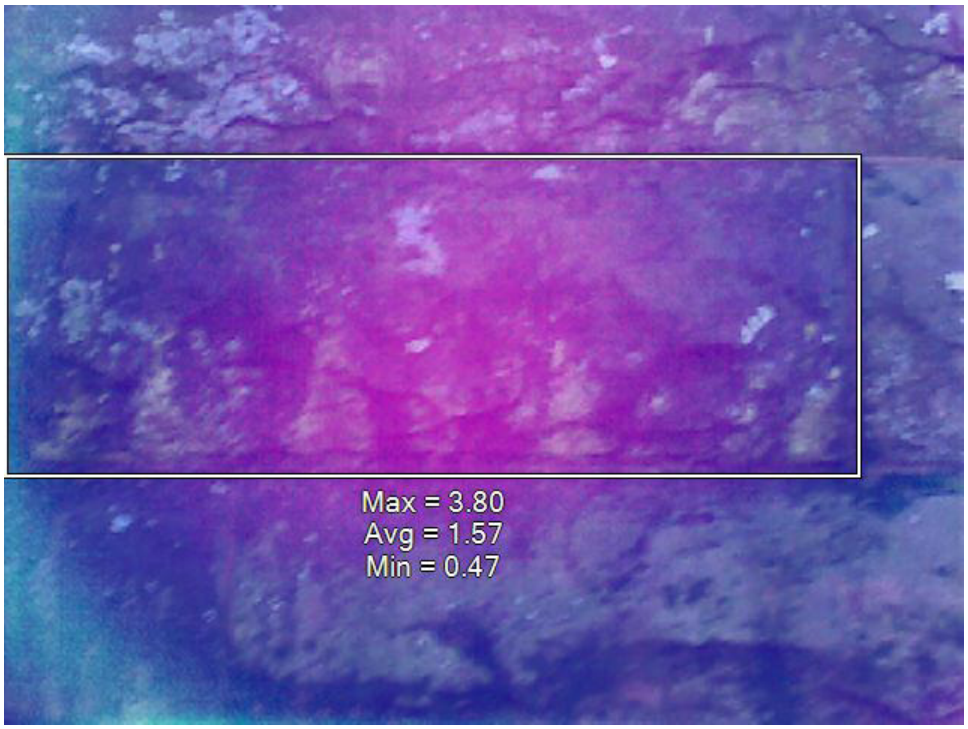


1 minute

Green biocolonization
Single stone

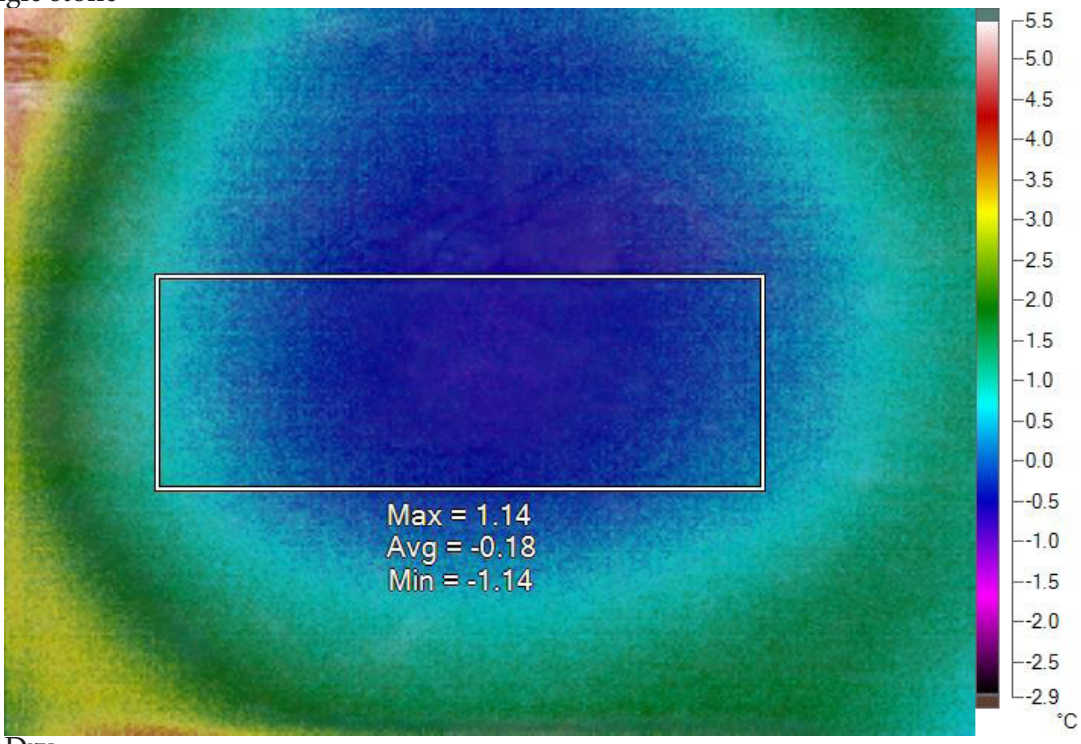


3 minutes

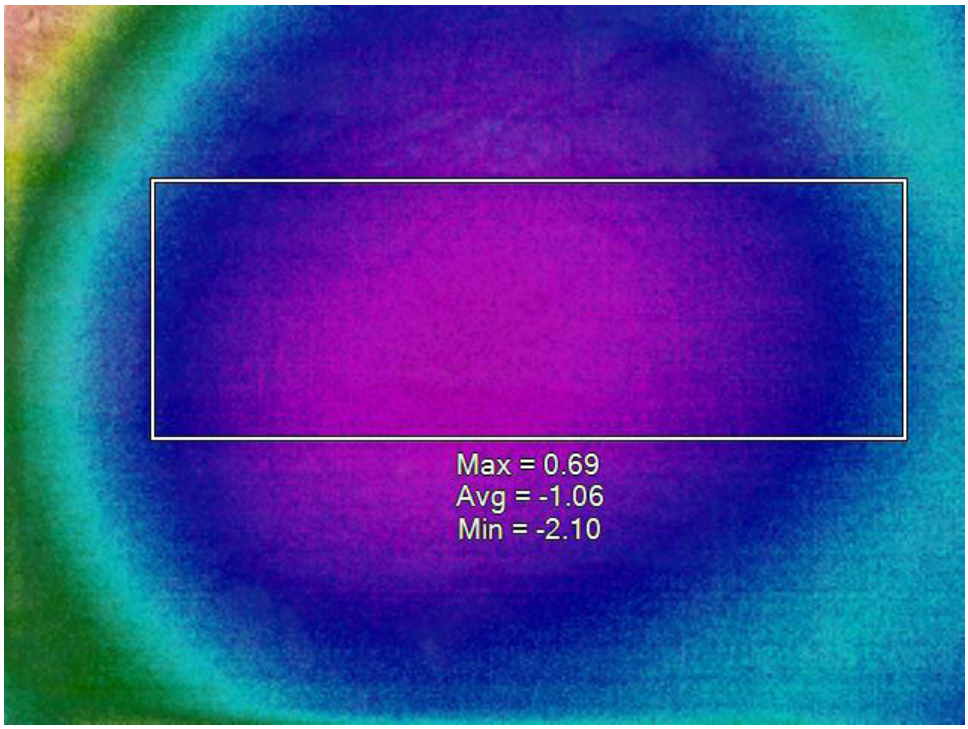


5 minutes

Black biocolonization
Single stone

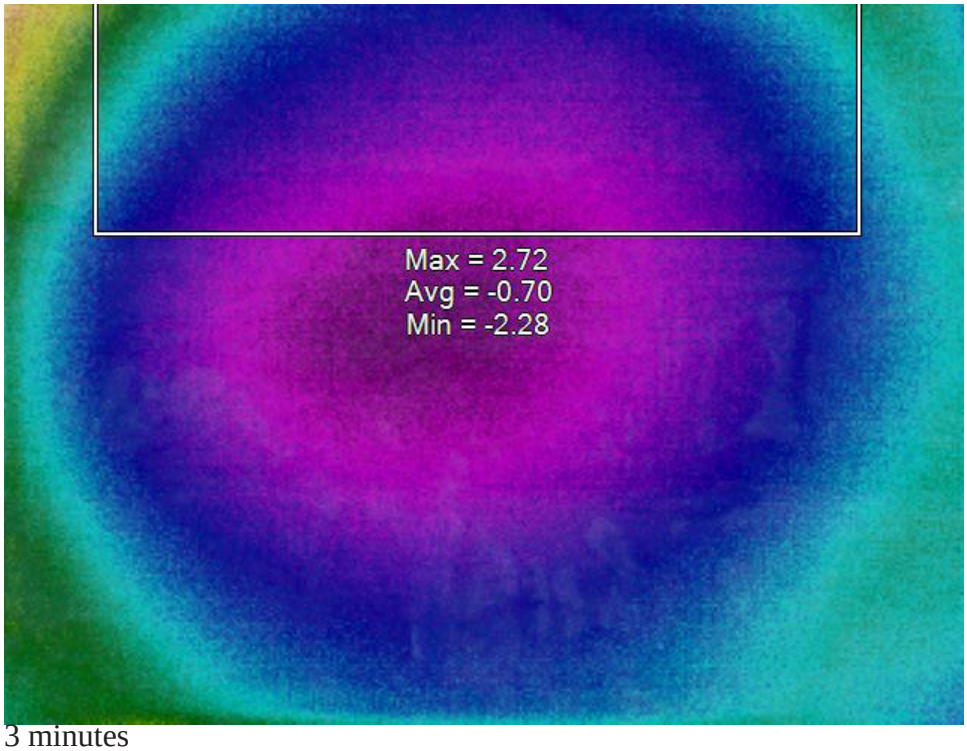


Dry

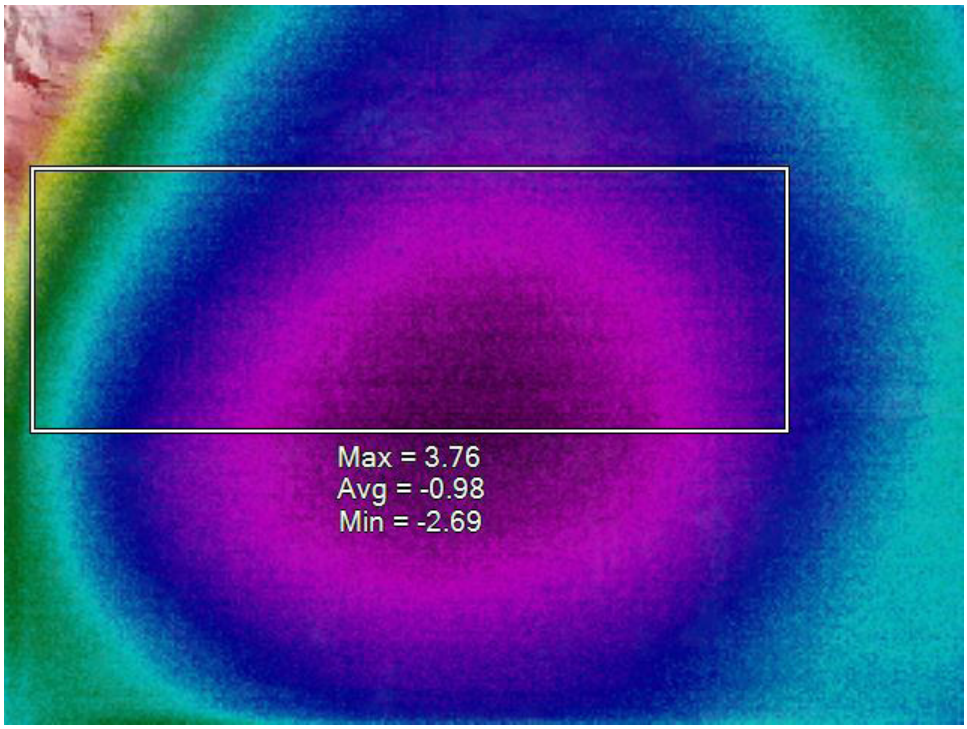


1 minute

Black biocolonization
Single stone

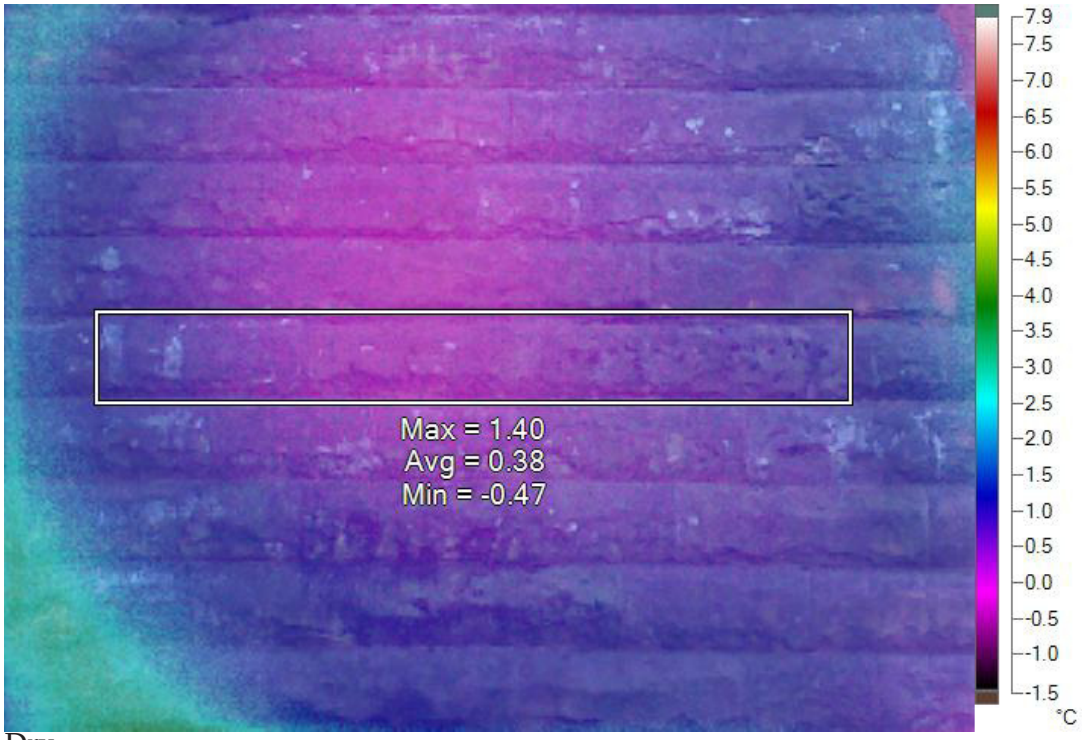


3 minutes

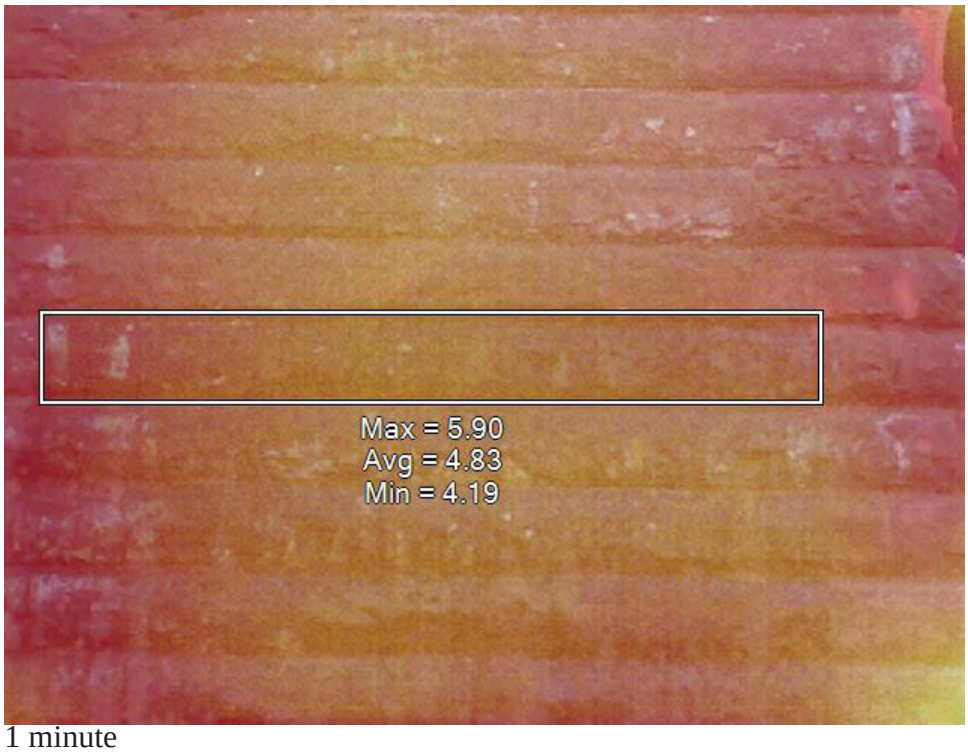


5 minutes

Green biocolonization
Three stones

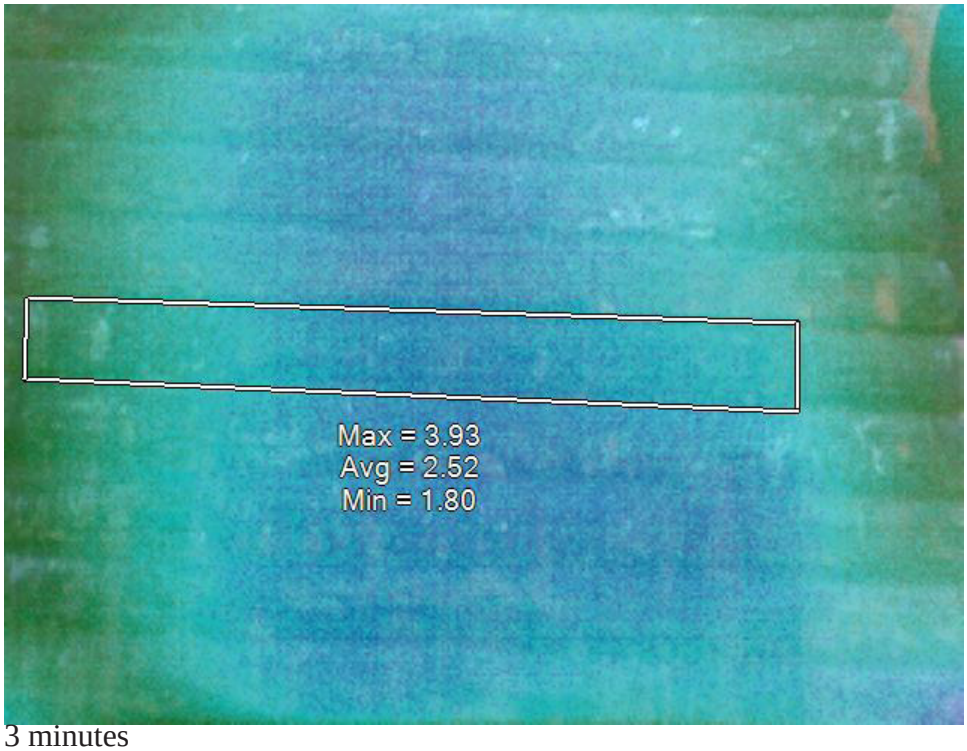


Dry

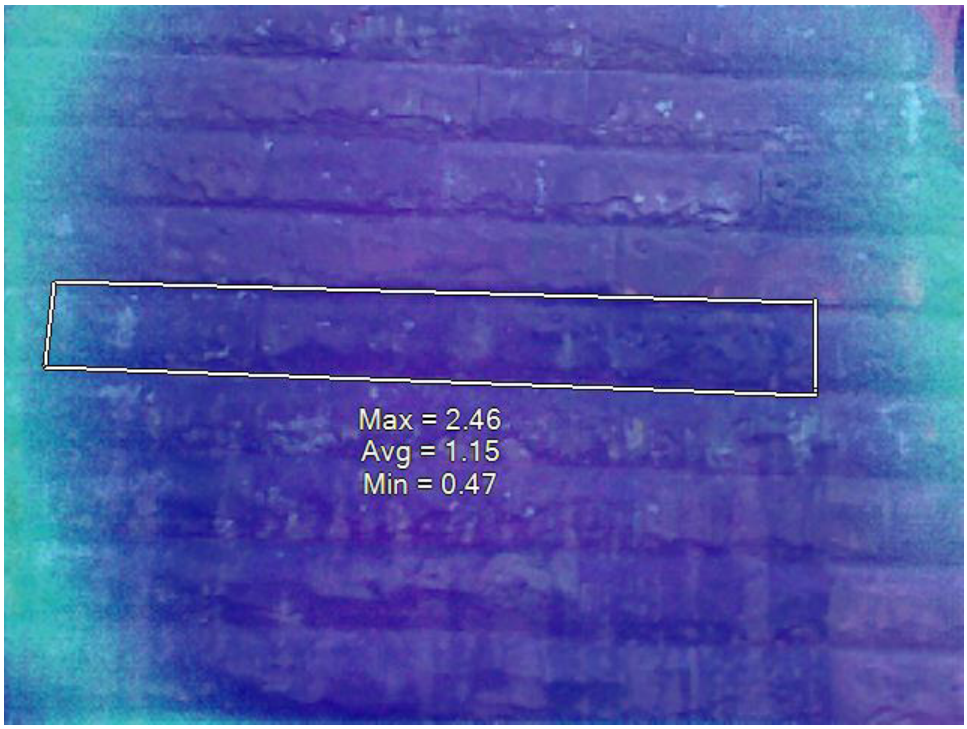


1 minute

Green biocolonization
Three stones



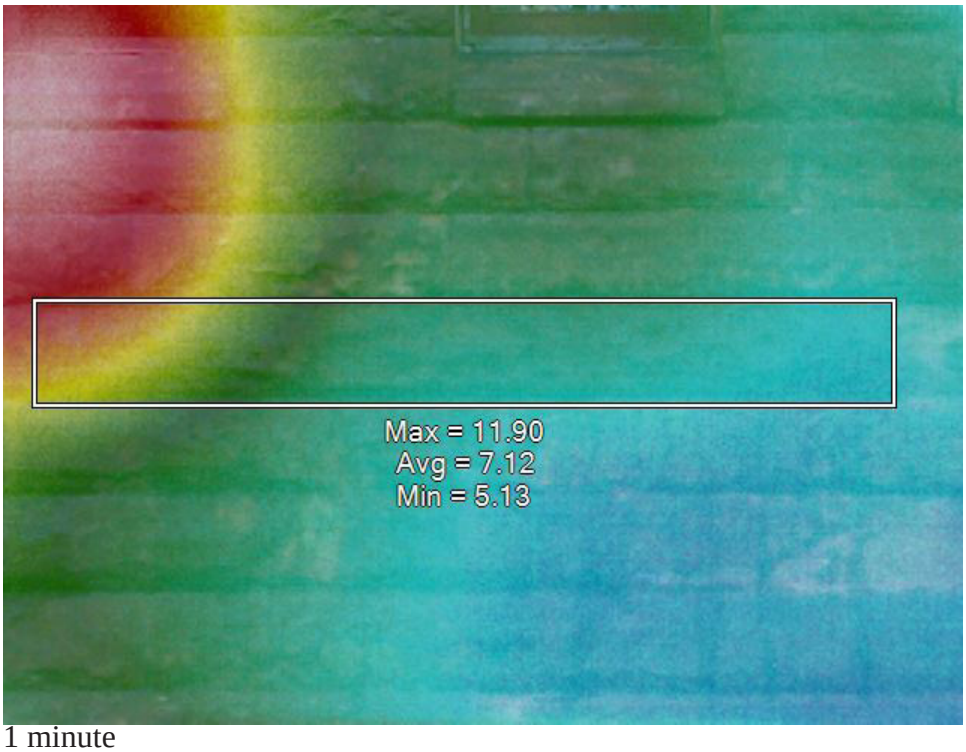
3 minutes



5 minutes

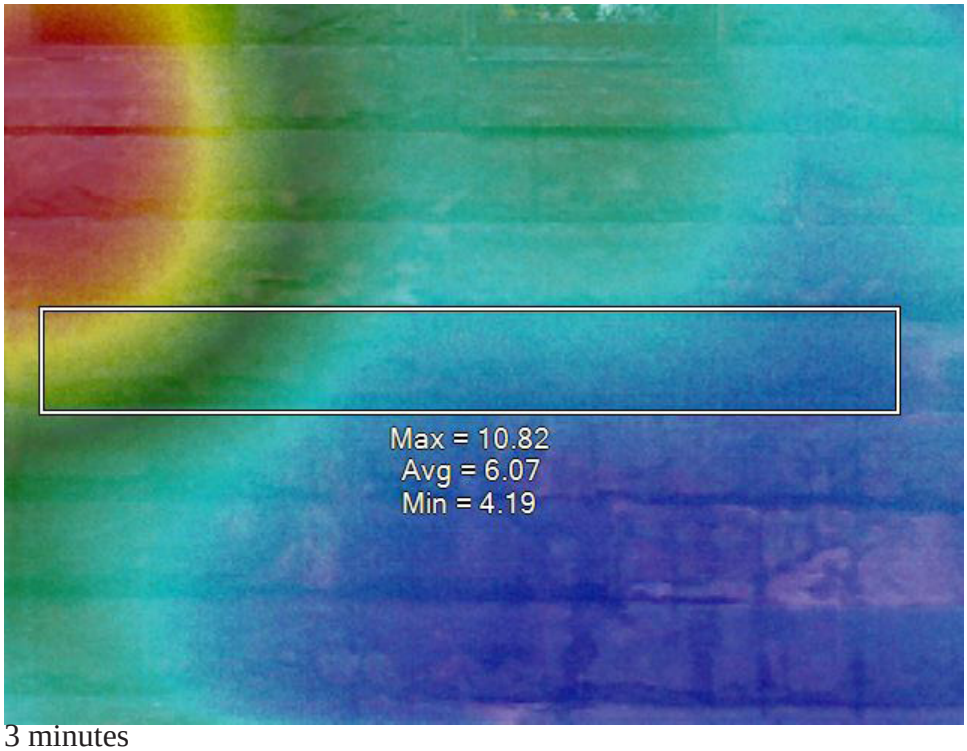
Black biocolonization
Three stones

Dry

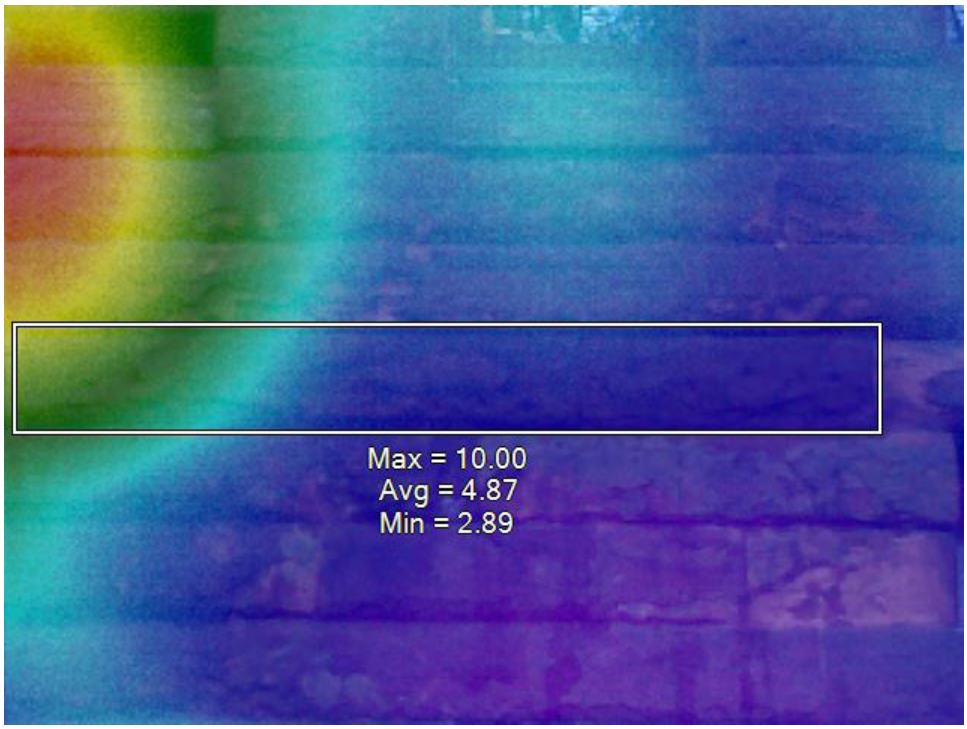


1 minute

Black biocolonization
Three stones



3 minutes

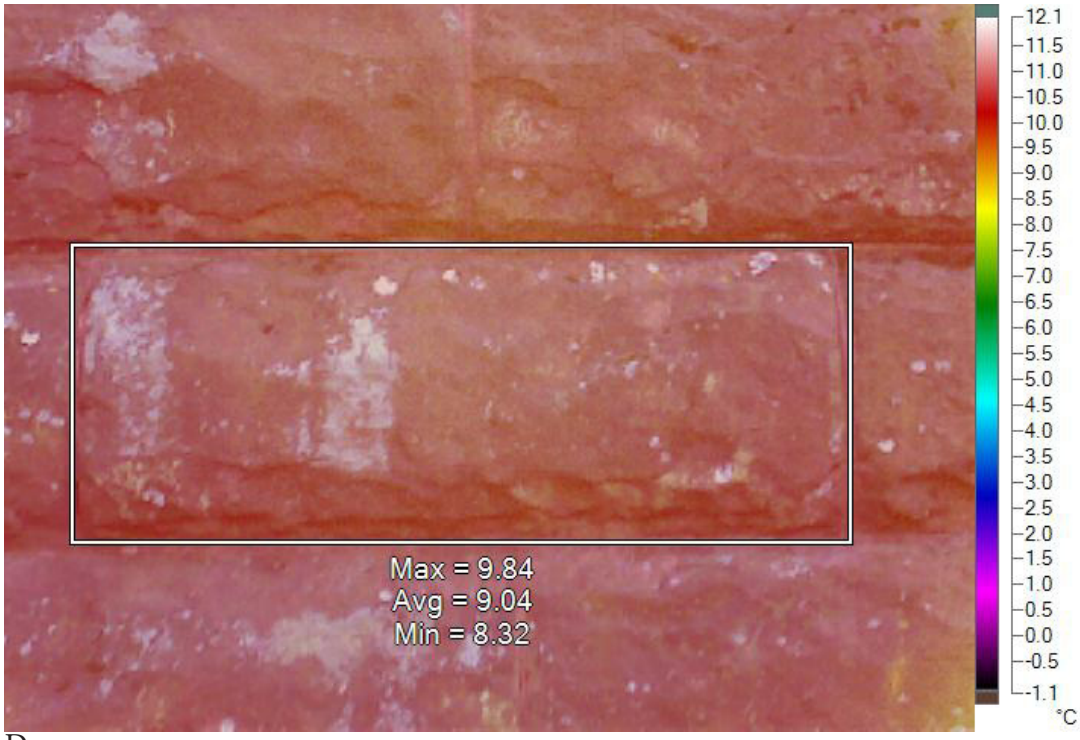


5 minutes

Appendix B. Thermal Imaging Data

			Average Stone Temperature (°C)					
	Time (min)	**DATE**	**Nov.19**	**Nov. 26**	**Dec. 4**	**Jan. 10**	**Feb. 7**	**Mar. 5**
Biocide: D/2	**0**	Dry	9.04	-1.18	20.32	0.72	-6.69	-4.90
	1		1.04	-1.60	16.36	2.09	-3.76	-1.84
	2		1.14	-1.51	15.90	0.96	-4.62	-2.66
	3		0.99	-2.38	15.26	0.84	-5.03	-3.05
	4		0.68	-2.13	14.64	0.26	-5.69	-3.63
	5		0.95	-1.89	13.82	-0.26	-5.97	-4.53
Biocide: none (control)	**0**	Dry	7.55	-1.77	18.97	0.76	-7.03	-4.99
	1		8.71	-1.71	16.56	3.63	-3.43	-0.82
	2		7.62	-1.37	16.21	3.07	-4.50	-1.39
	3		6.42	-2.35	15.28	2.68	-4.88	-2.78
	4		5.62	-2.62	14.44	1.38	-5.46	-3.42
	5		4.61	-2.49	13.90	0.46	-6.09	-3.75
Biocide: BioWash	**0**	Dry	4.50	-3.96	17.62	-1.55	-9.26	-6.05
	1		7.23	-3.57	16.47	3.06	-3.58	-1.01
	2		6.30	-4.44	16.07	2.80	-4.39	-1.61
	3		4.59	-3.30	15.42	1.51	-5.40	-2.29
	4		3.76	-3.19	14.52	0.52	-6.28	-4.33
	5		2.88	-3.72	13.75	0.08	-7.30	-4.56
Environmental Conditions	**Temp (°C)**		11.6	8.8	18.6	8.6	4.0	4.7
	RH (%)		51	31	59	37	30	43
	Wind		+	+	+	++	+	+
	Sunny (s); partly cloudy (pc); cloudy (c)		s	s	pc	s	c	s
Block Condition	**Sunny (O); partly shaded (ps); shaded (s)**		s	s	s	s	s	s
Temperature (°C)	**Water**		20	21	21	21	17	22
	D/2		21	-	-	-	-	-
	BioWash		21	-	-	-	-	-

Green biocolonization treated with D/2
November 19, 2012



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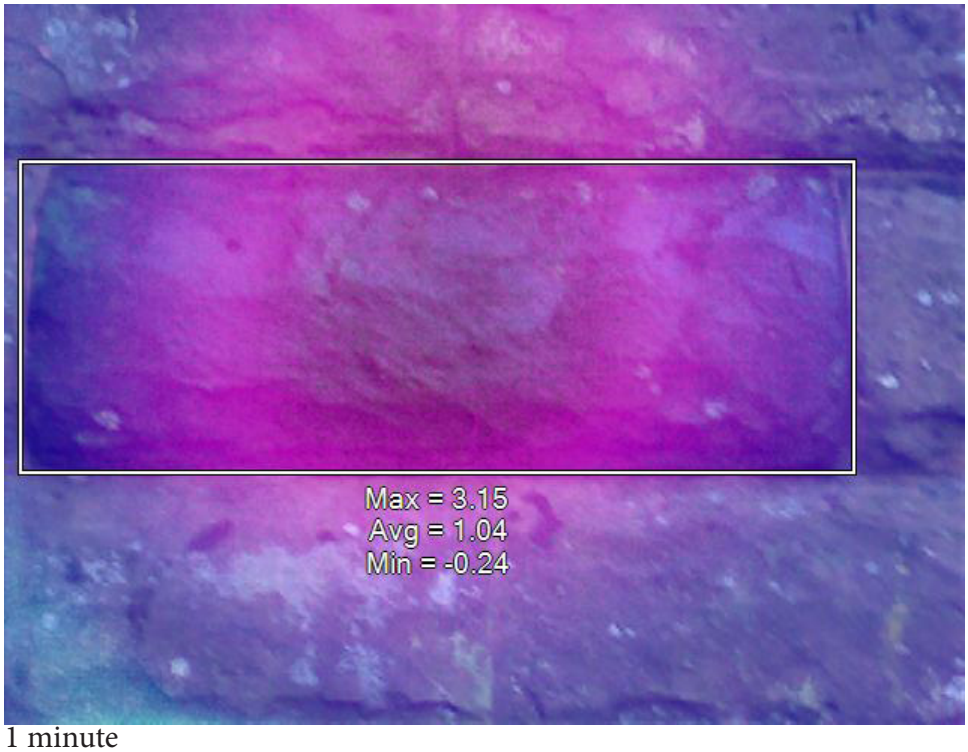
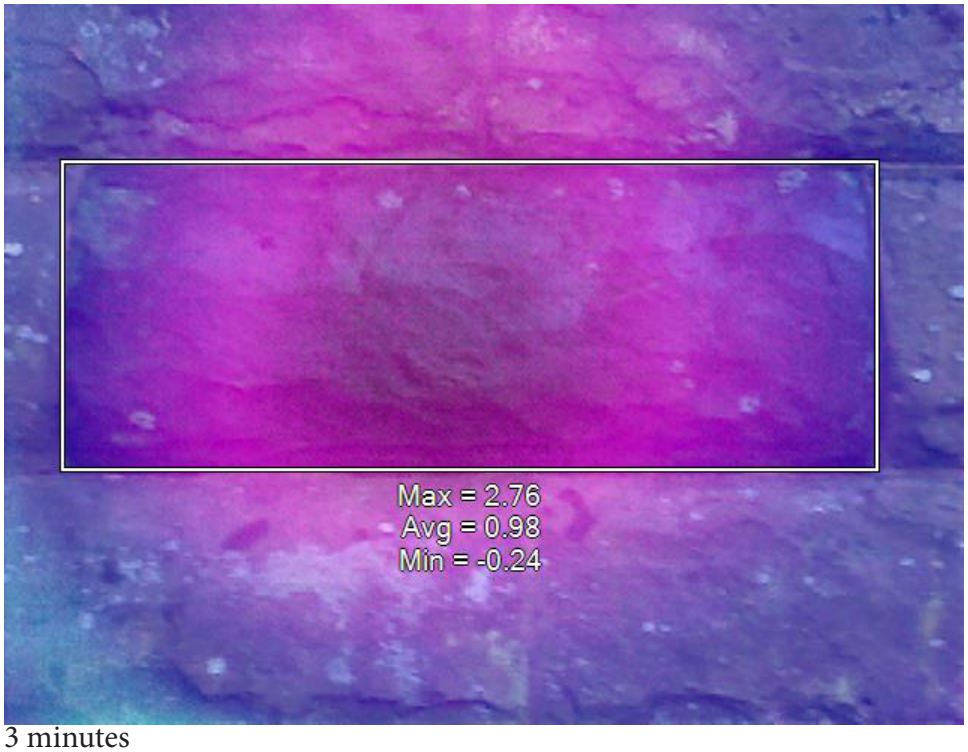


1 minute

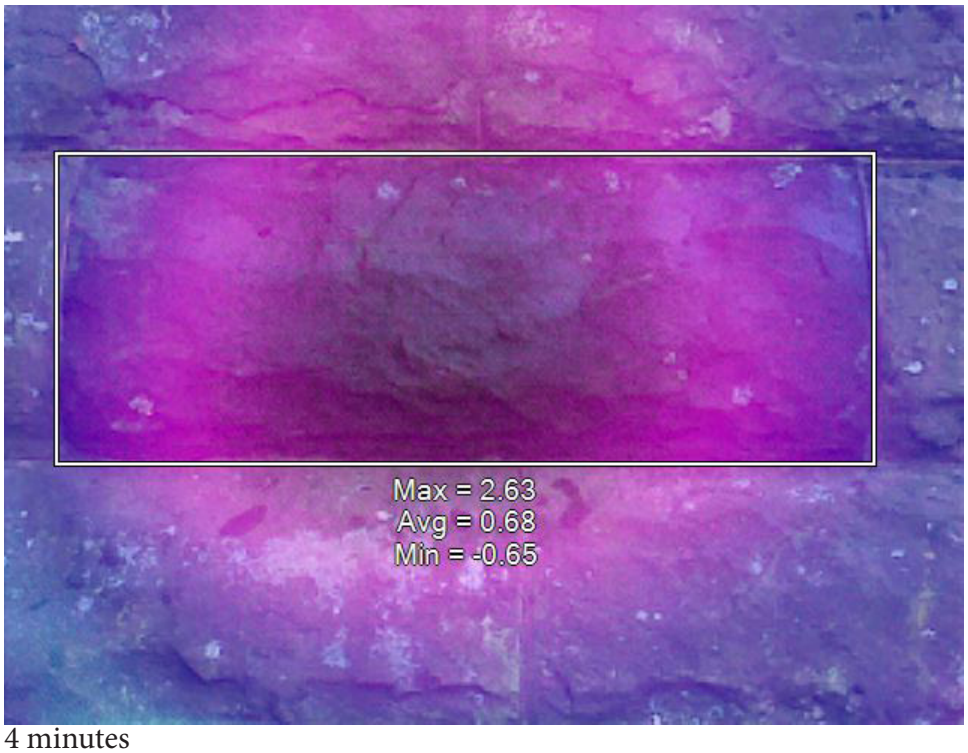
Green biocolonization treated with D/2
November 19, 2012

2 minutes



3 minutes

Green biocolonization treated with D/2
November 19, 2012



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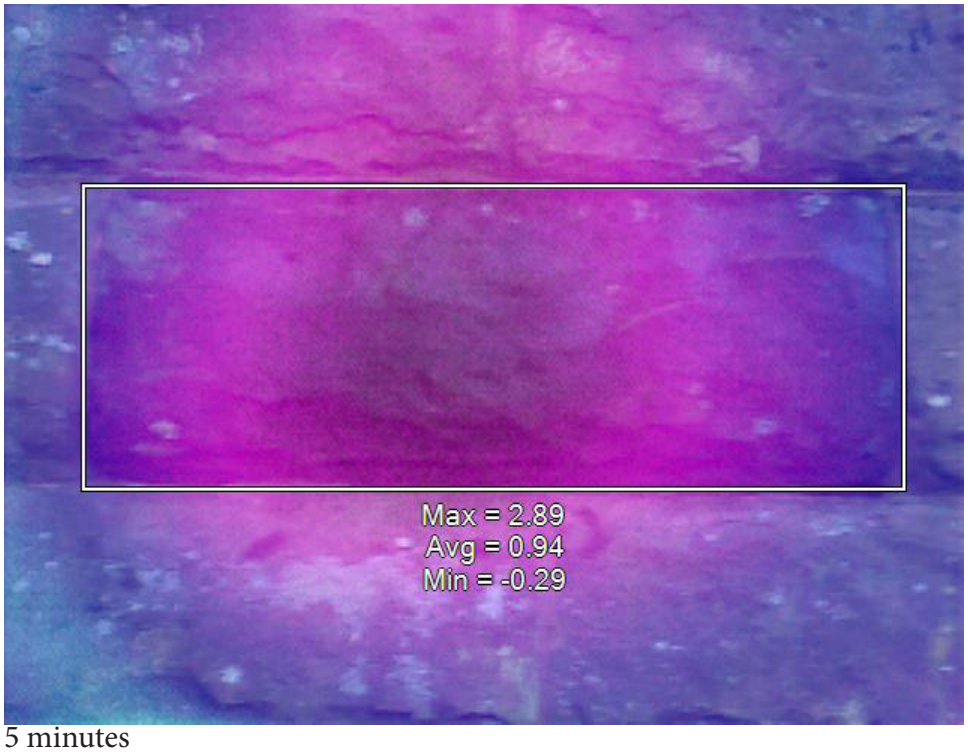
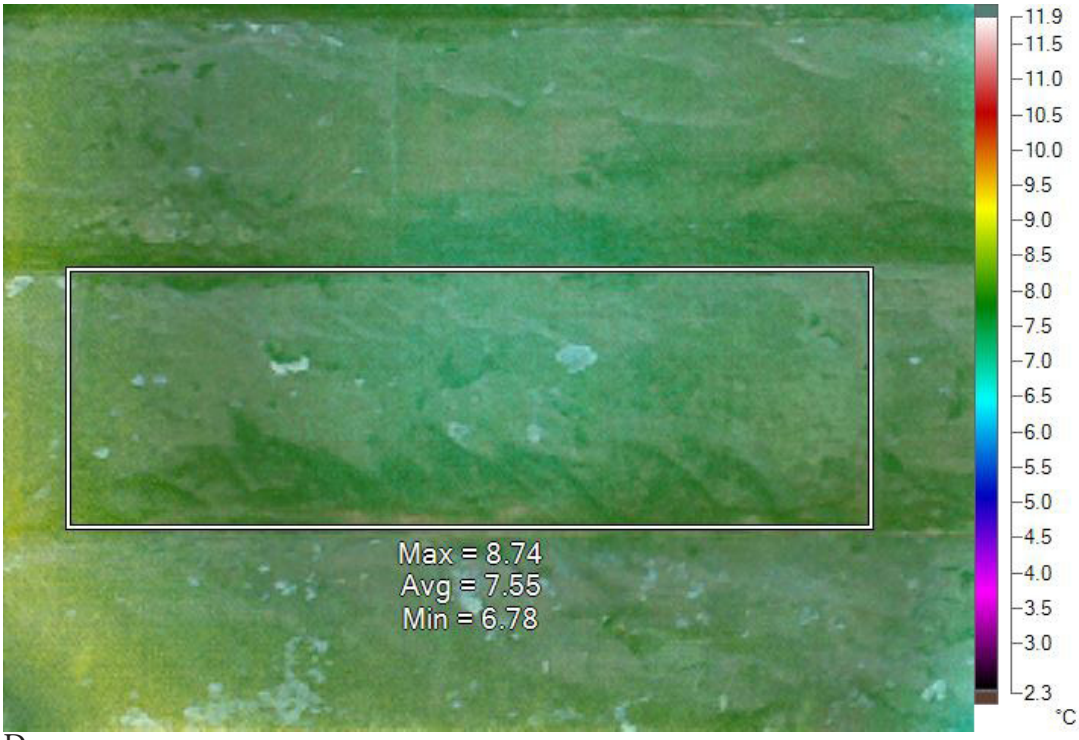


5 minutes

Green biocolonization control
November 19, 2012



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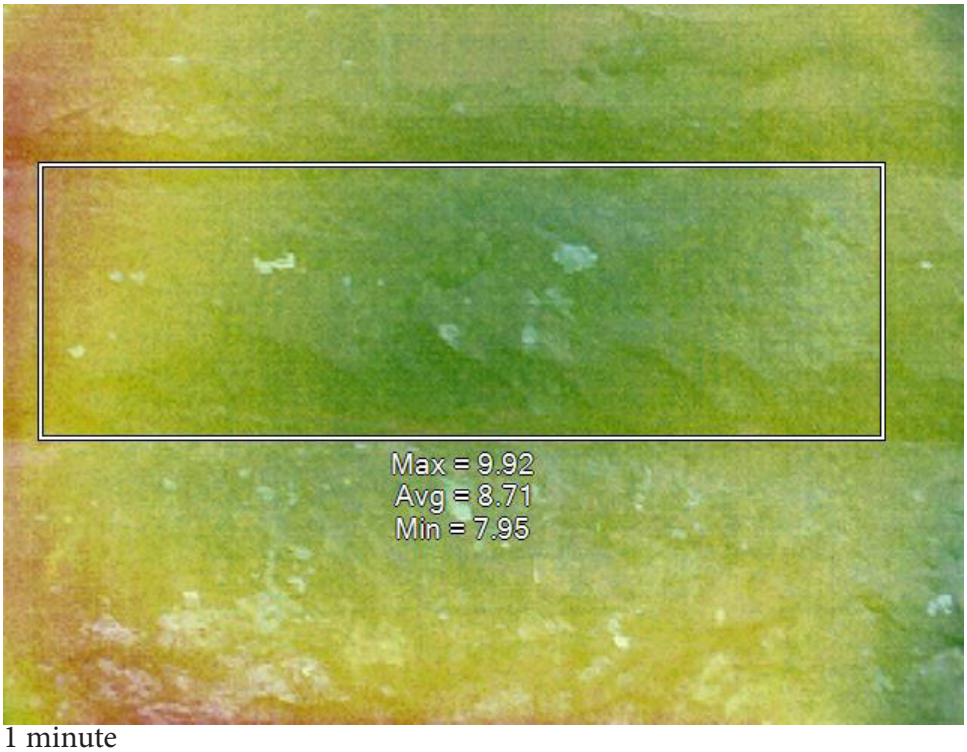

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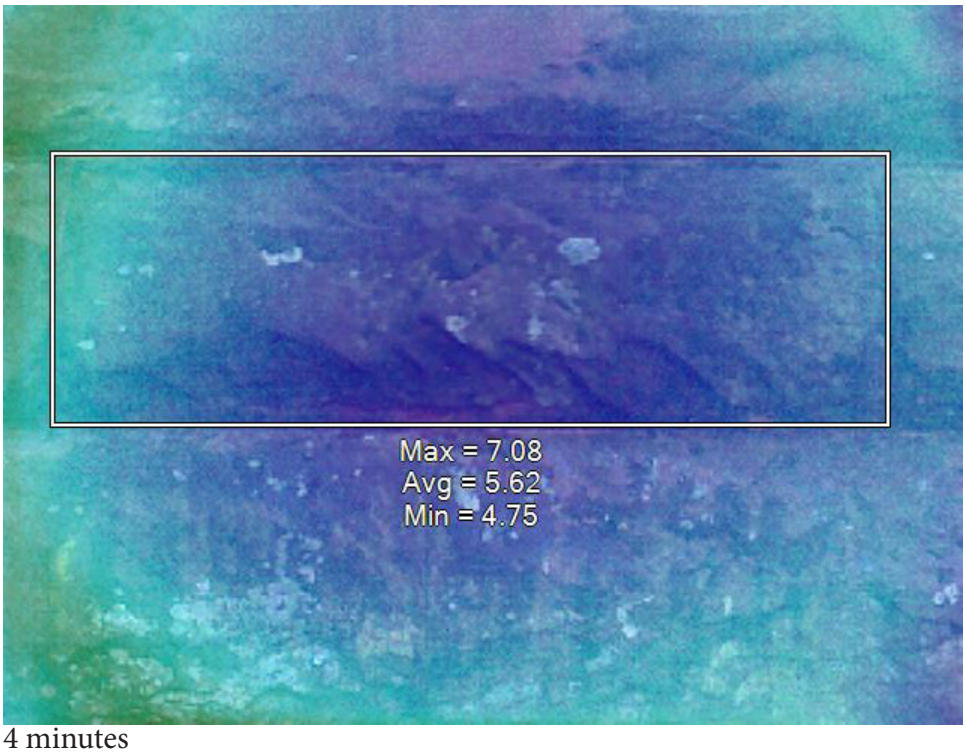
2 minutes

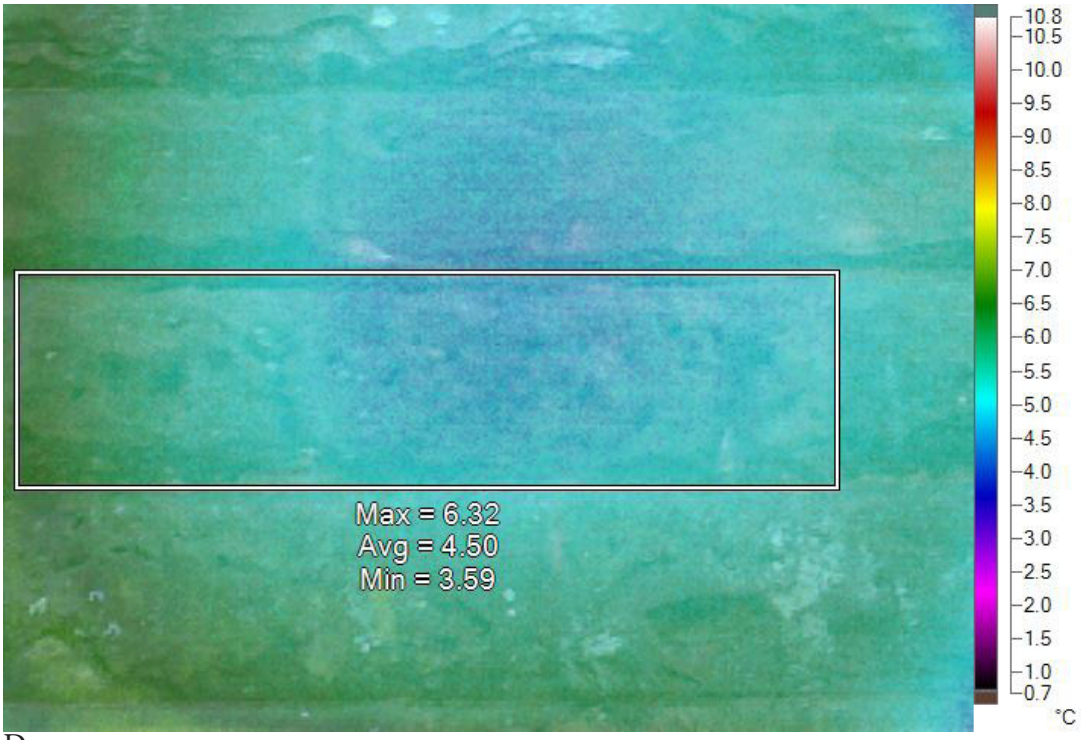
3 minutes



4 minutes

5 minutes

Green biocolonization treated with BioWash
November 19, 2012



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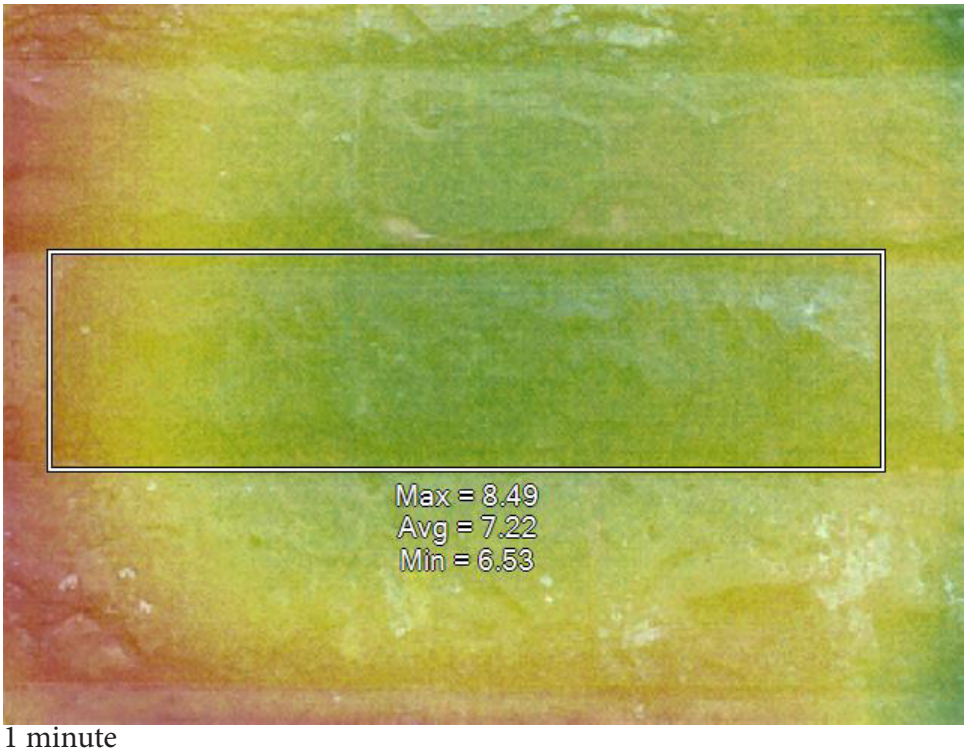

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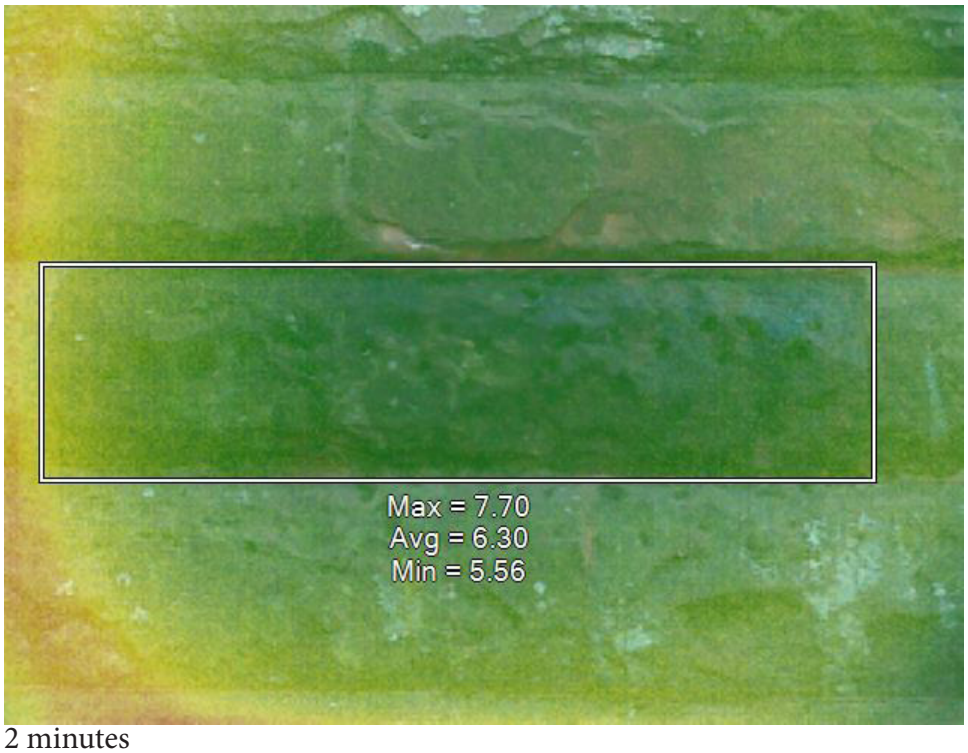
Green biocolonization treated with BIoWash
November 19, 2012



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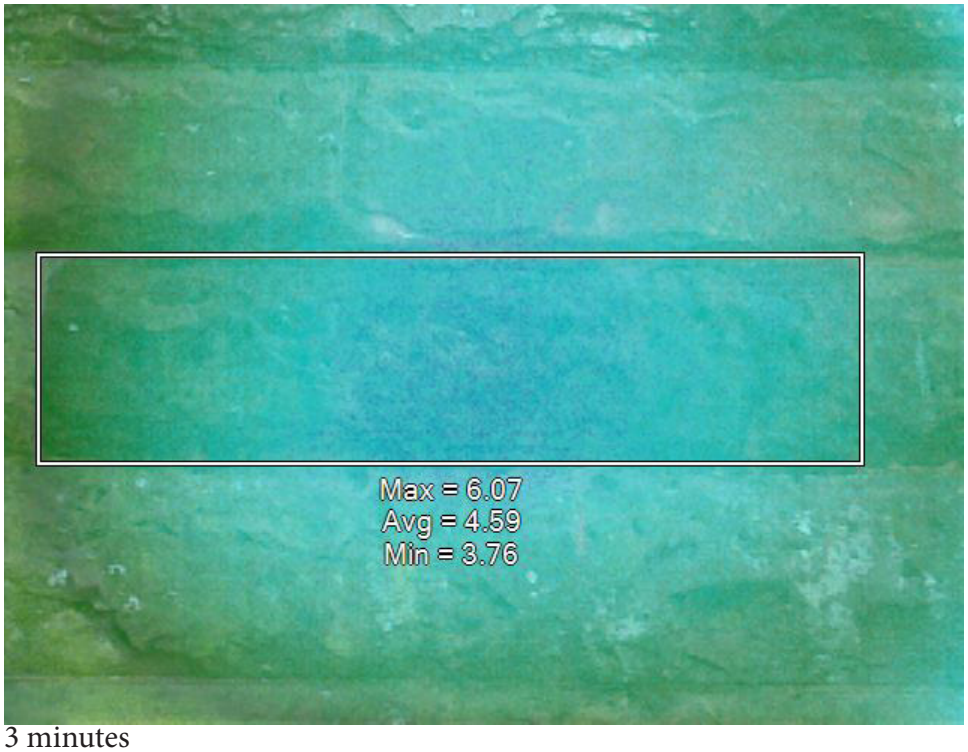

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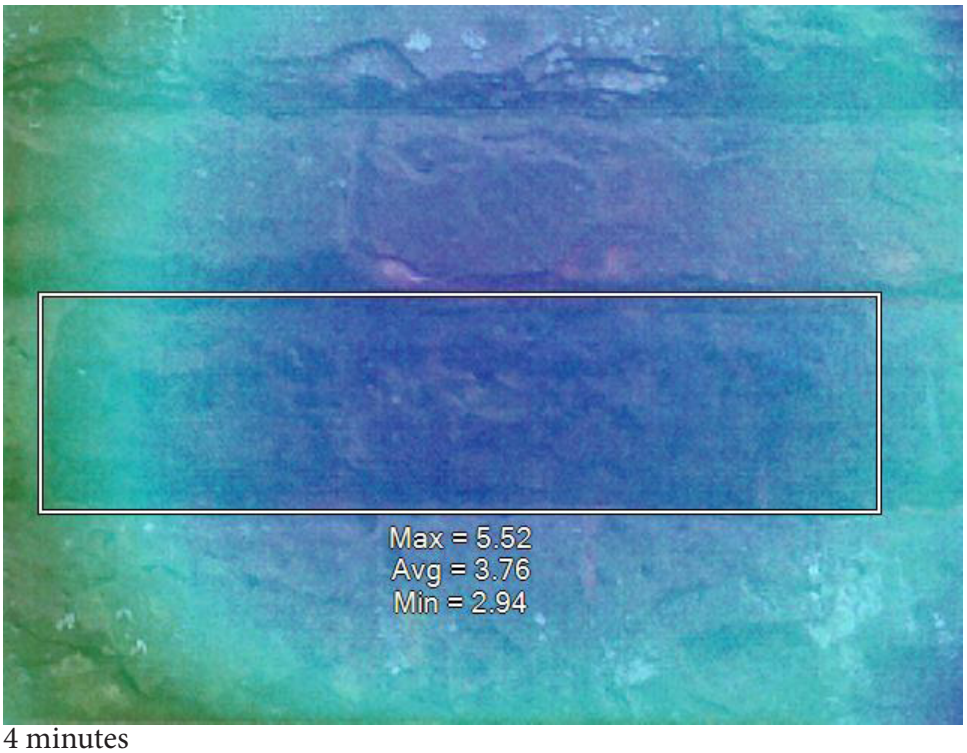
Green biocolonization treated with BioWash
November 19, 2012



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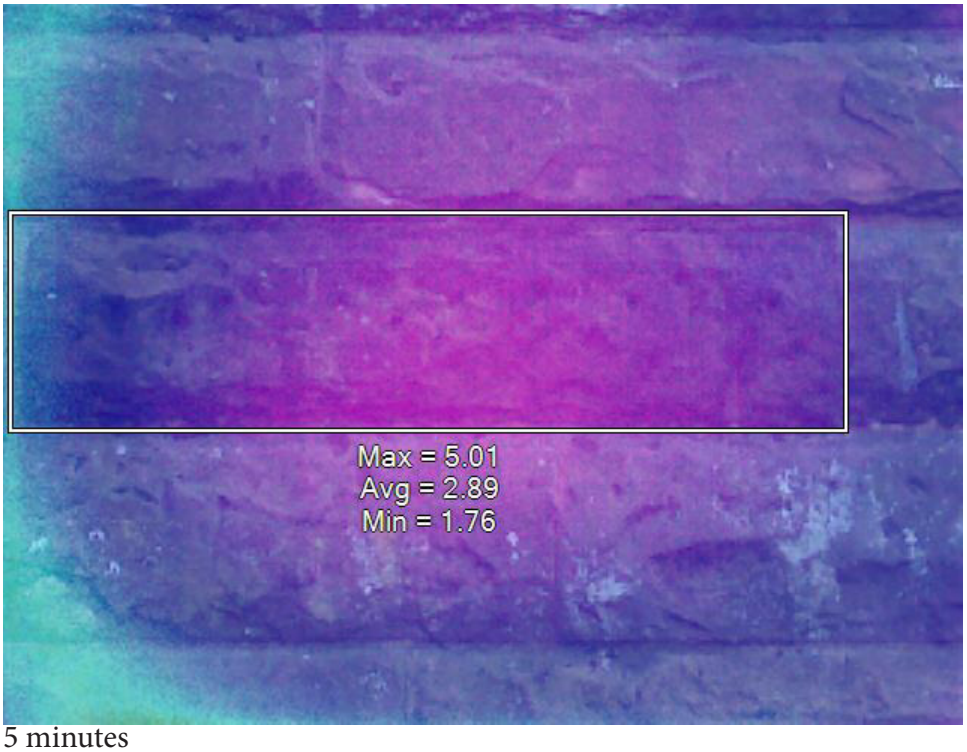

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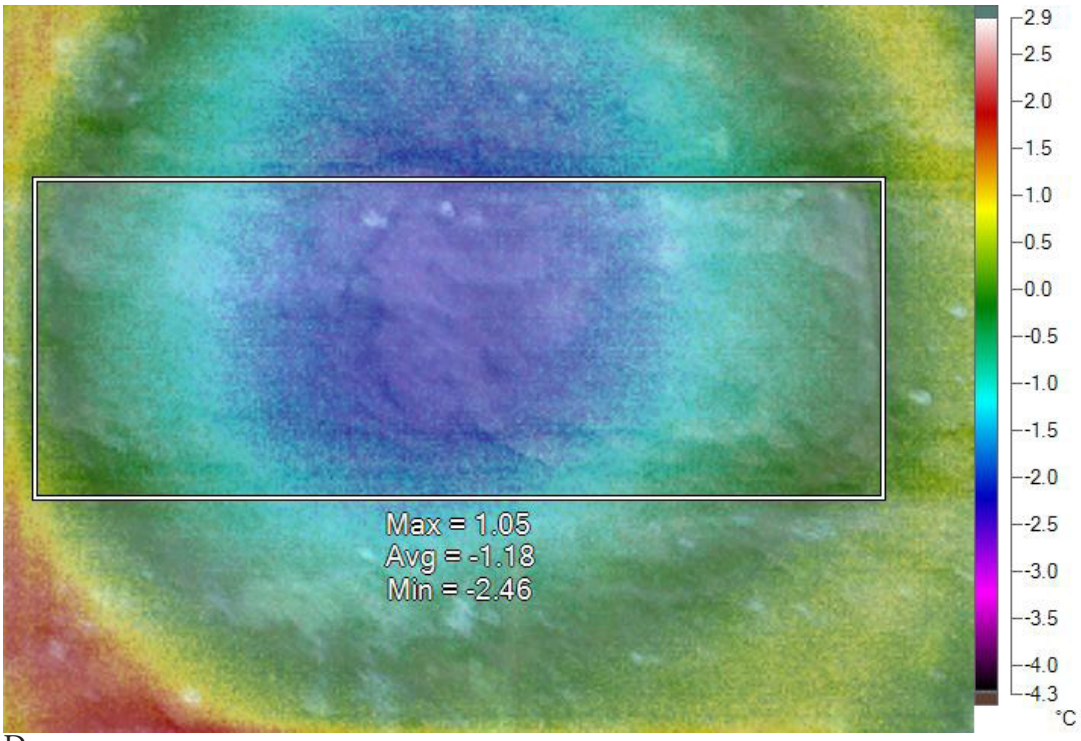
Green biocolonization treated with D/2
November 26, 2012



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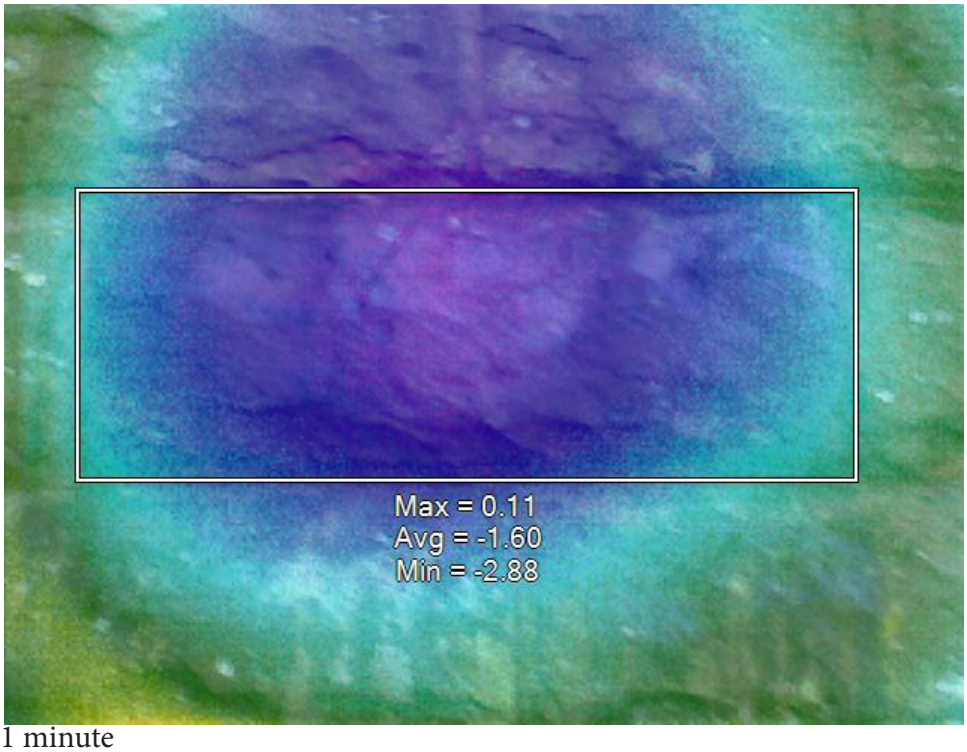

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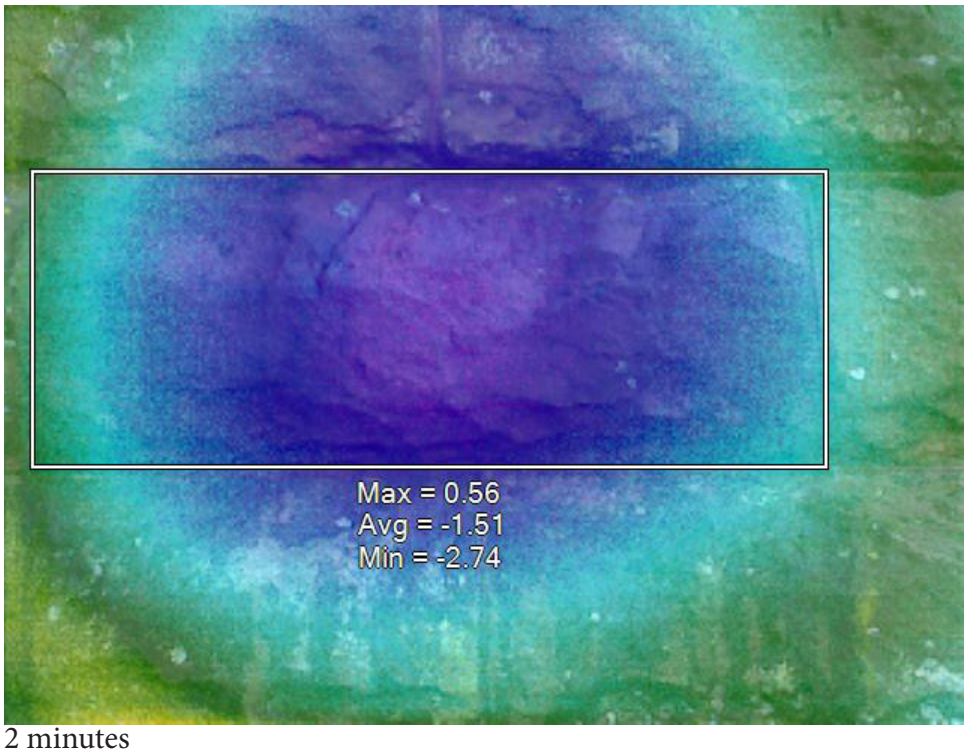
Green biocolonization treated with D/2
November 26, 2012



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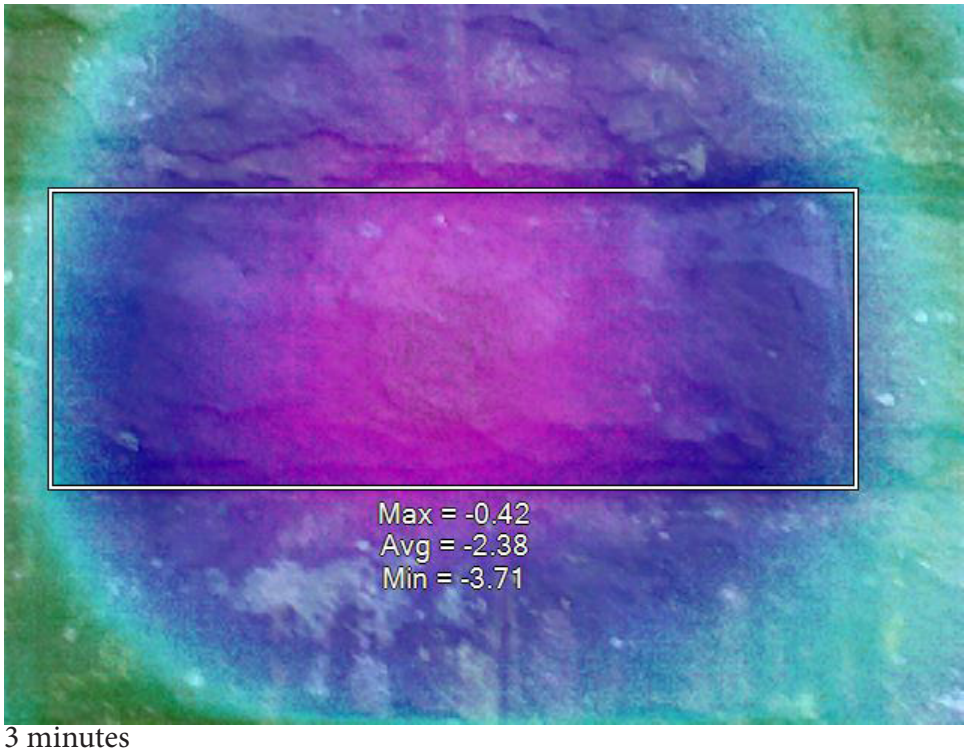

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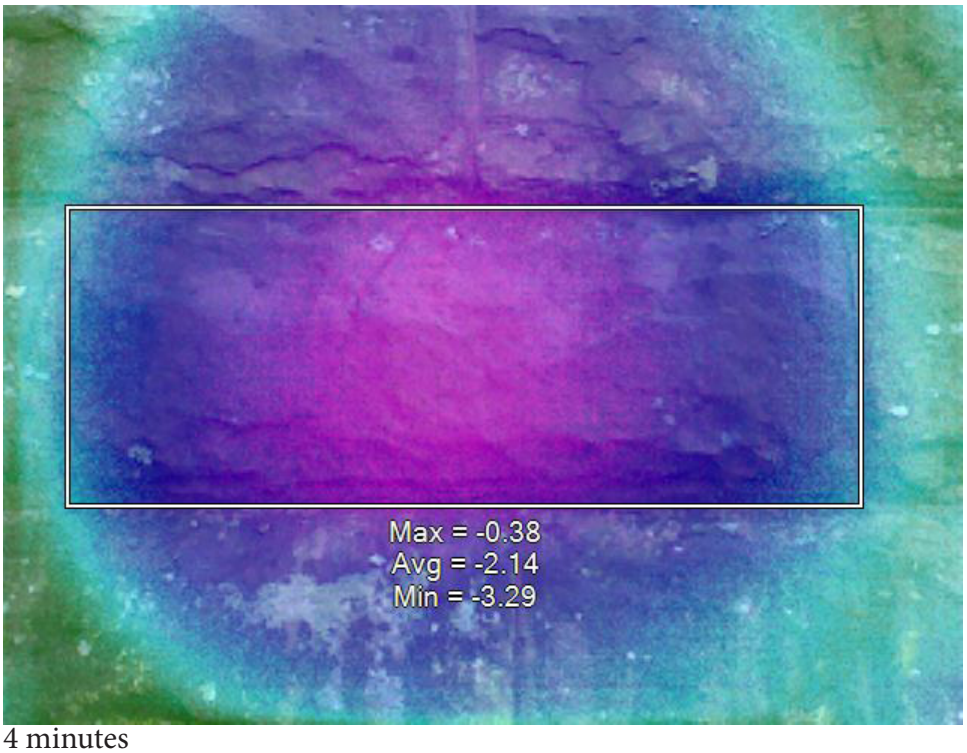
November 26, 2012



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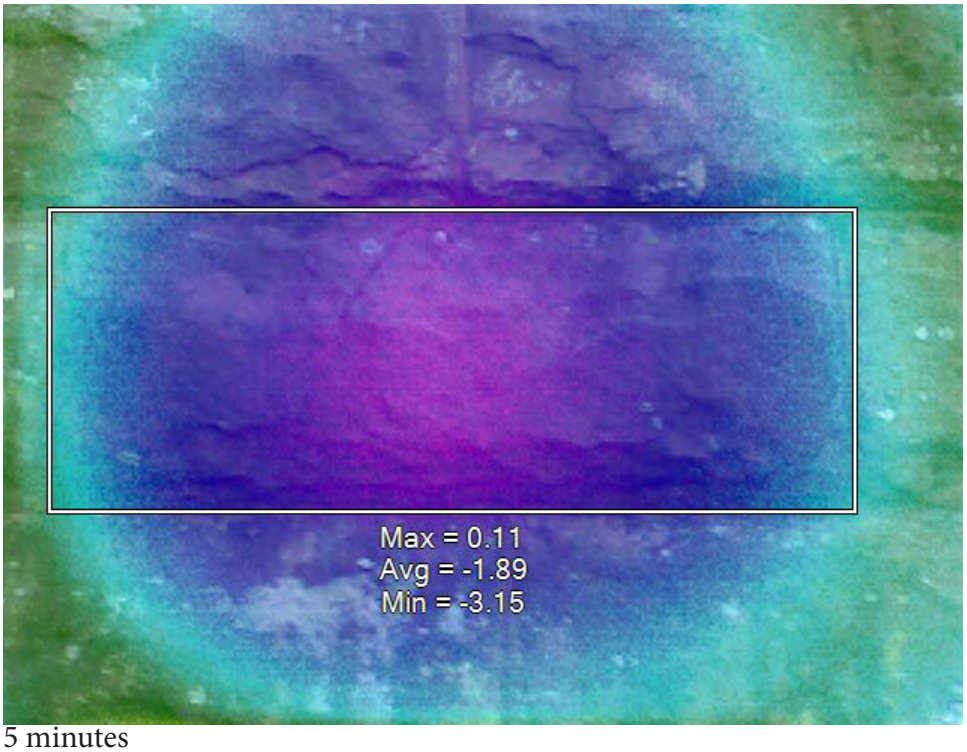

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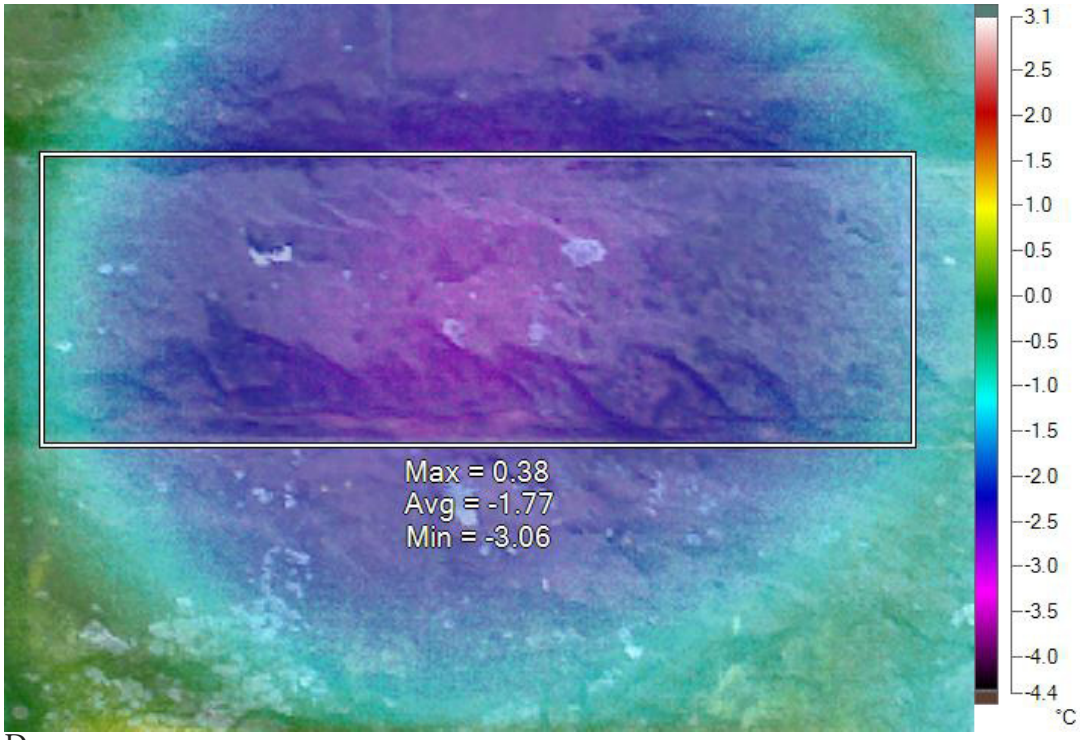
Green biocolonization control
November 26, 2012



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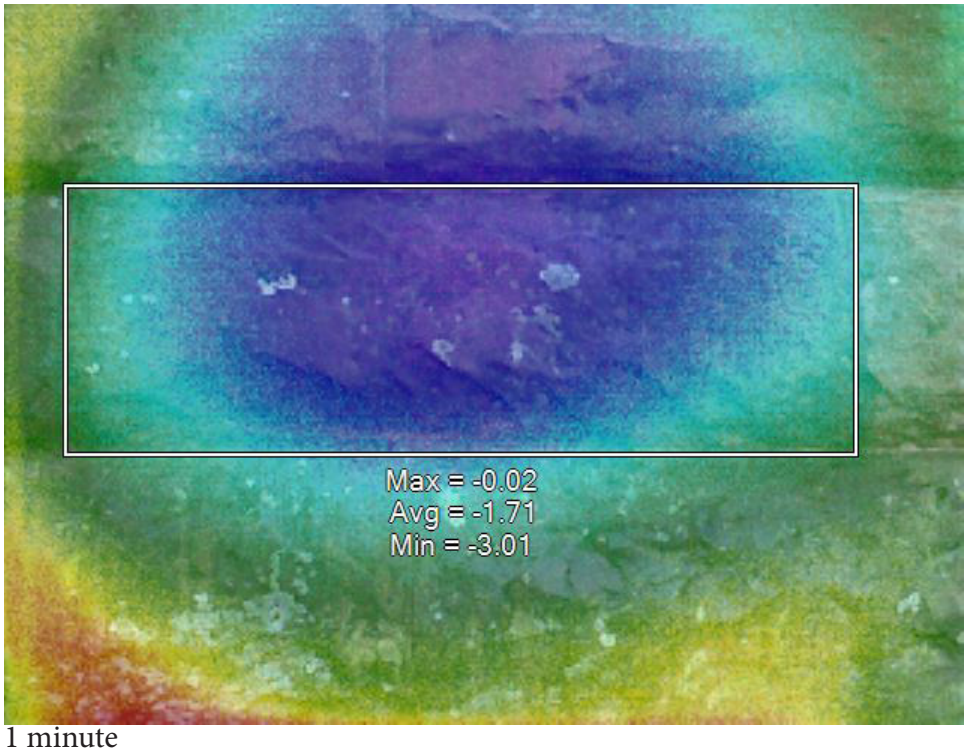

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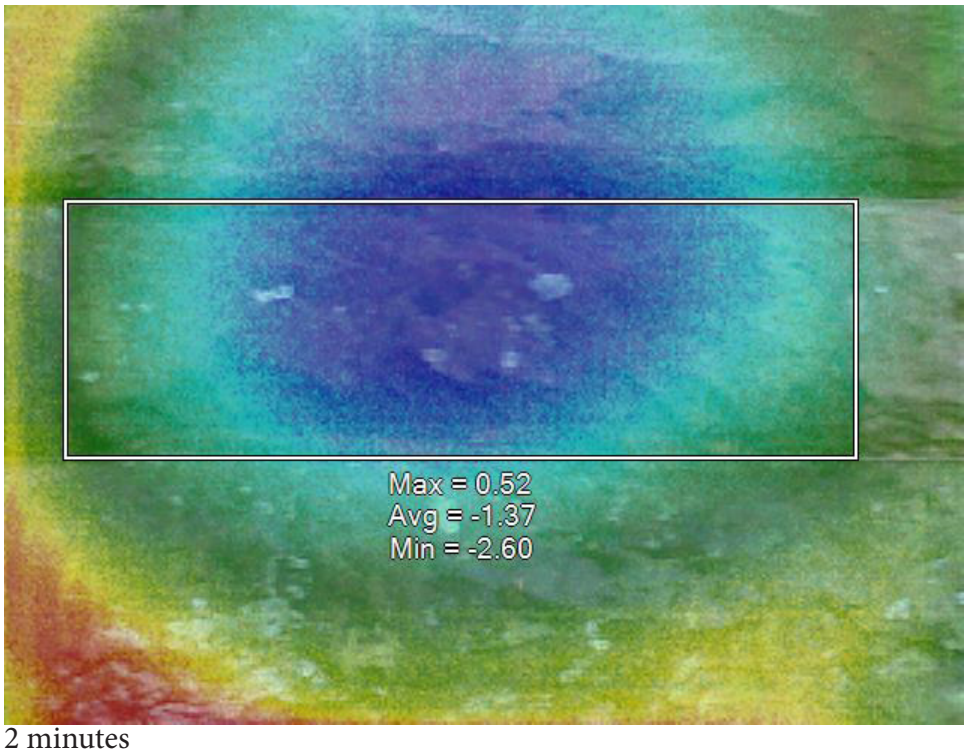

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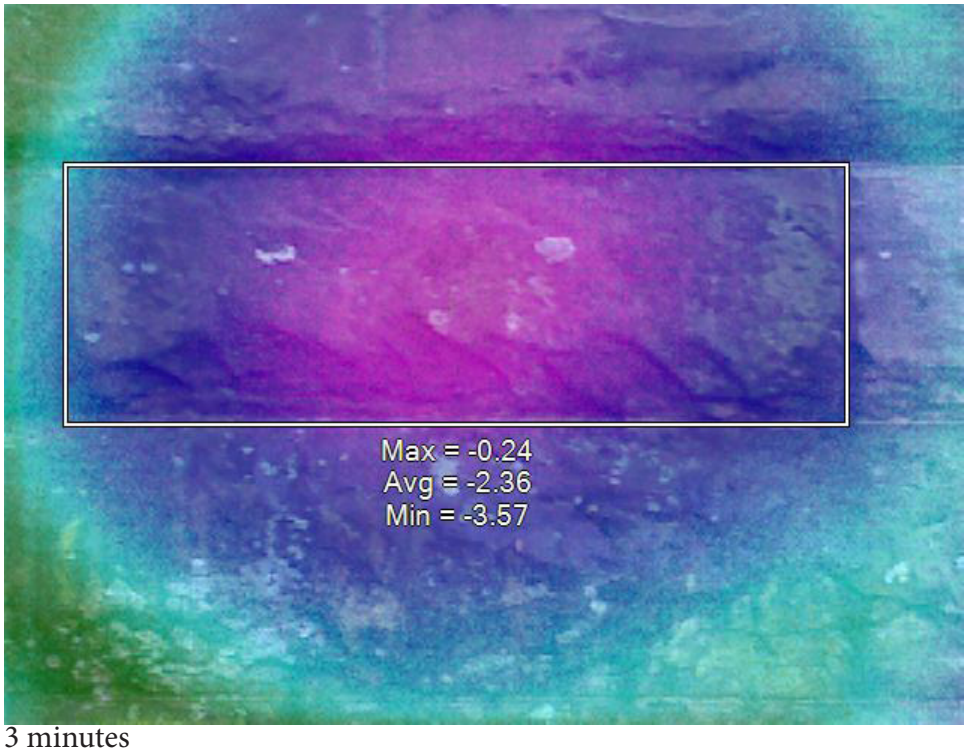

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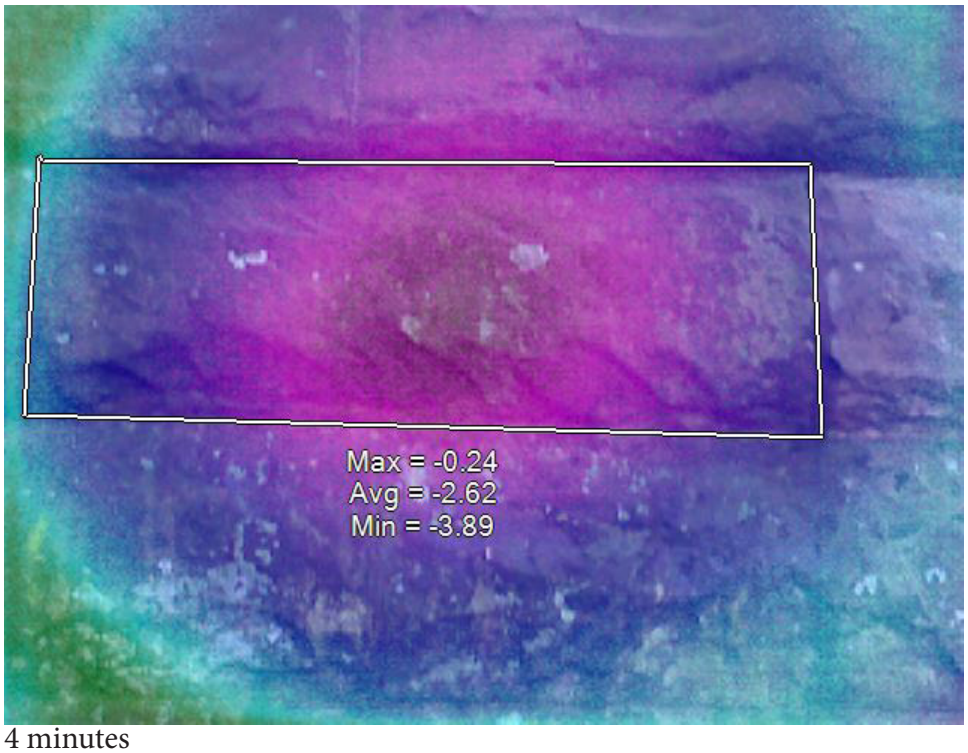

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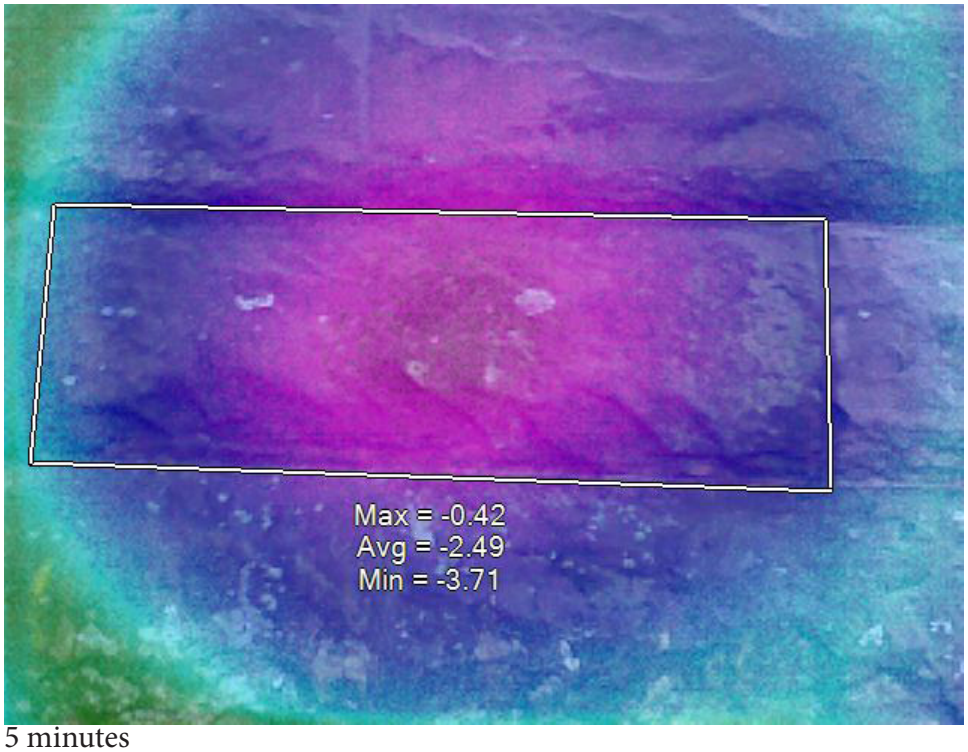

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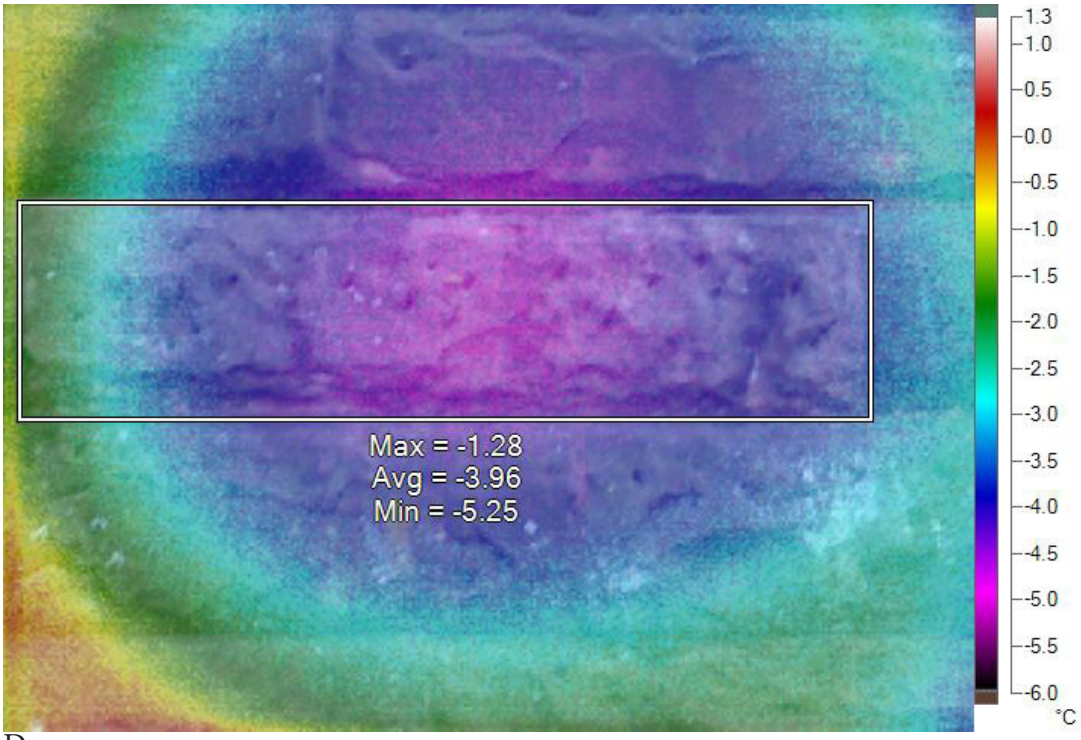
Green biocolonization treated with BioWash
November 26, 2012



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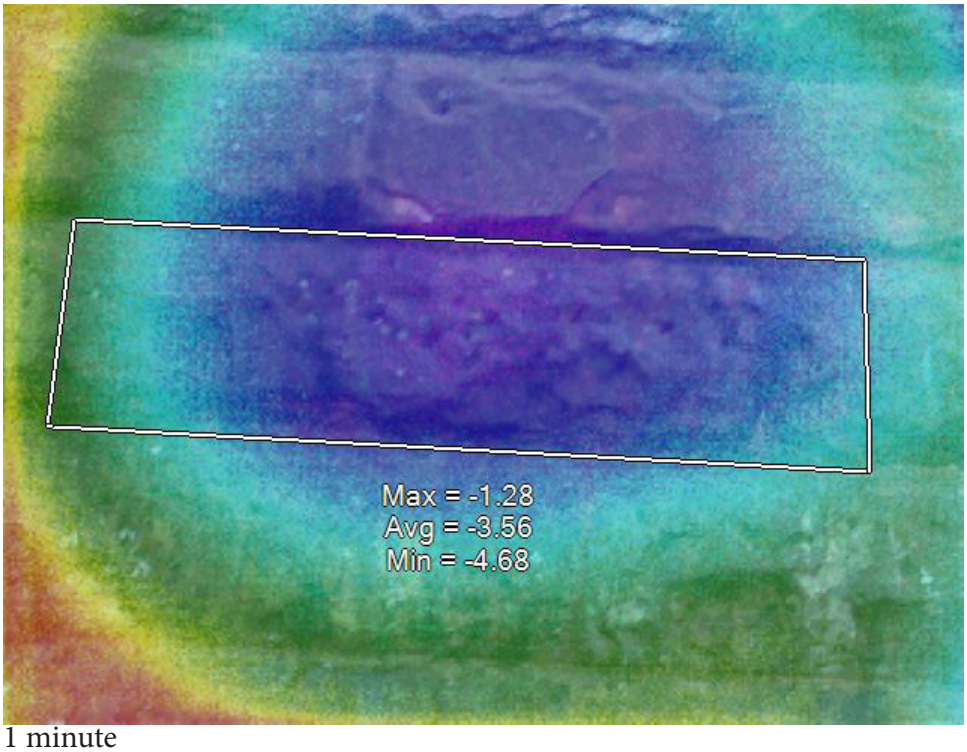

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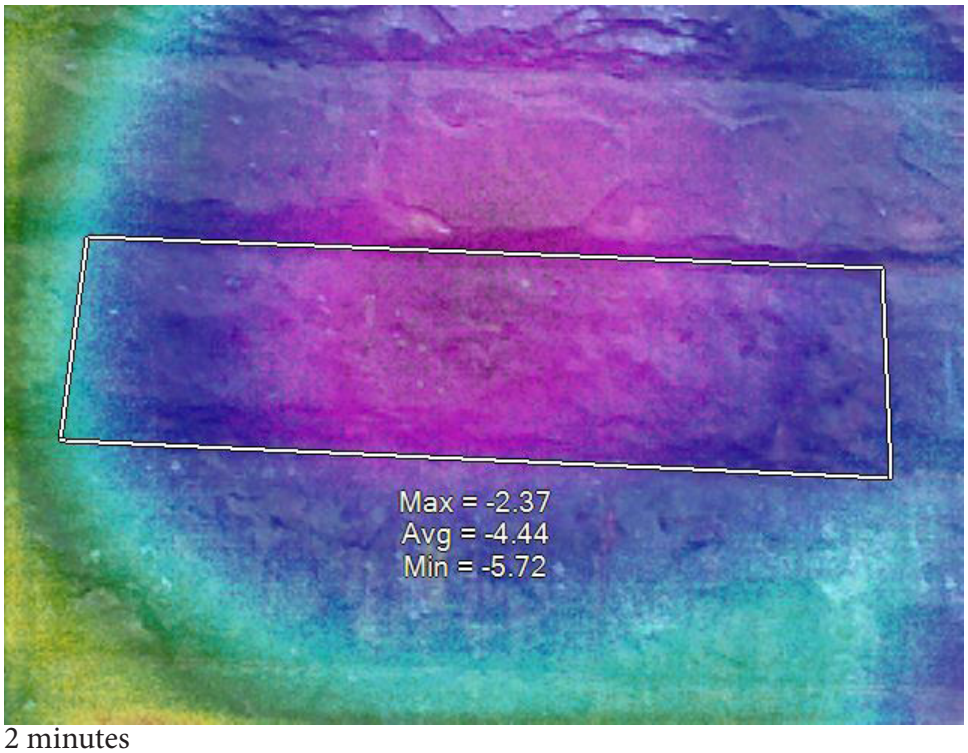
Green biocolonization treated with BIoWash
November 26, 2012



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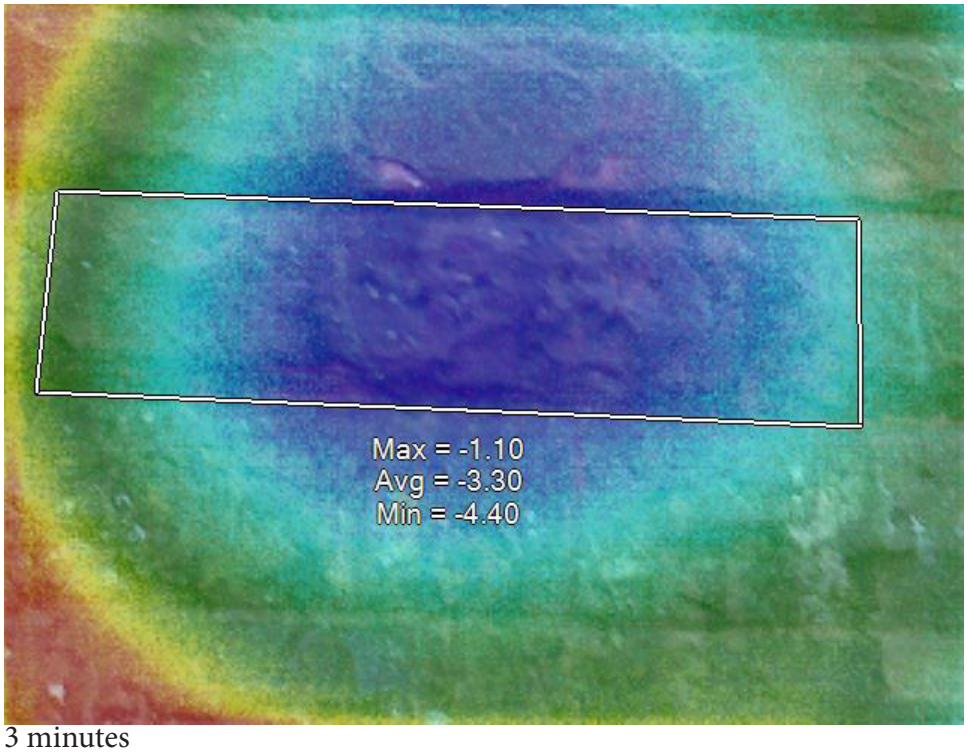

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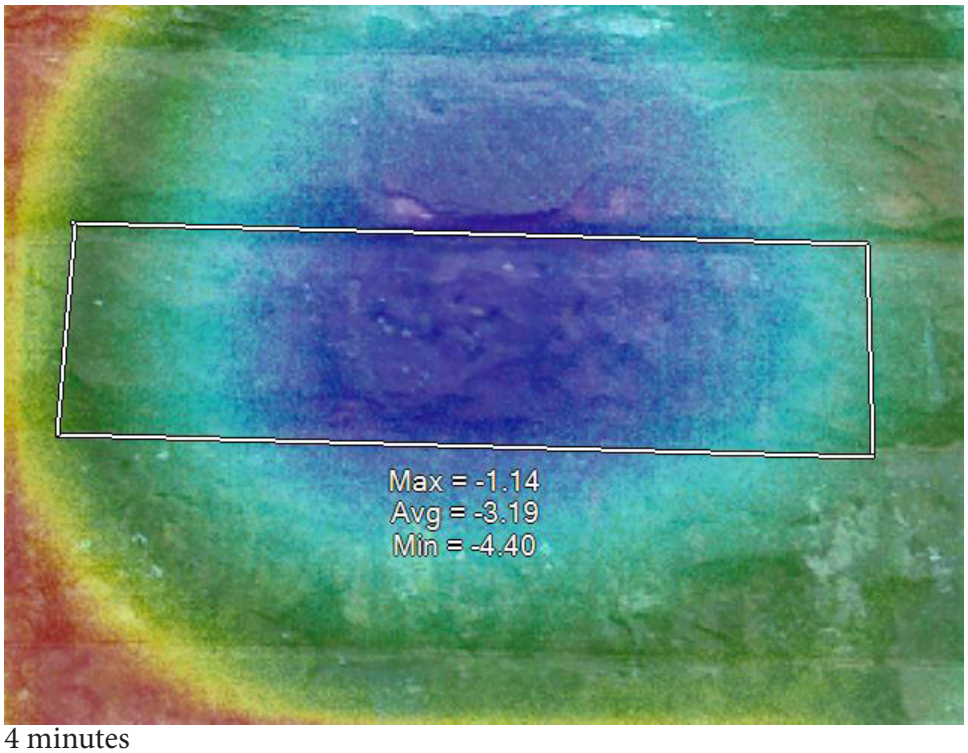
Green biocolonization treated with BioWash
November 26, 2012



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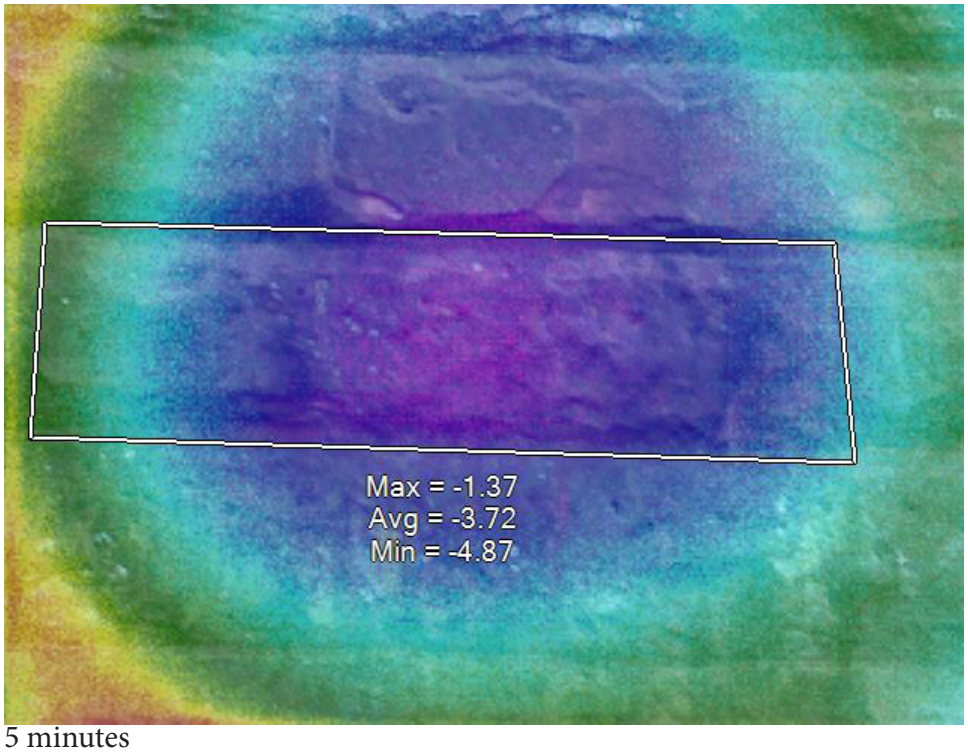
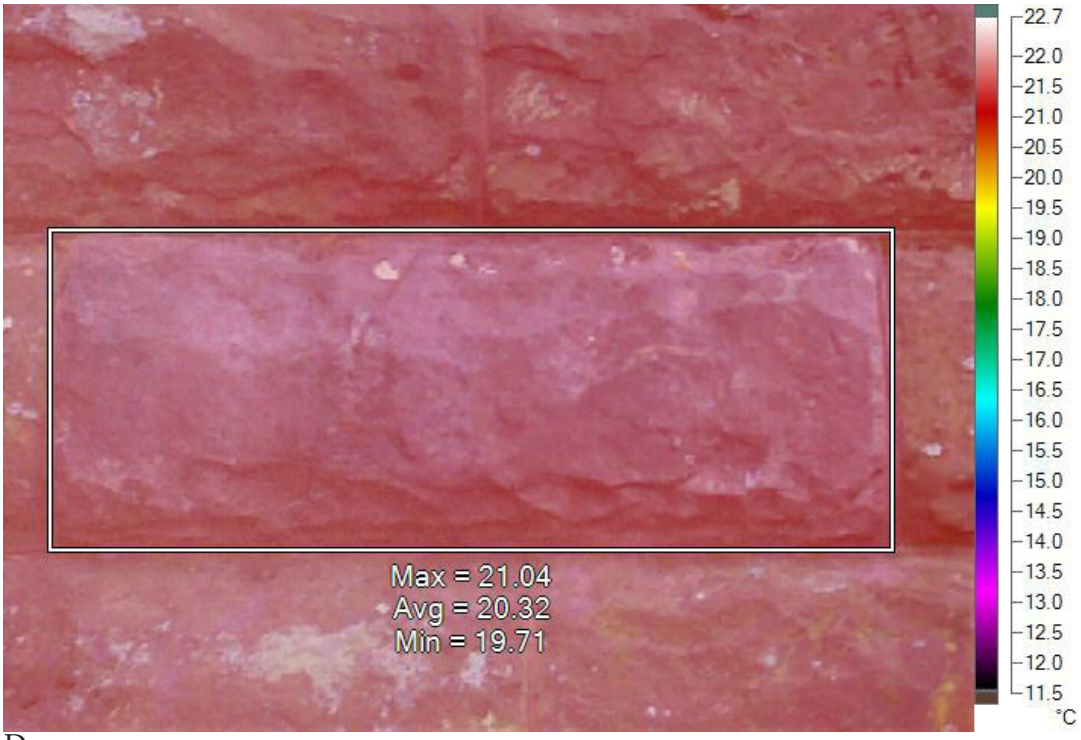


5 minutes

Green biocolonization treated with D/2
December 4, 2012



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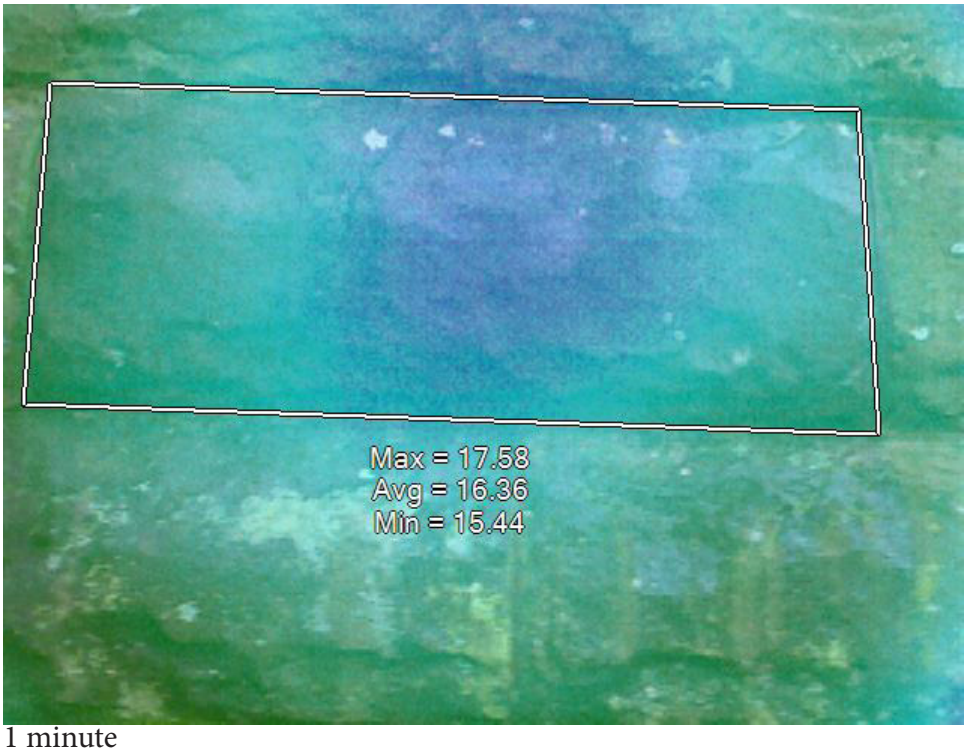

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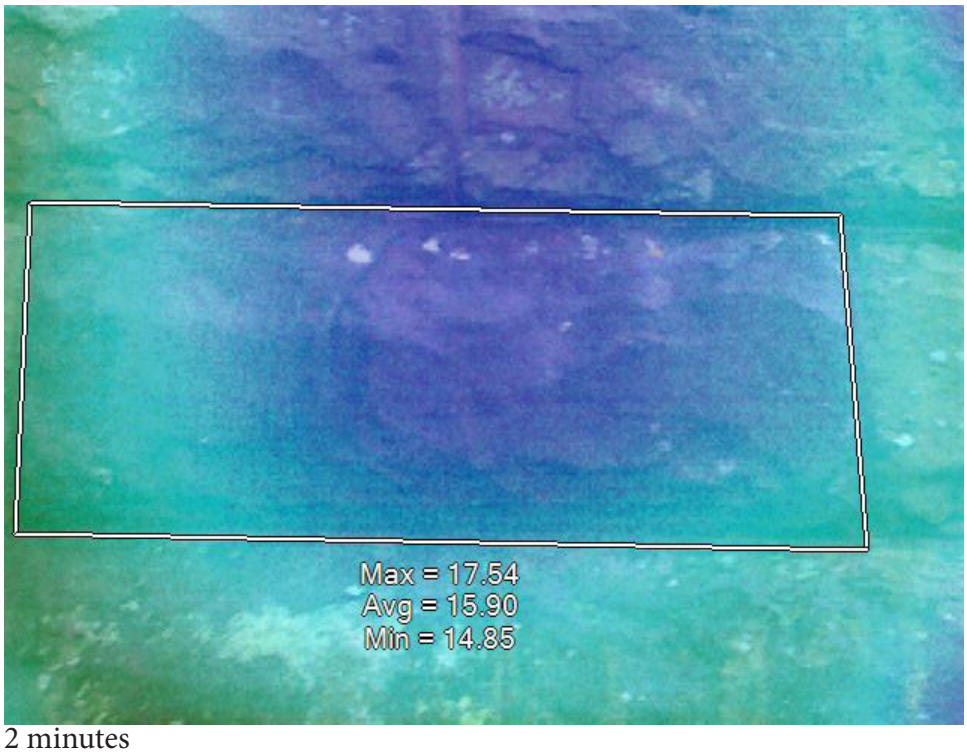
Green biocolonization treated with D/2
December 4, 2012



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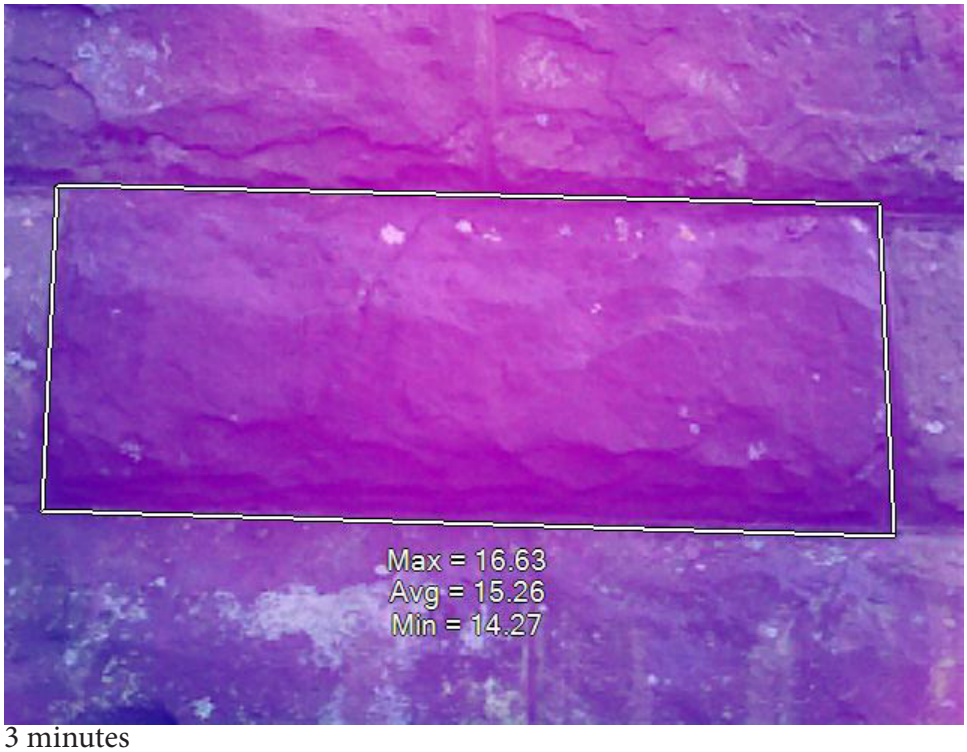

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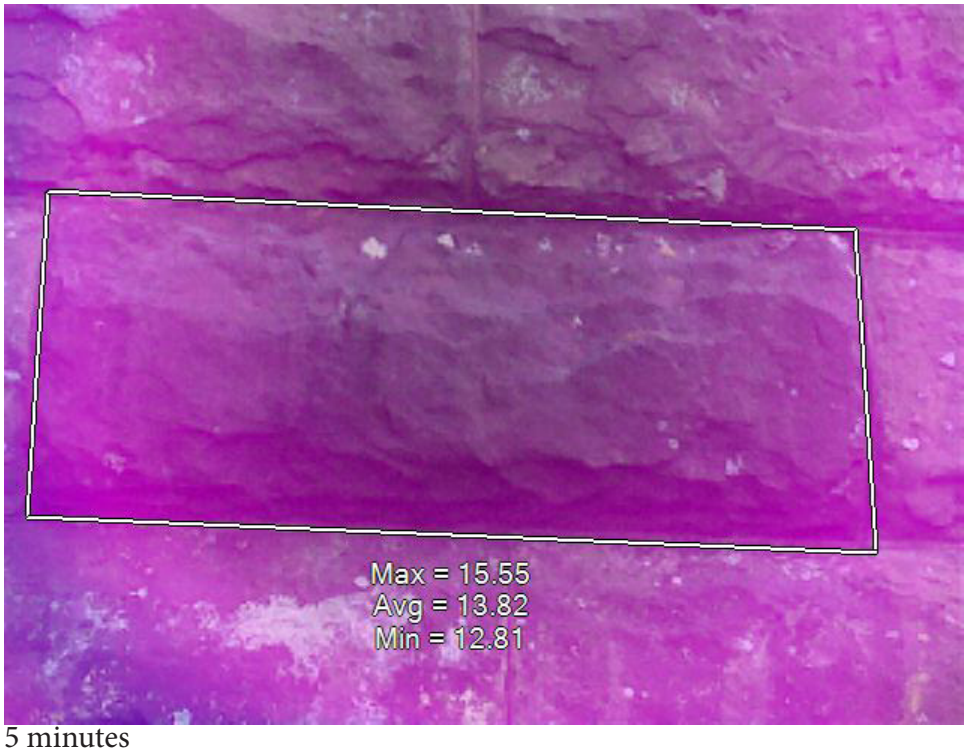
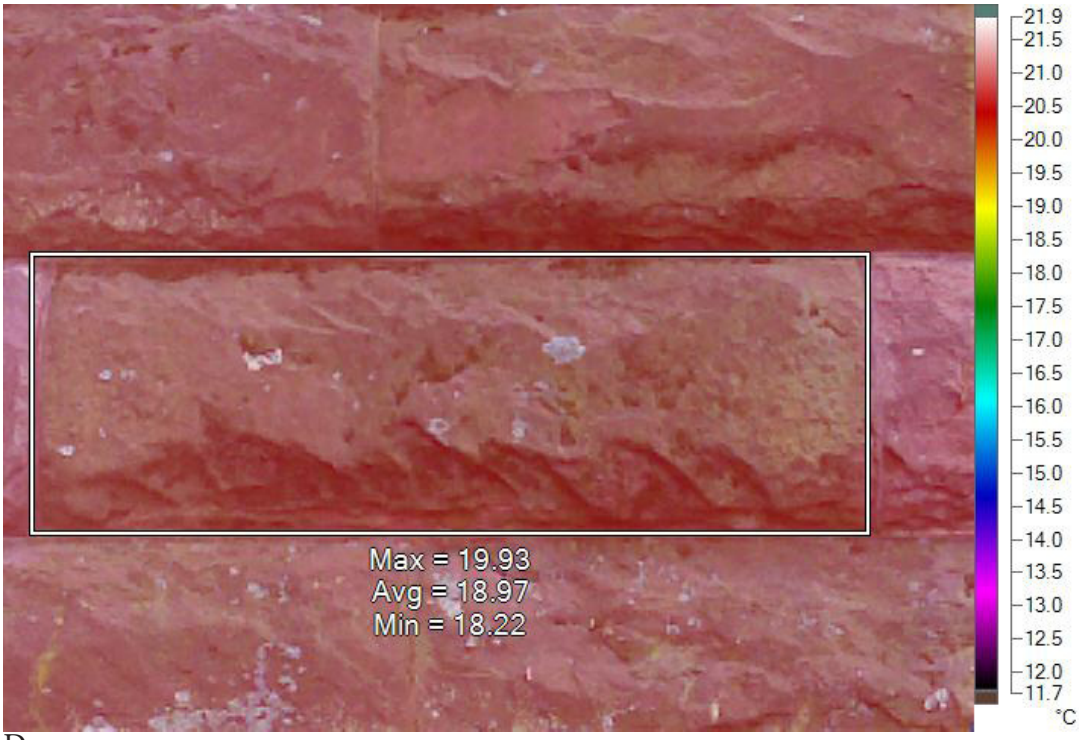
Green biocolonization treated with D/2
December 4, 2012

4 minutes



5 minutes

Green biocolonization control
December 4, 2012



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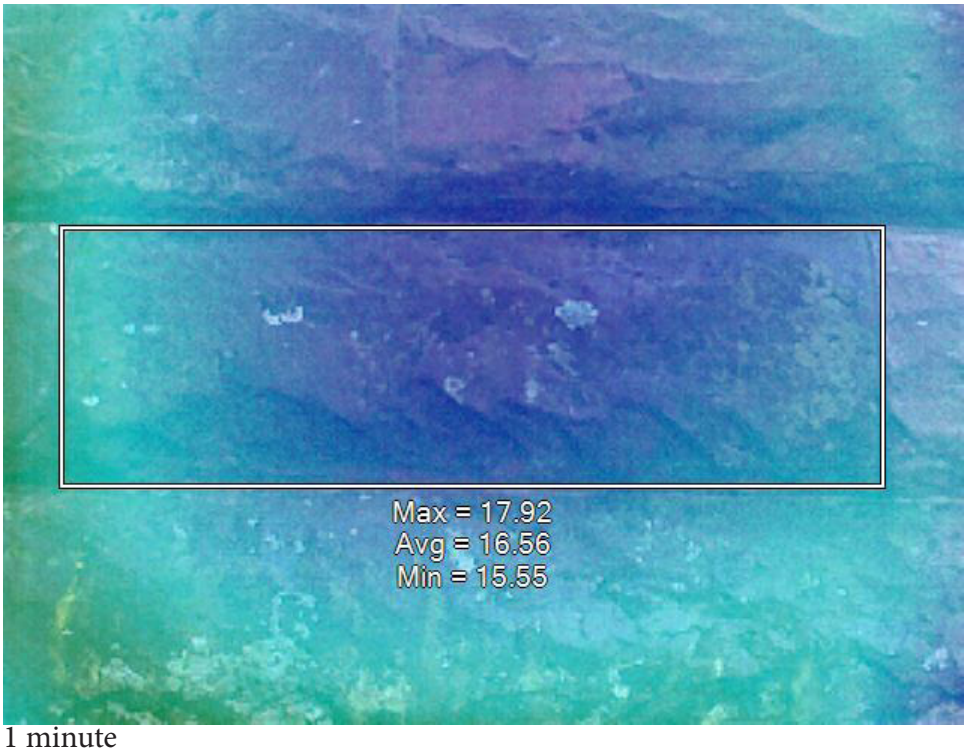

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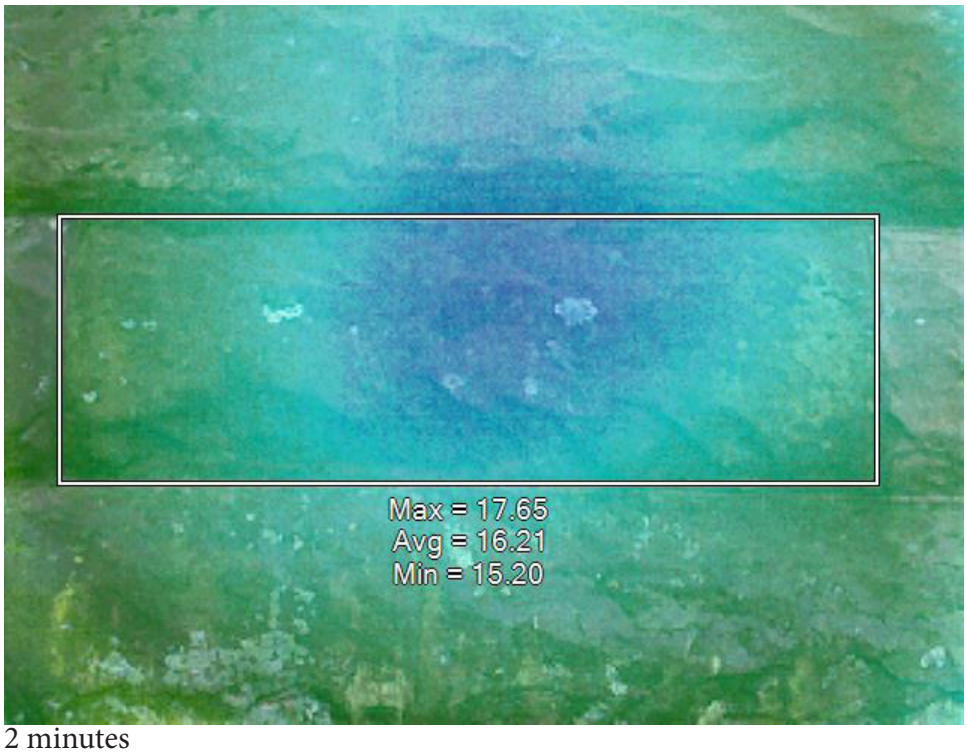

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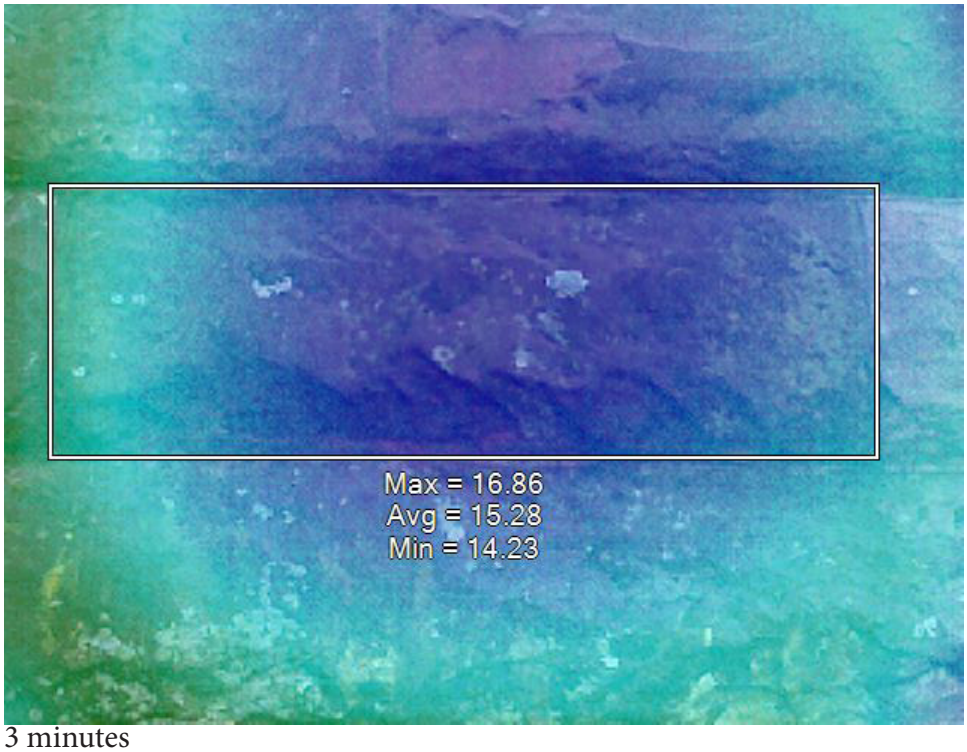

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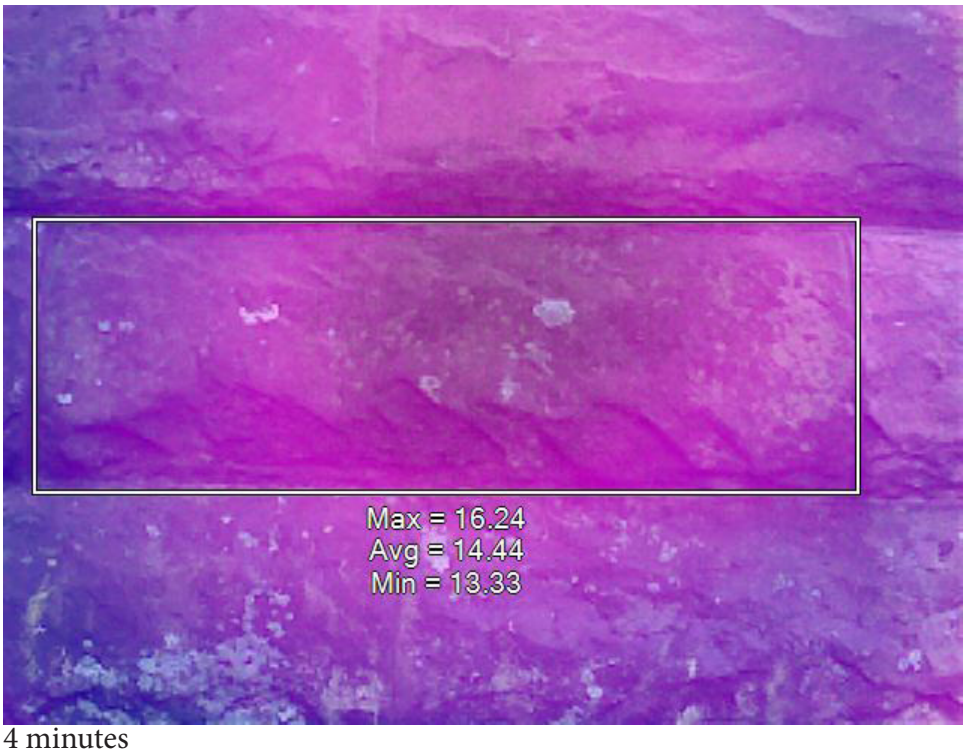

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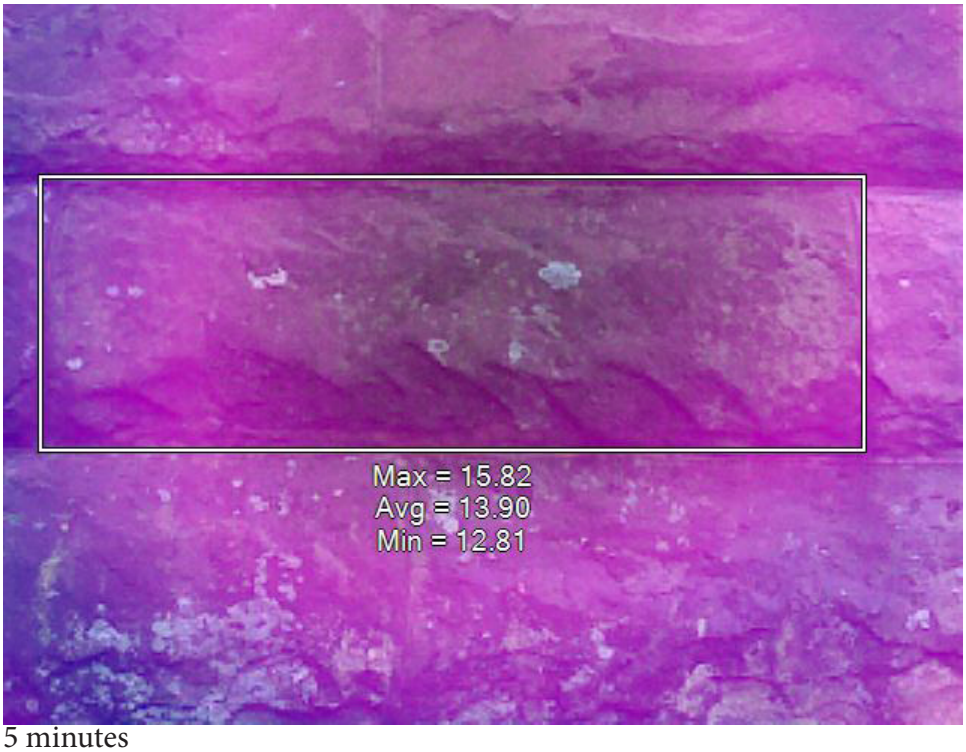
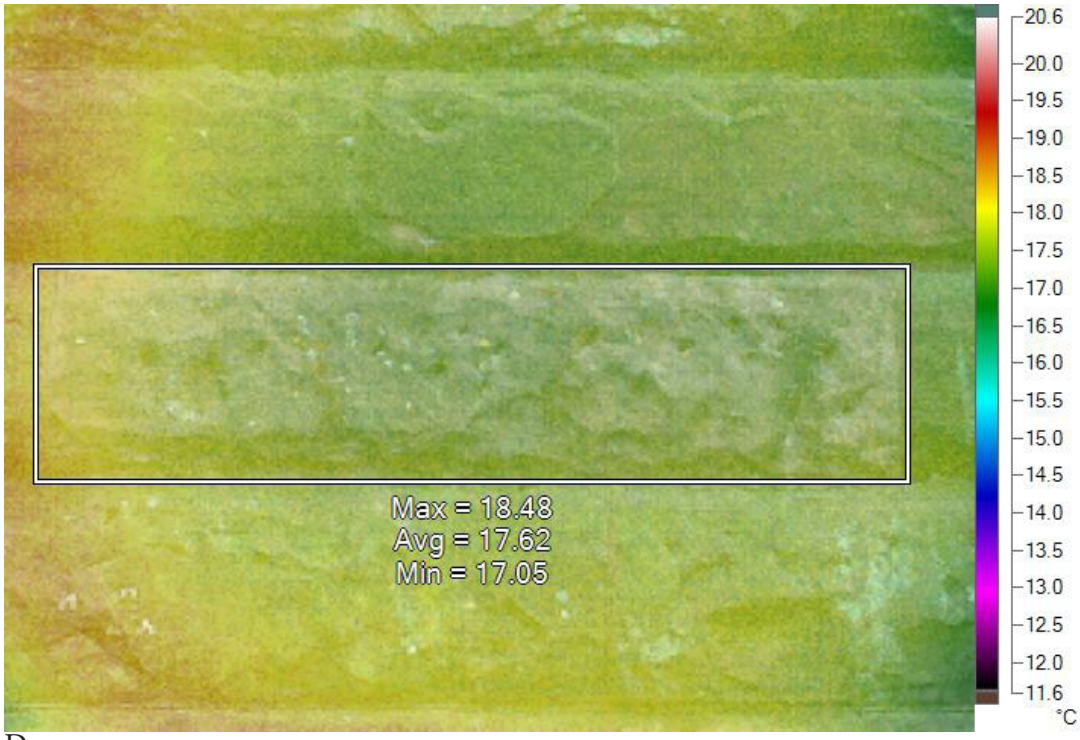


5 minutes

Green biocolonization treated with BioWash
December 4, 2012



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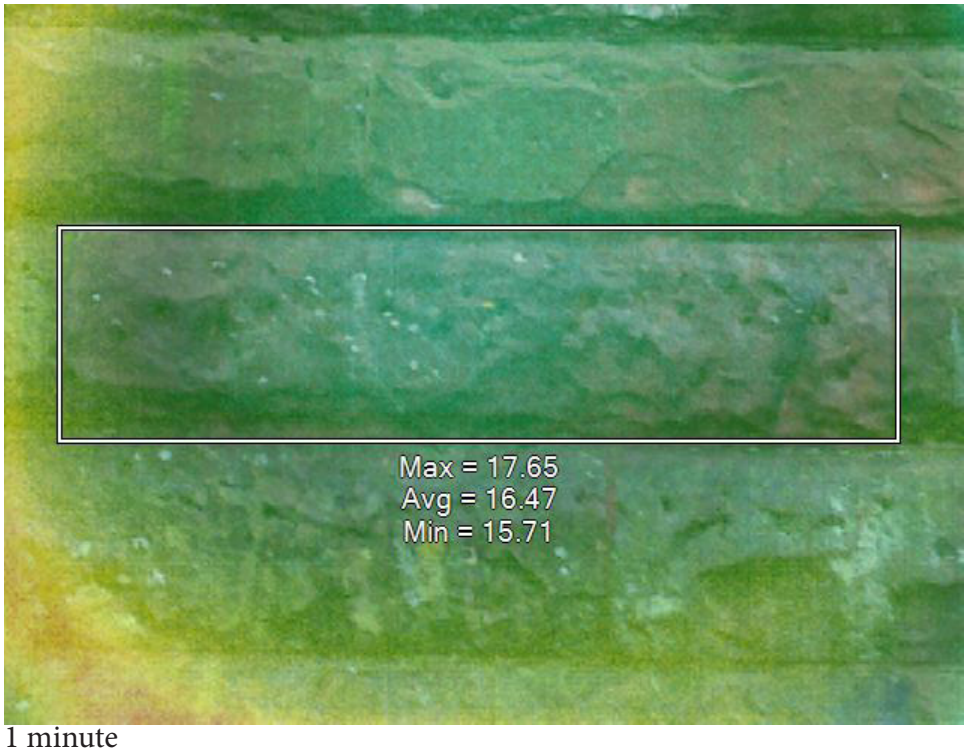


1 minute

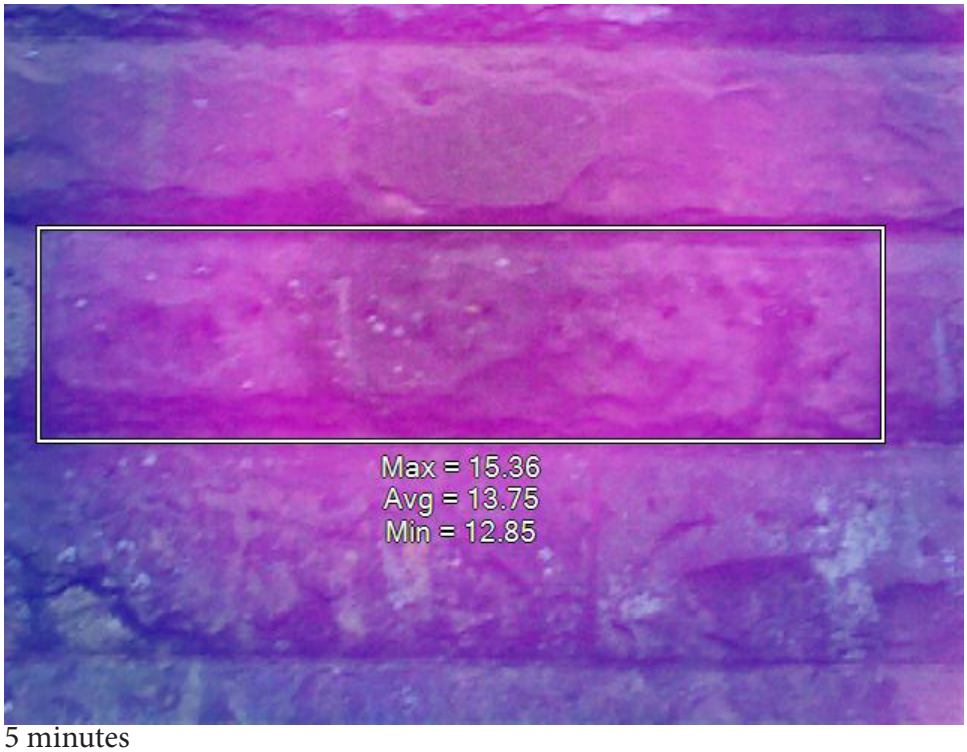
Green biocolonization treated with BIoWash
December 4, 2012

2 minutes

3 minutes

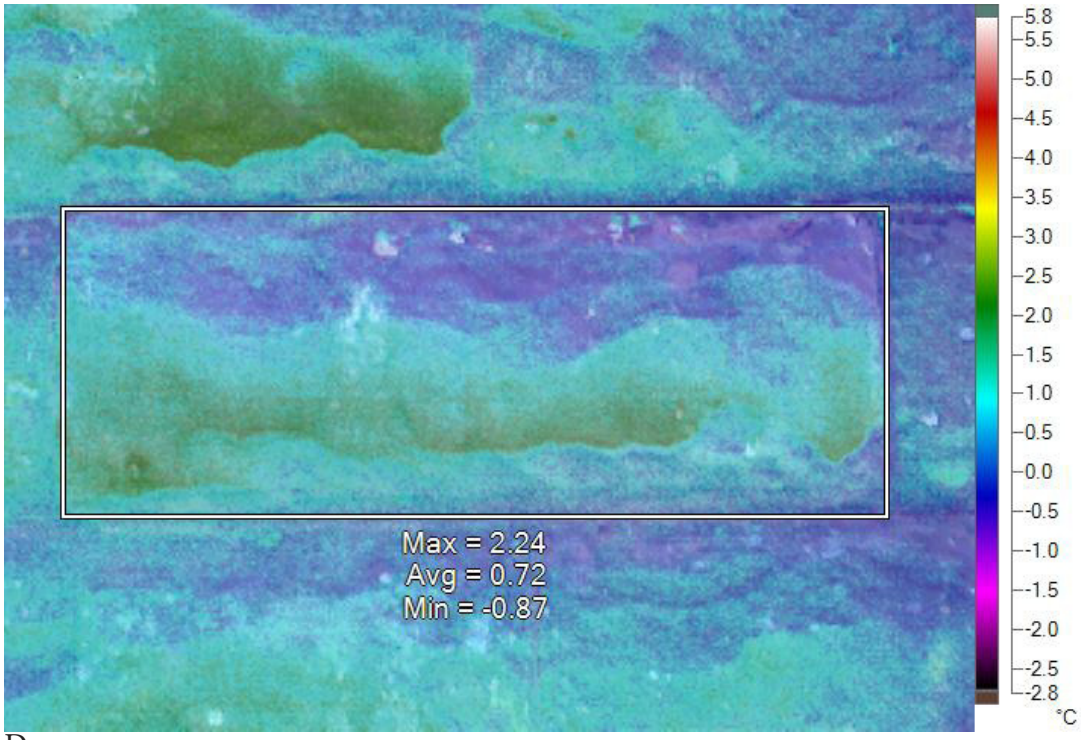
Green biocolonization treated with BioWash
December 4, 2012

4 minutes

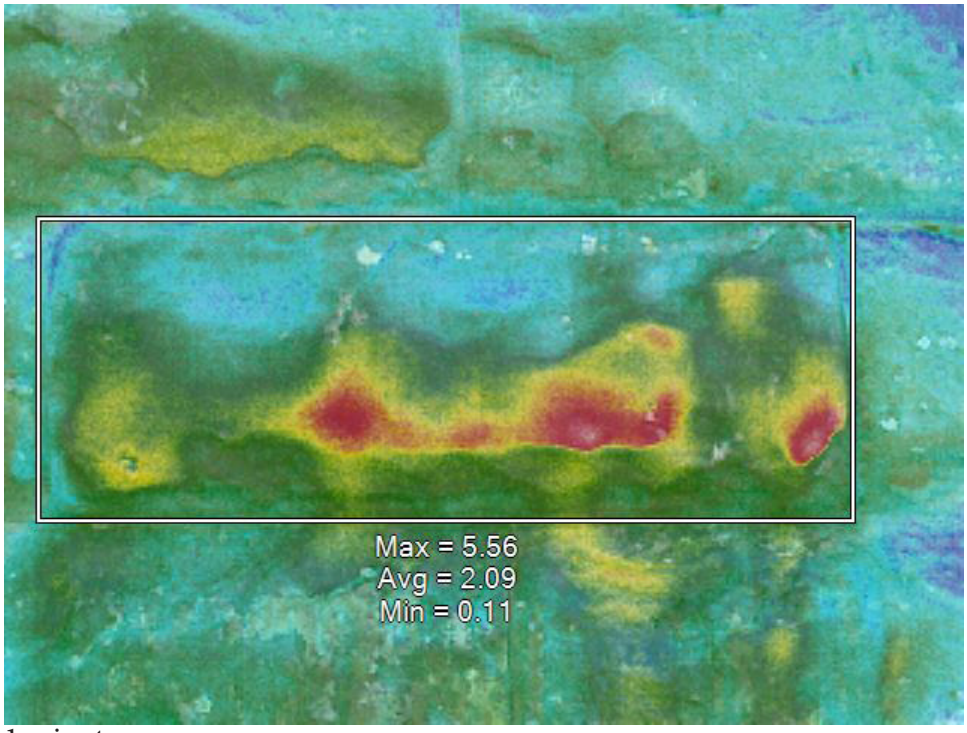


5 minutes

Green biocolonization treated with D/2
January 10, 2013

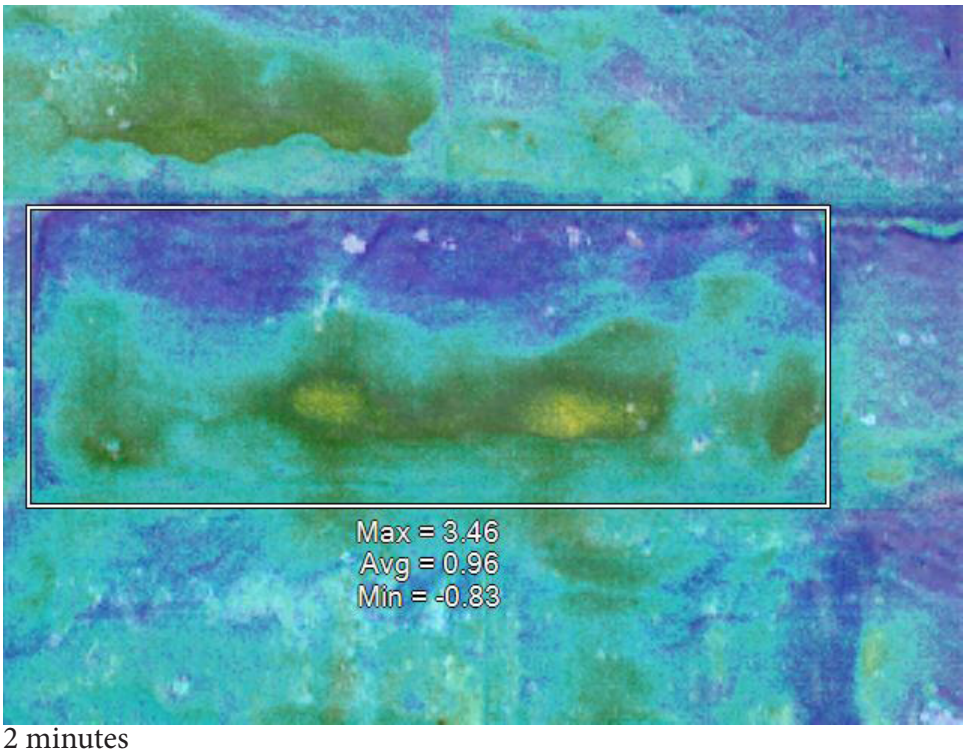


Dry

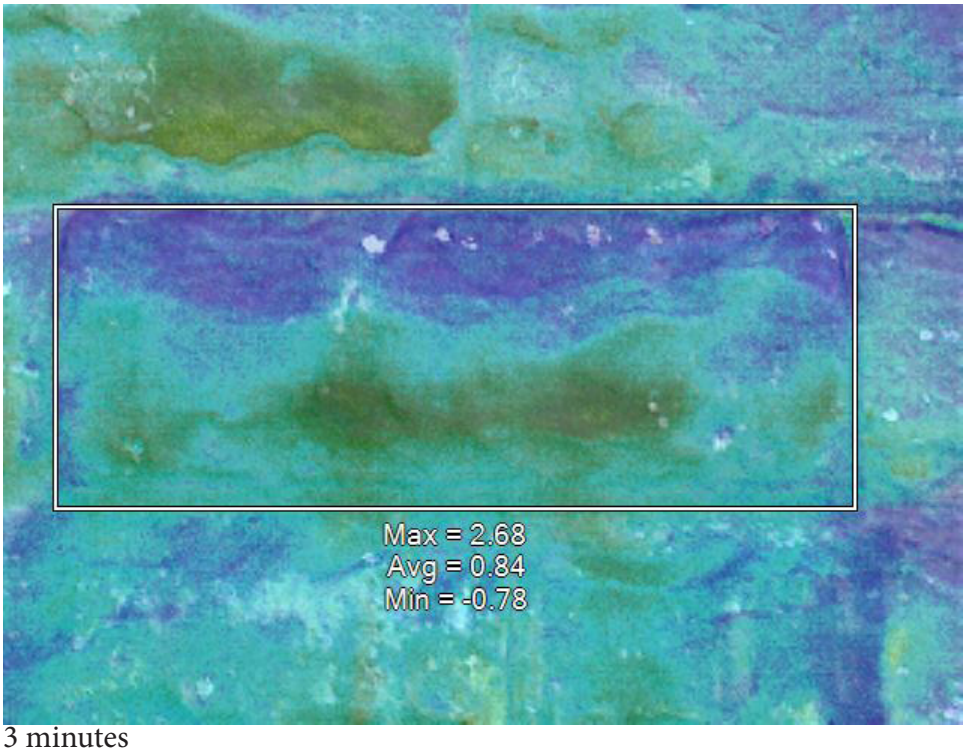


1 minute

Green biocolonization treated with D/2
January 10, 2013

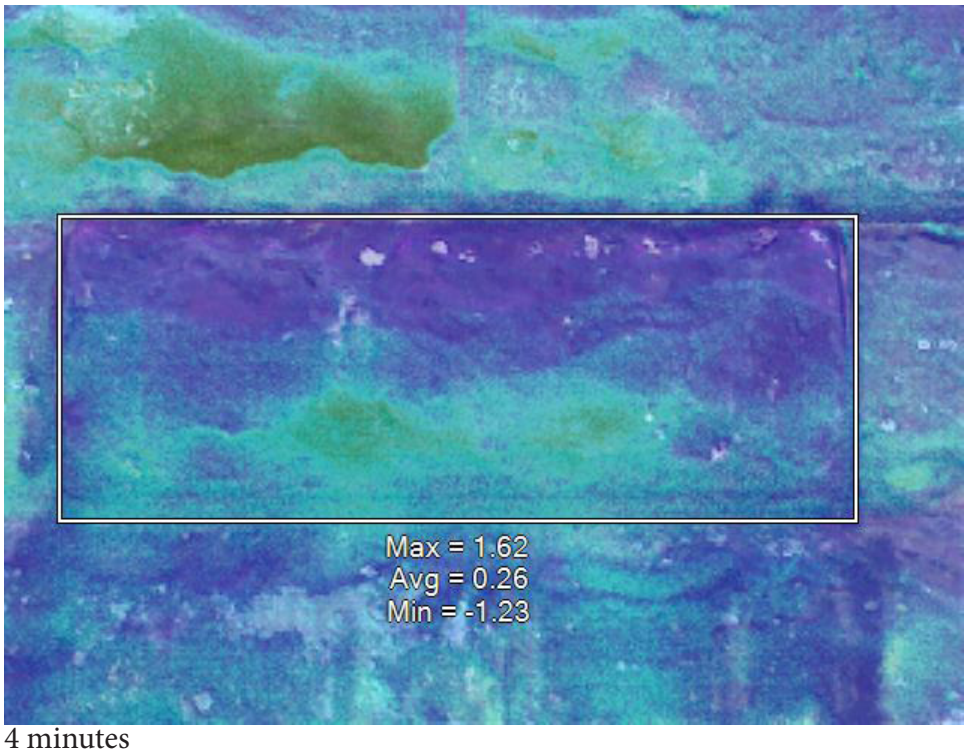


2 minutes

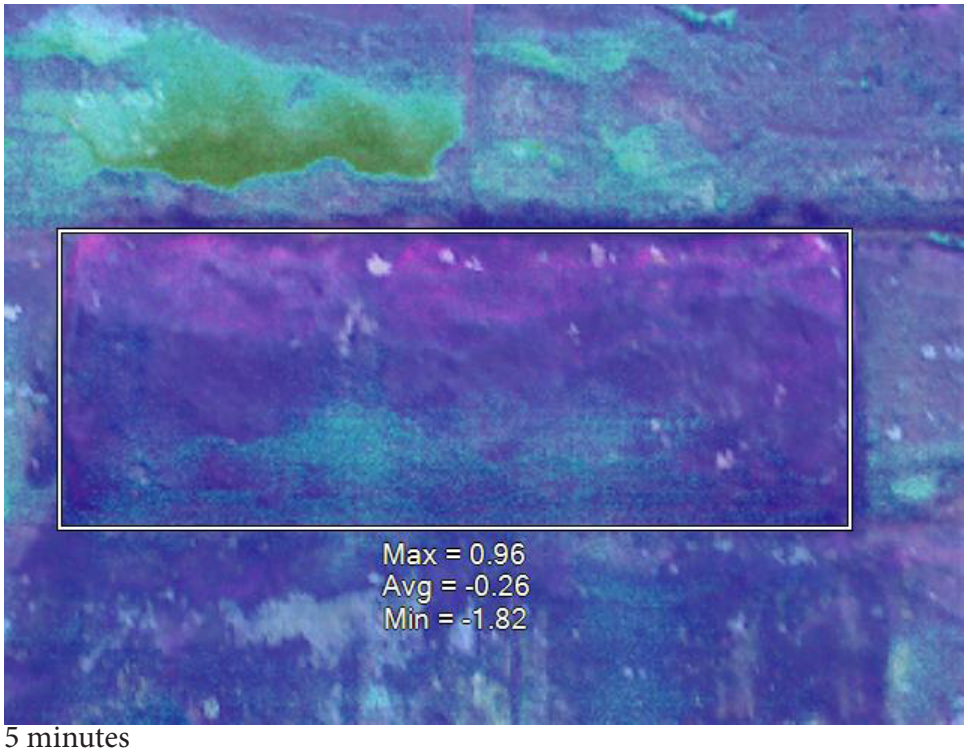


3 minutes

Green biocolonization treated with D/2
January 10, 2013

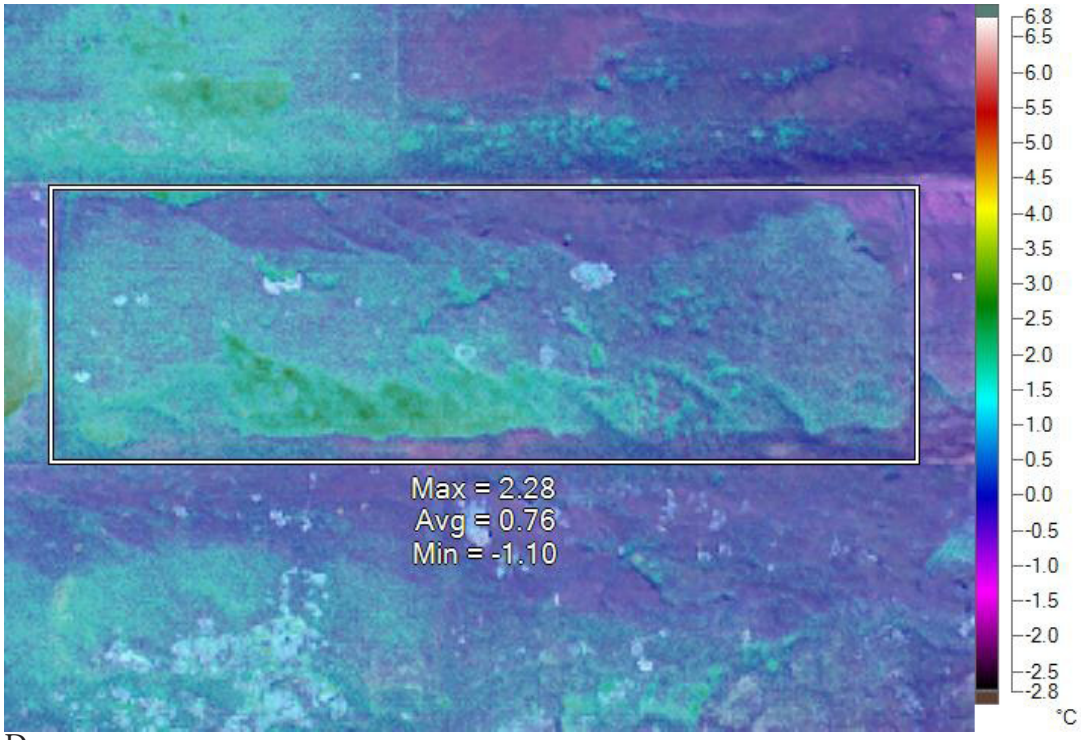


4 minutes

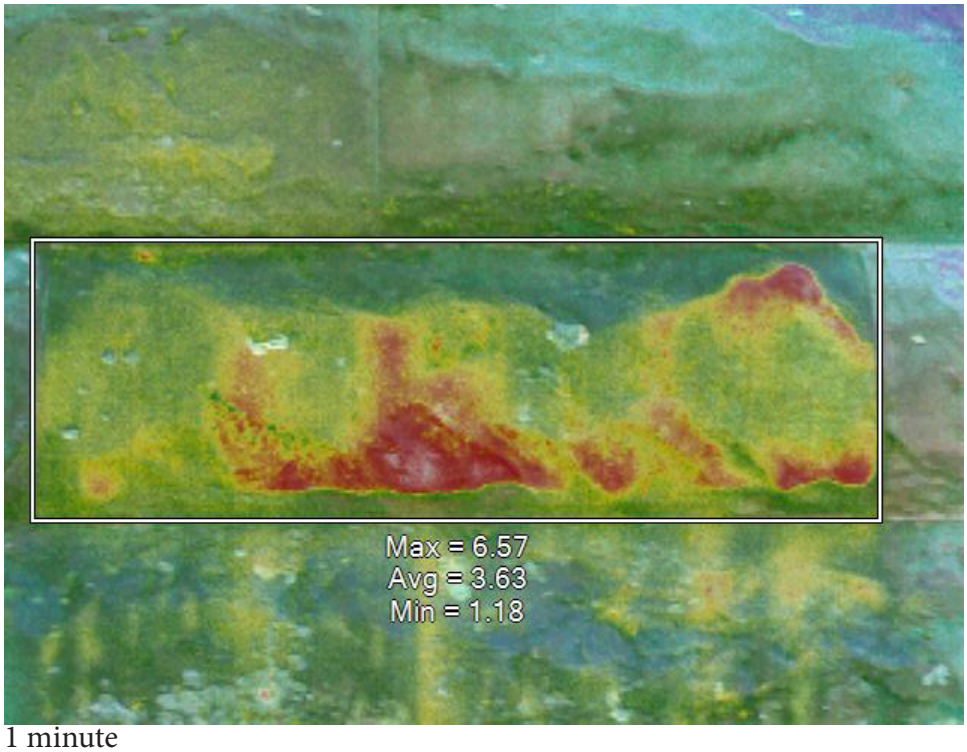


5 minutes

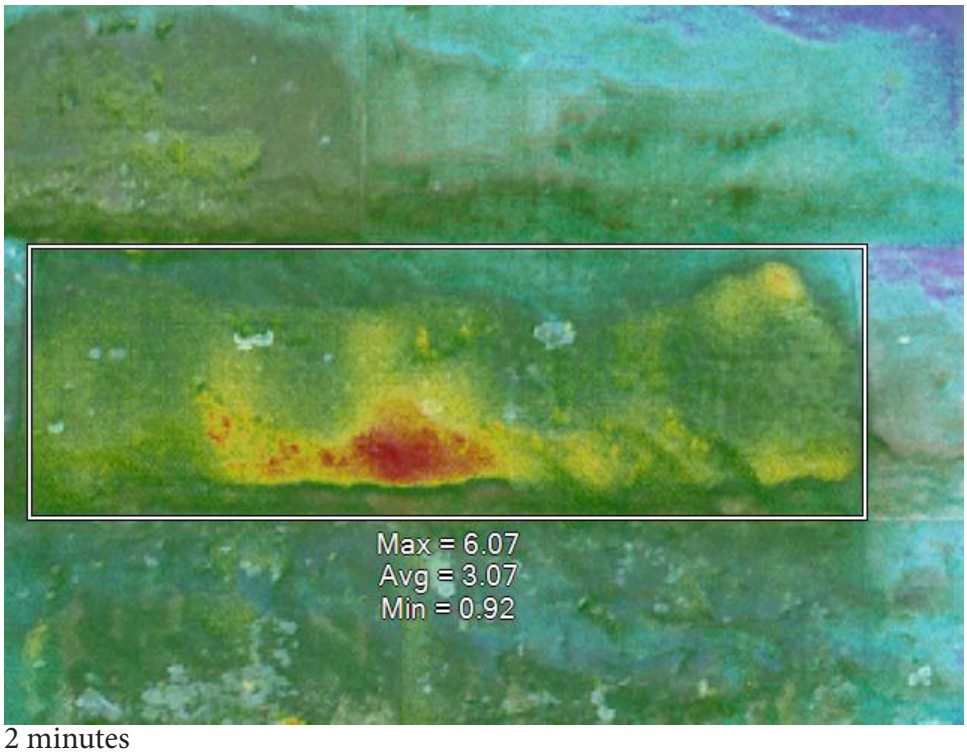
Green biocolonization control
January 10, 2013



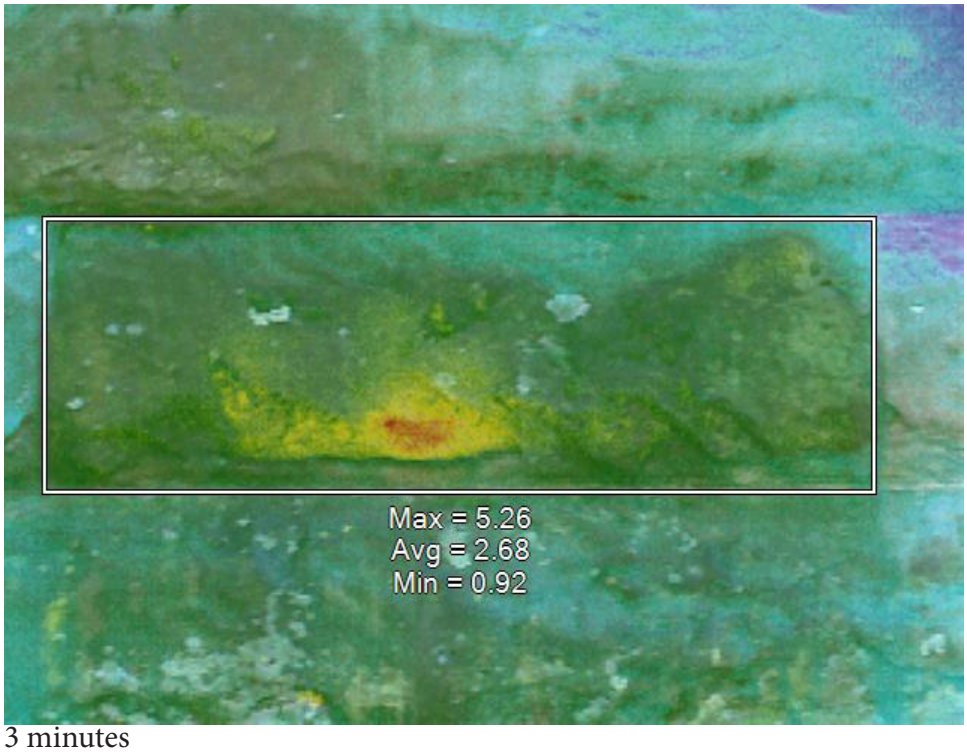
Dry



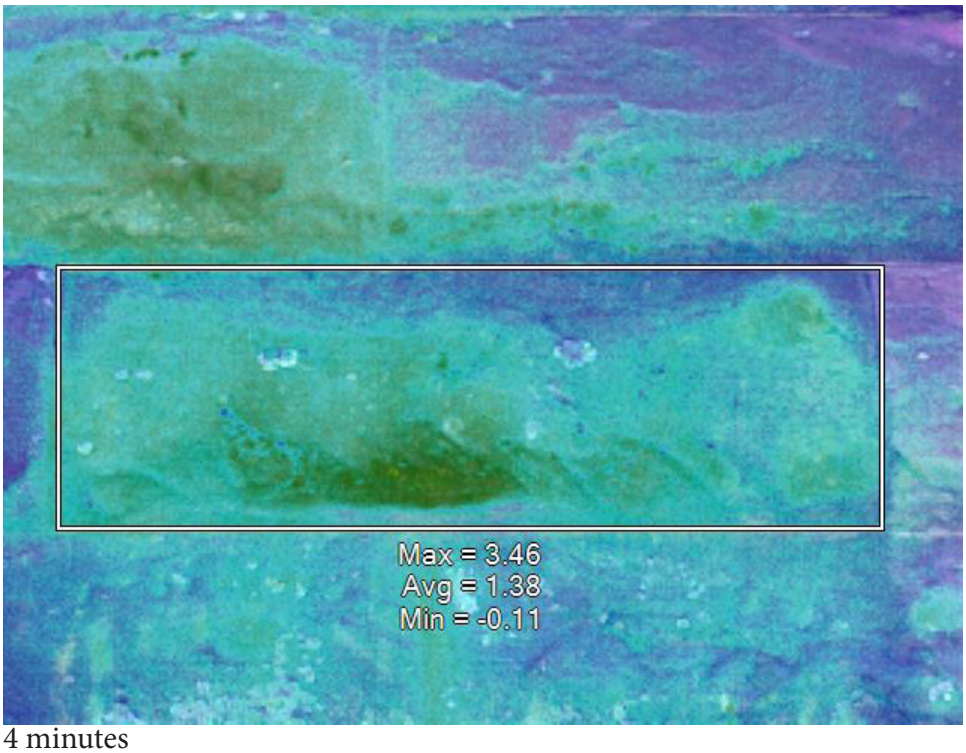
1 minute



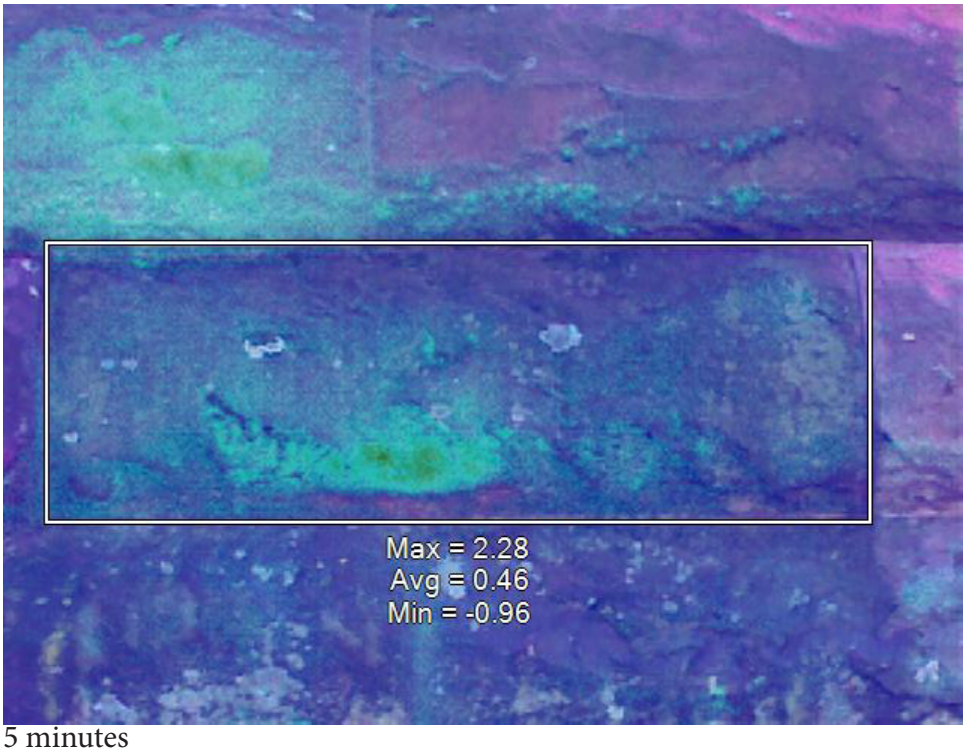
2 minutes



3 minutes

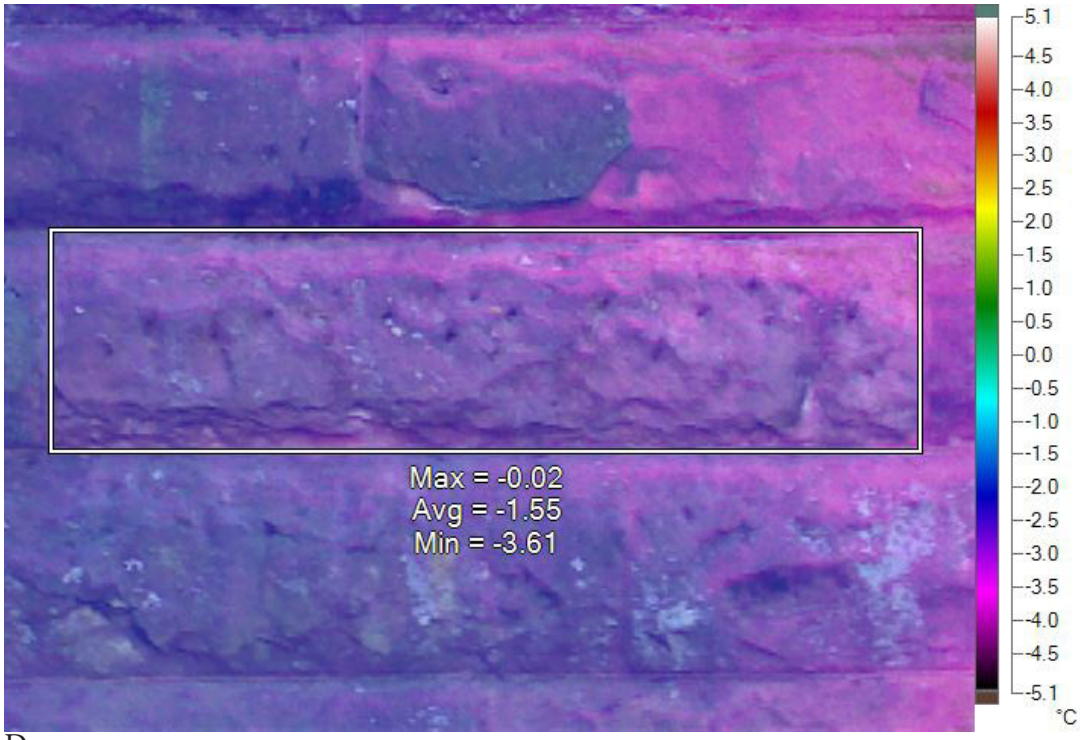


4 minutes

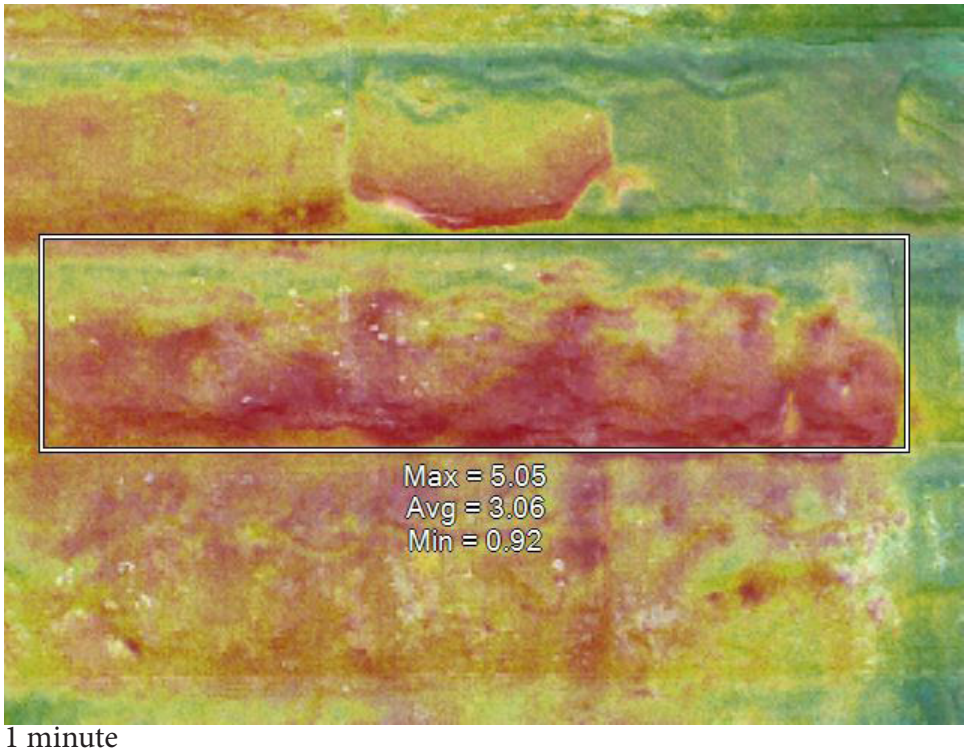


5 minutes

Green biocolonization treated with BioWash
January 10, 2013

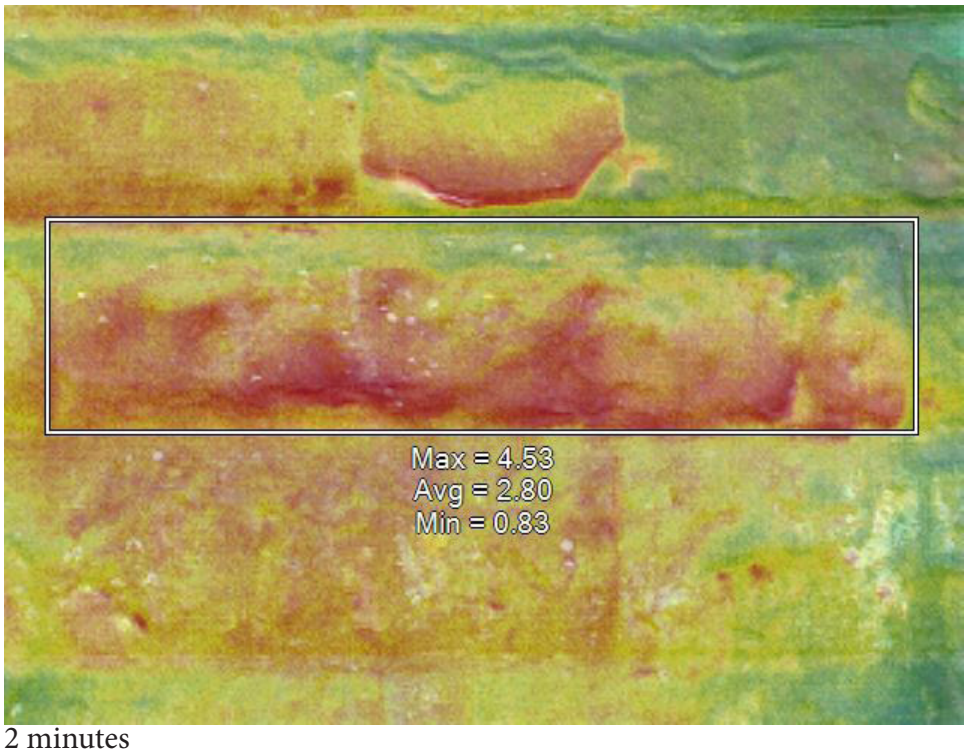


Dry

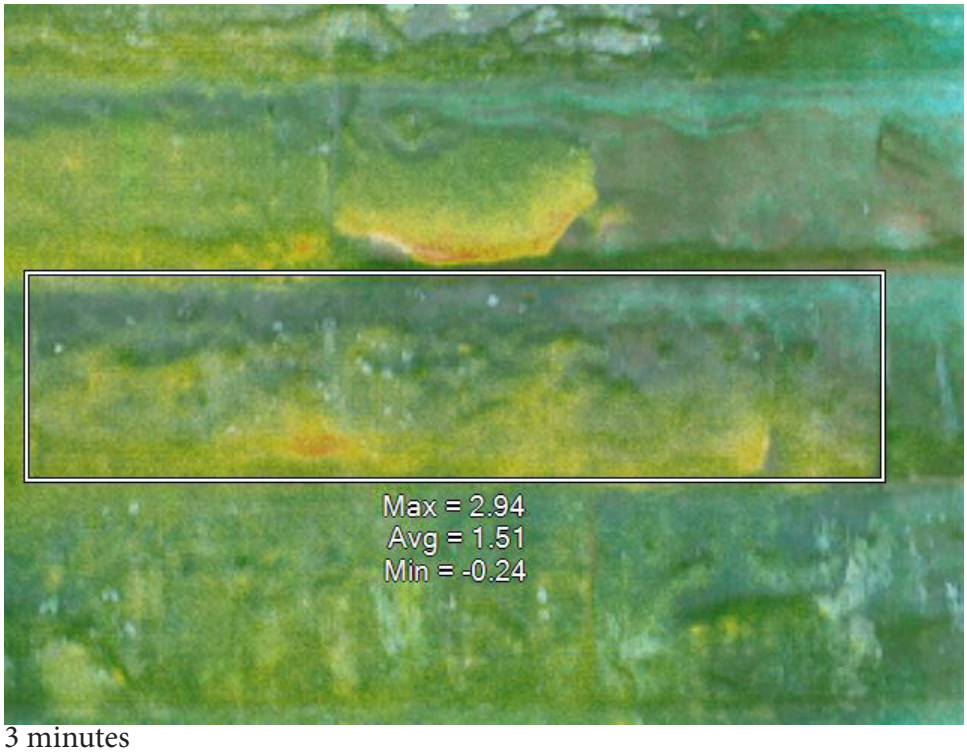


1 minute

Green biocolonization treated with BIoWash
January 10, 2013

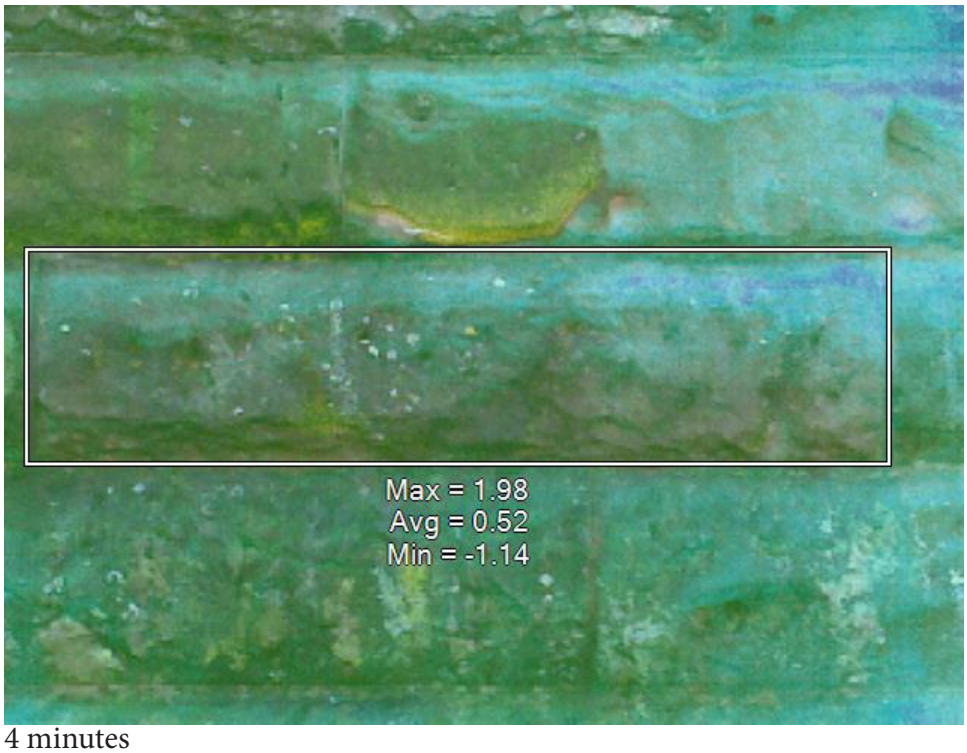


2 minutes

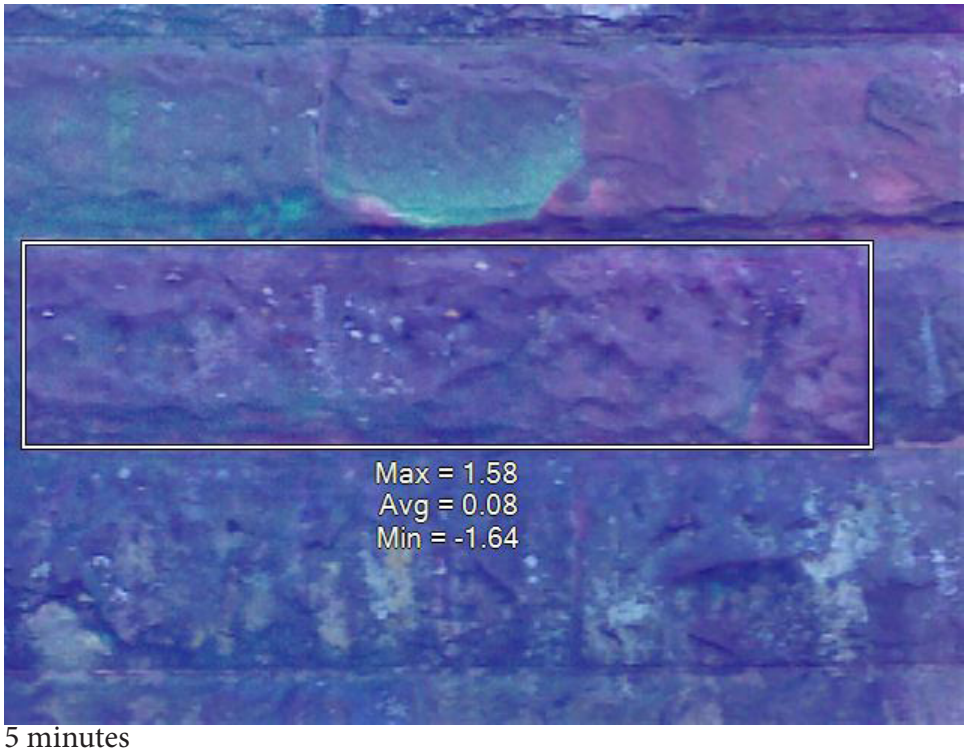


3 minutes

Green biocolonization treated with BioWash
January 10, 2013

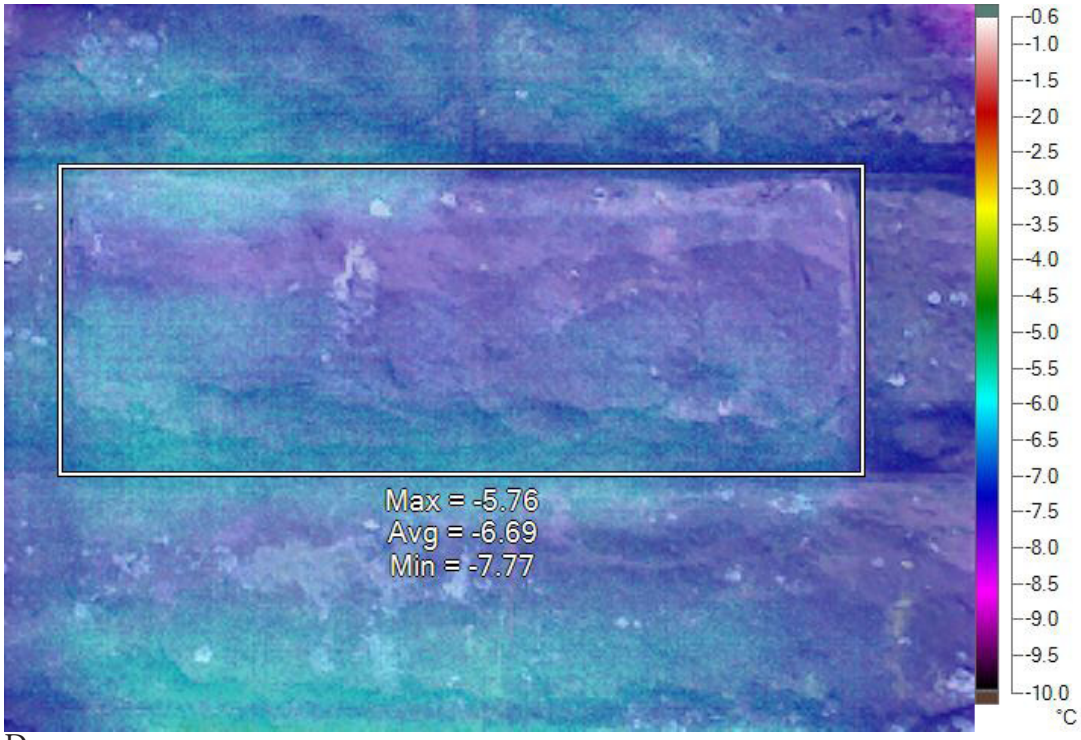


4 minutes

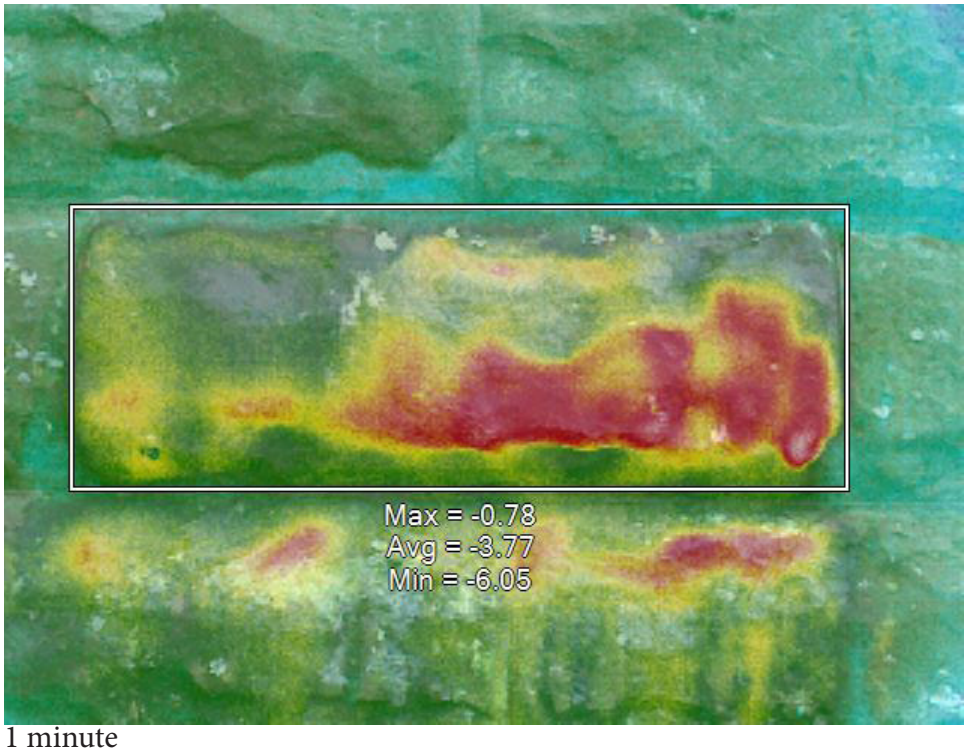


5 minutes

Green biocolonization treated with D/2
February 7, 2013

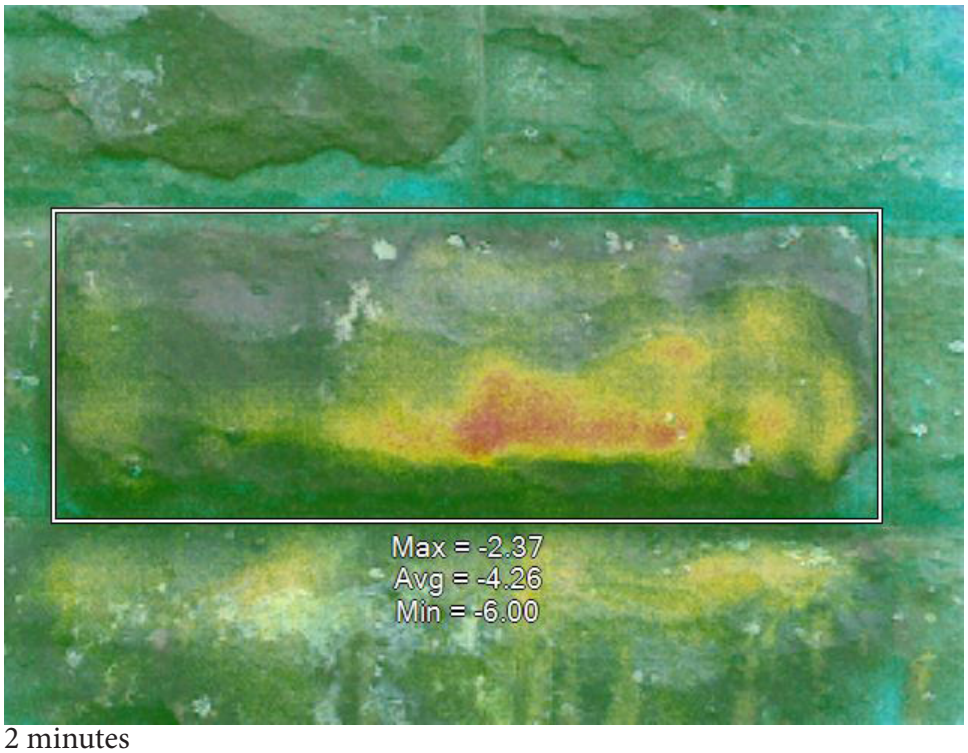


Dry

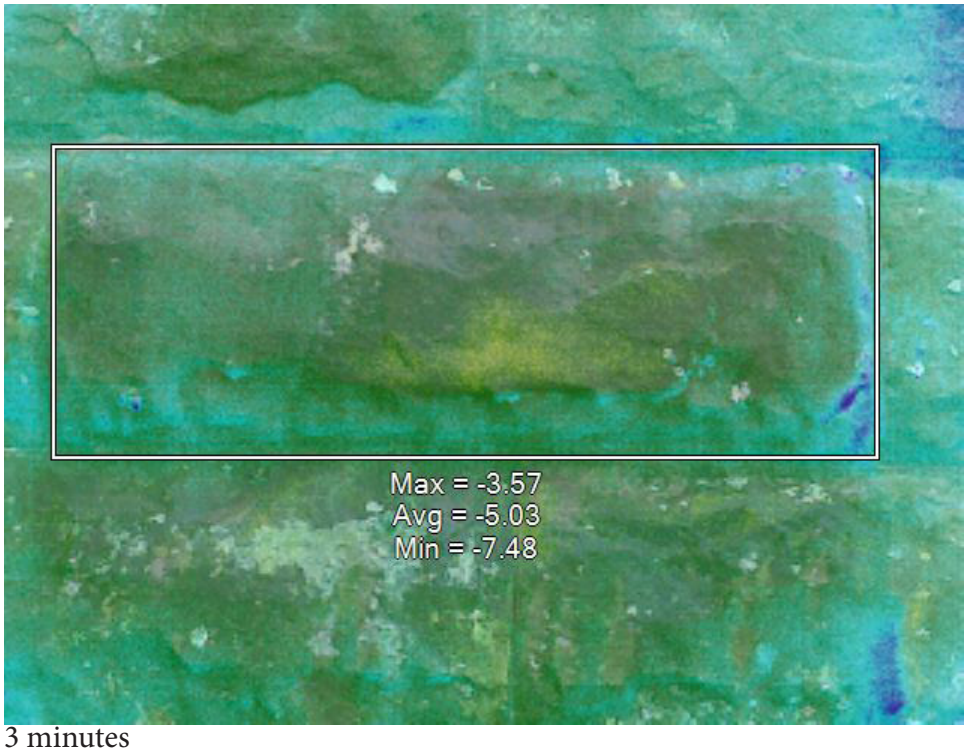


1 minute

Green biocolonization treated with D/2
February 7, 2013

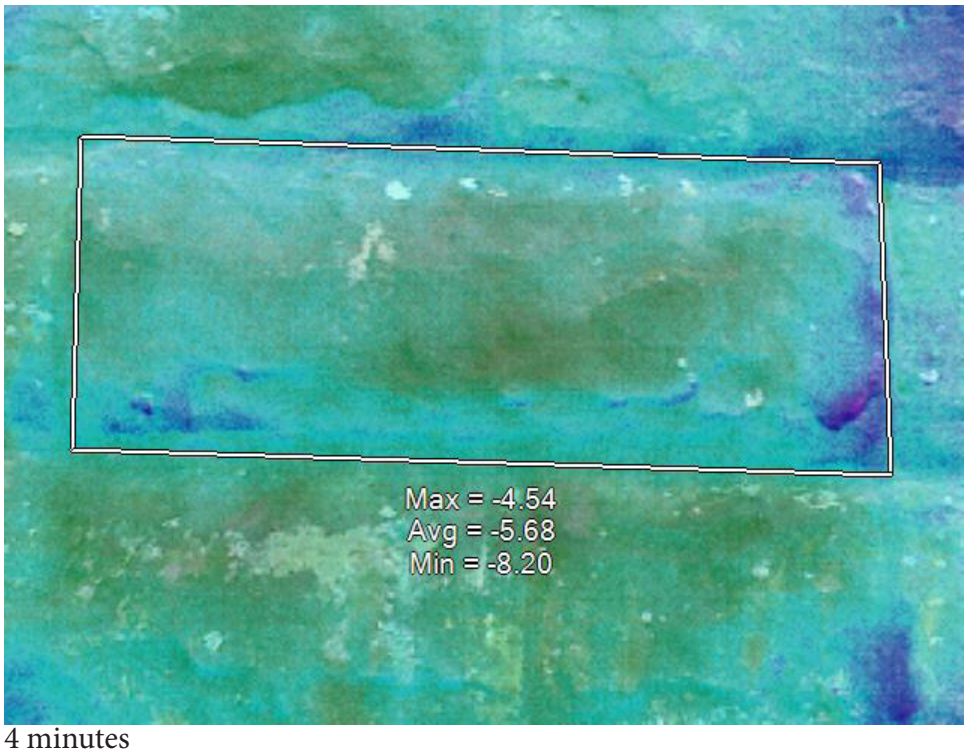


2 minutes

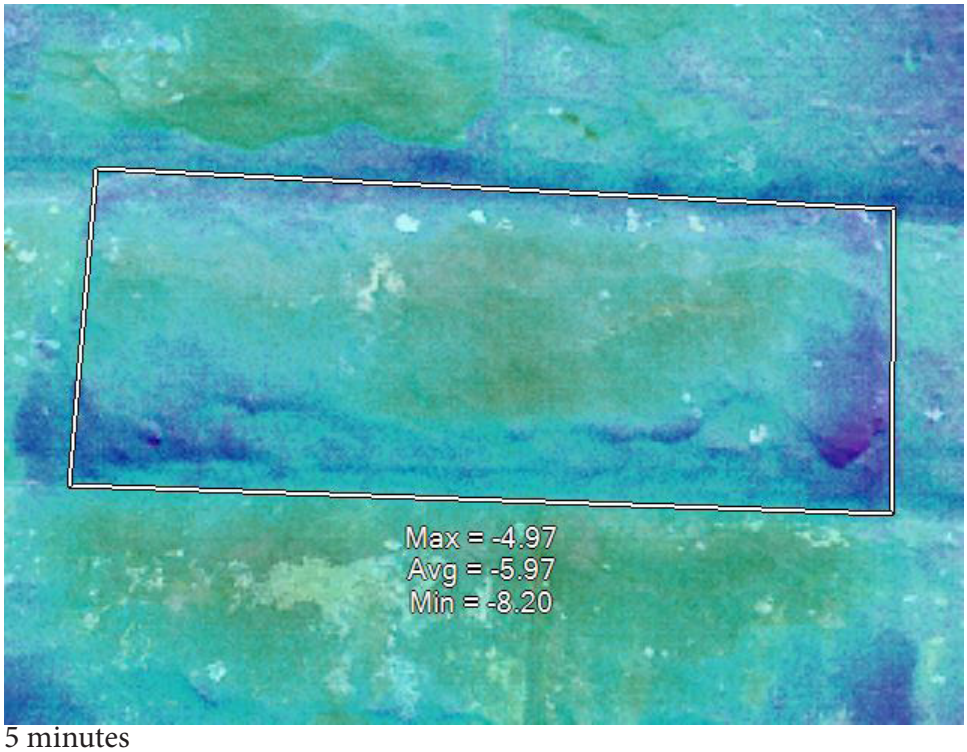


3 minutes

Green biocolonization treated with D/2
February 7, 2013

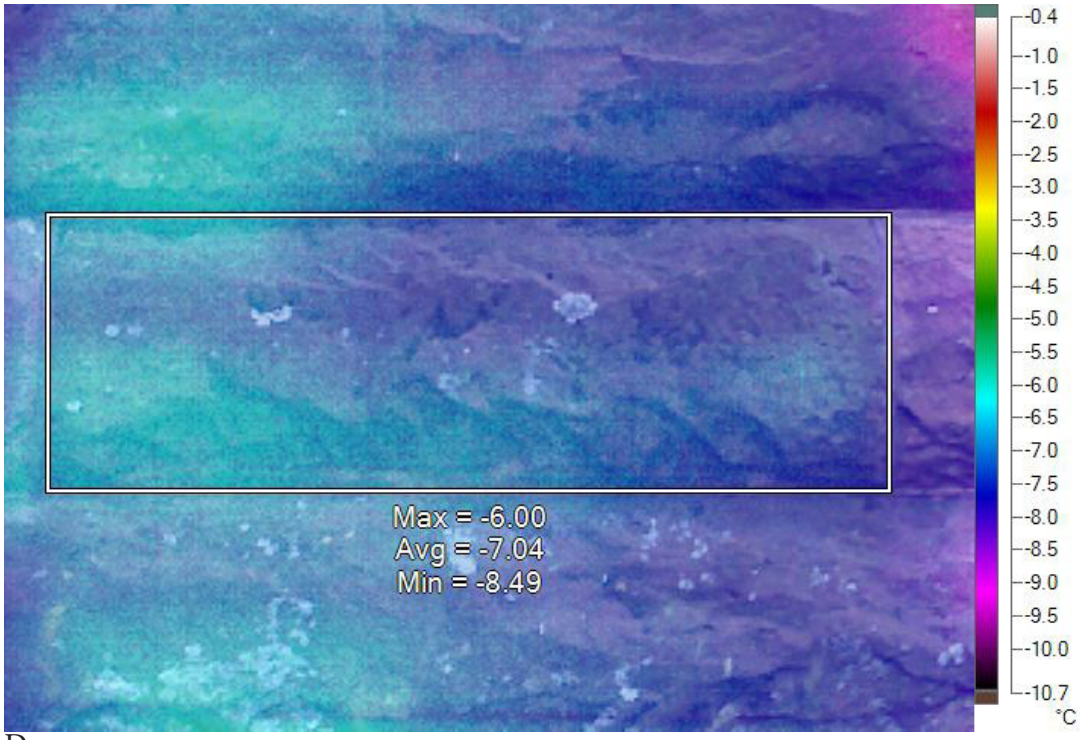


4 minutes

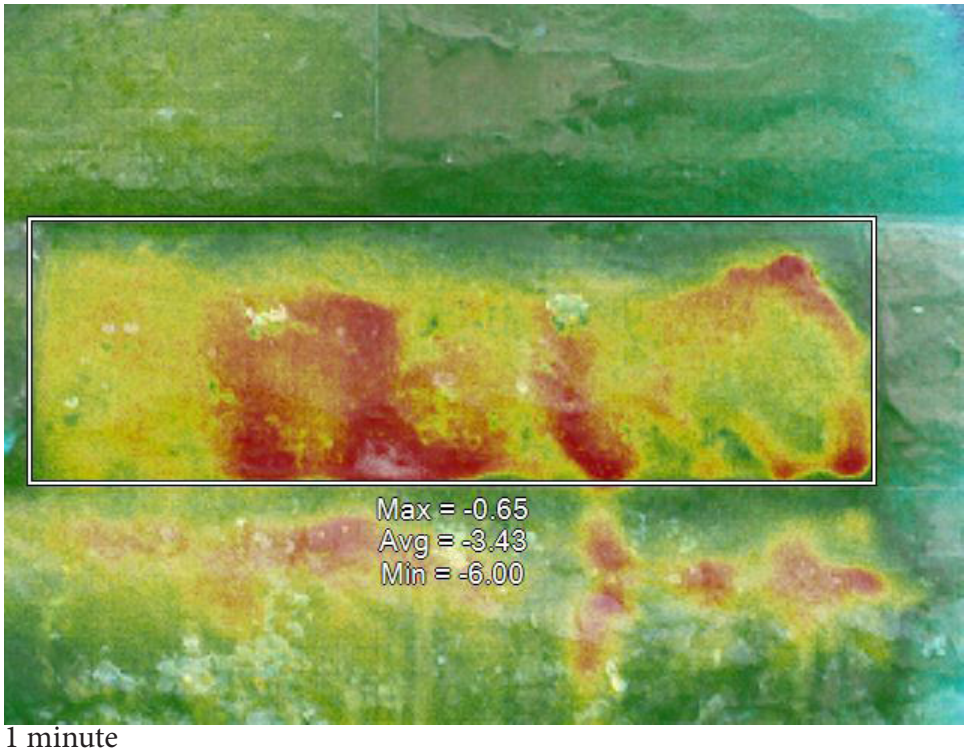


5 minutes

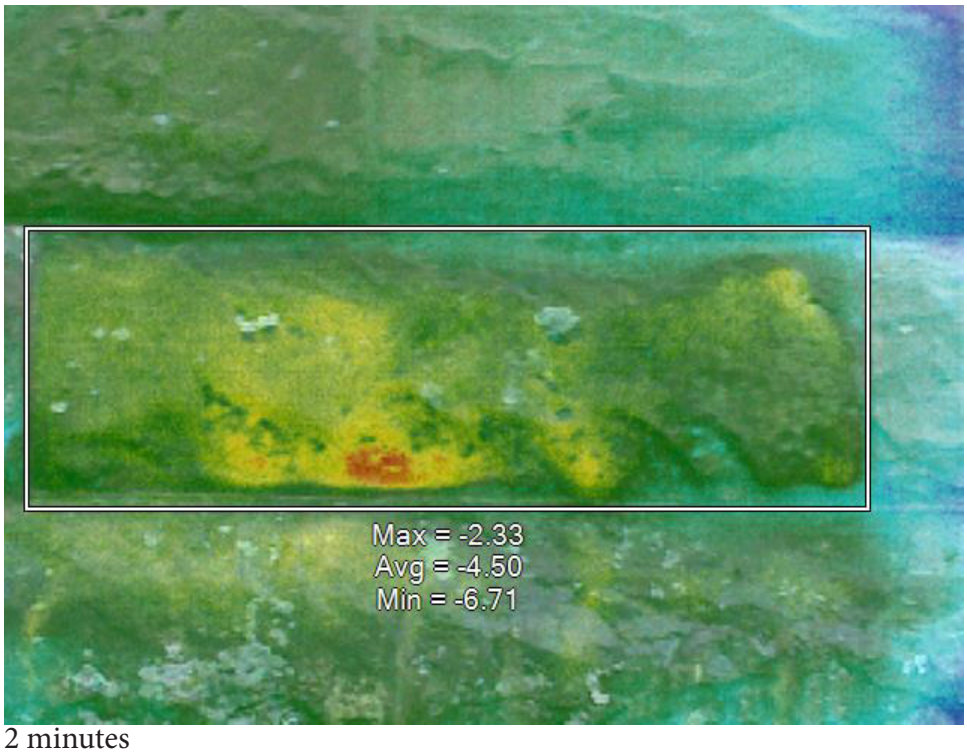
Green biocolonization control
February 7, 2013



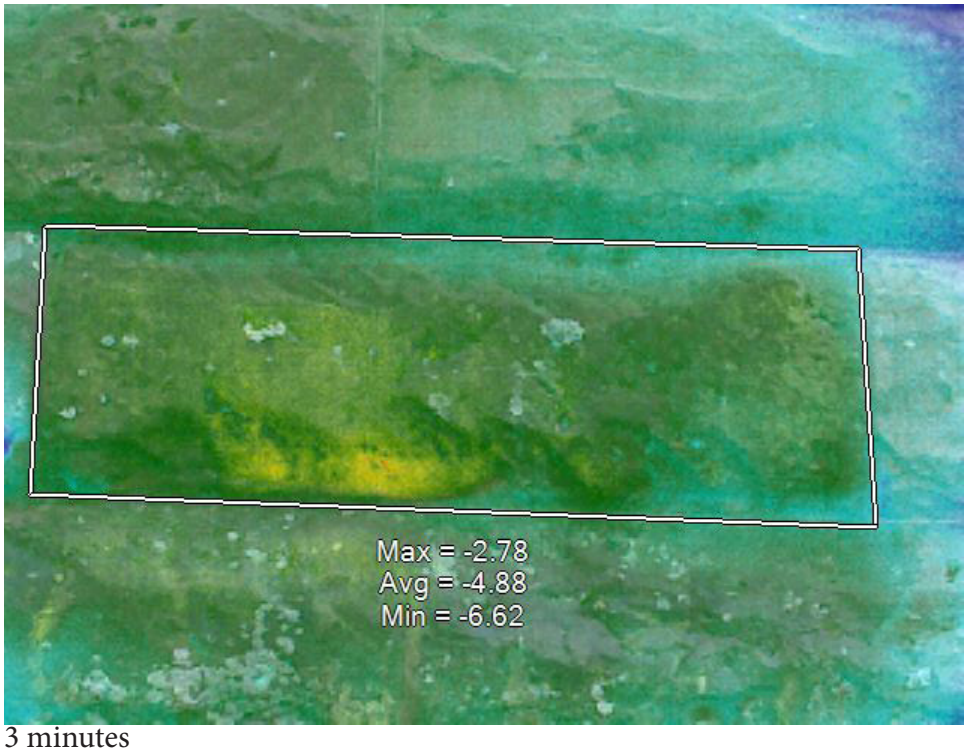
Dry



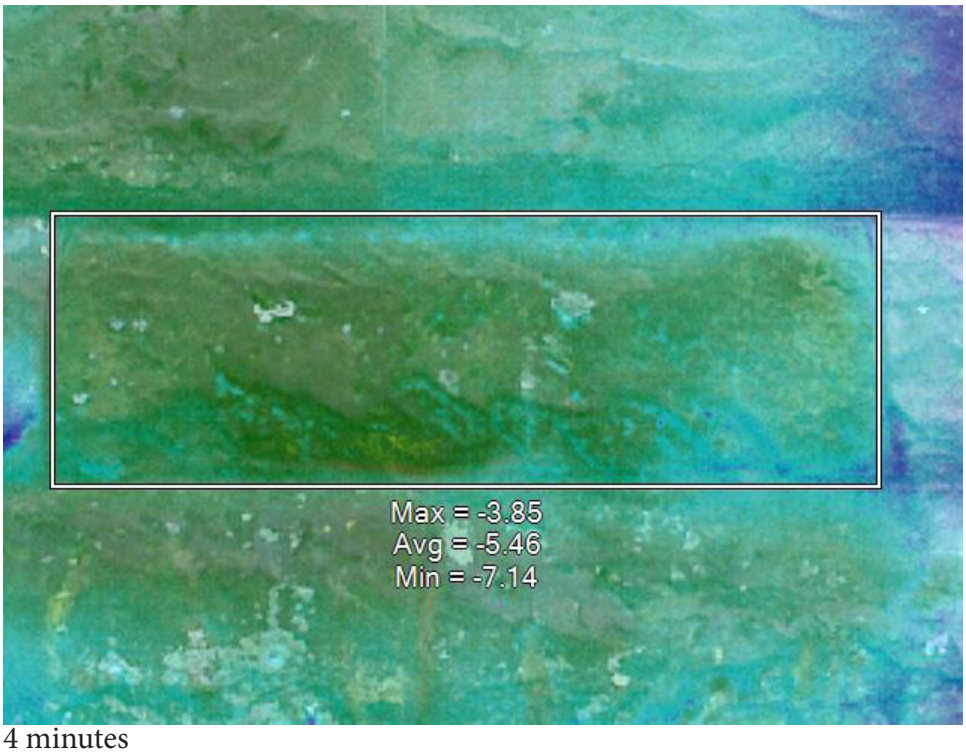
1 minute



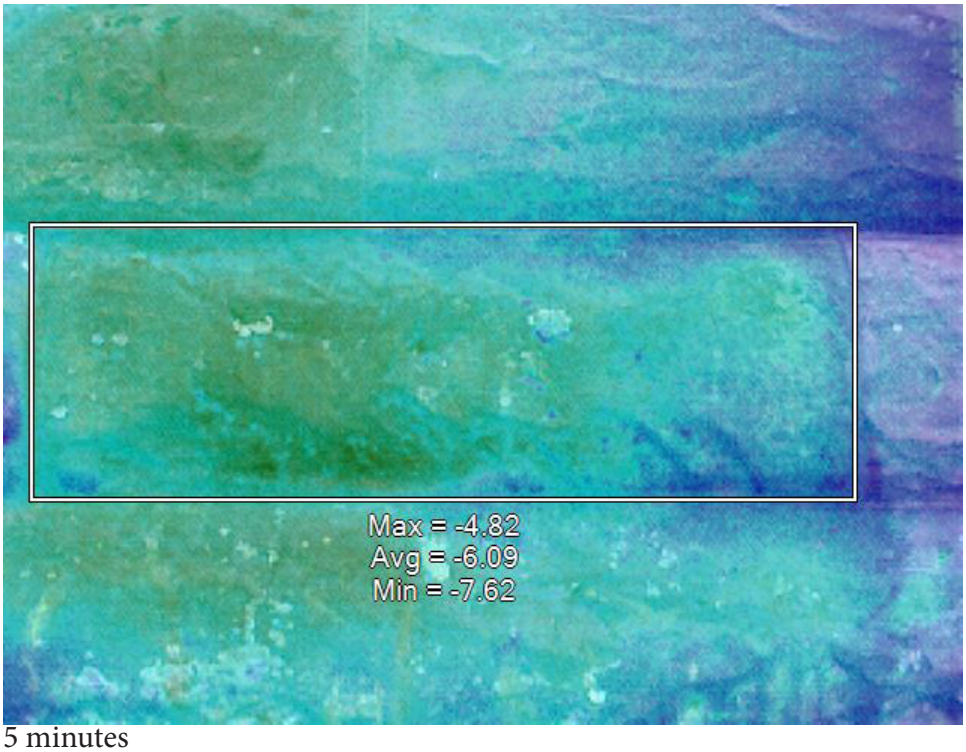
2 minutes



3 minutes

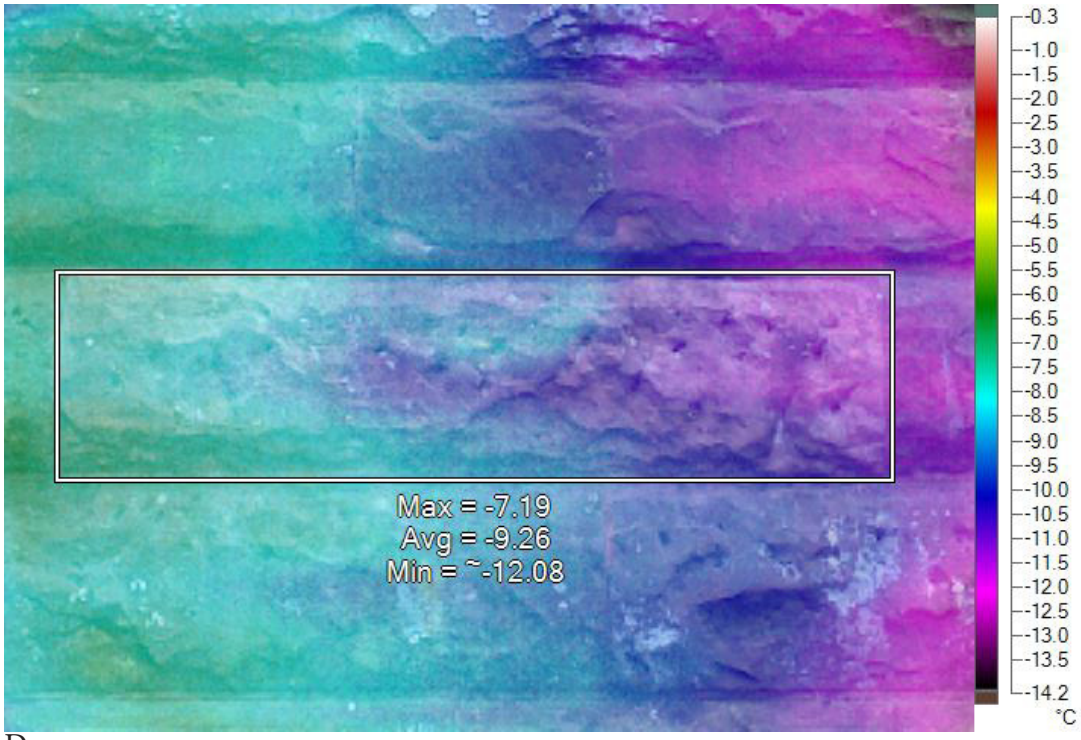


4 minutes

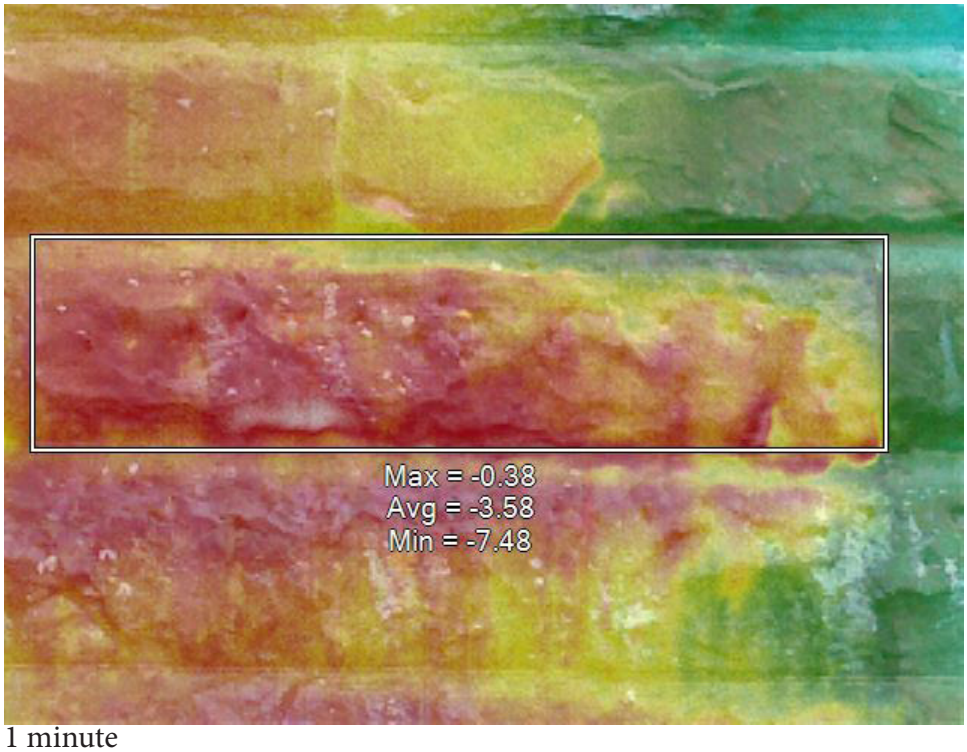


5 minutes

Green biocolonization treated with BioWash
February 7, 2013

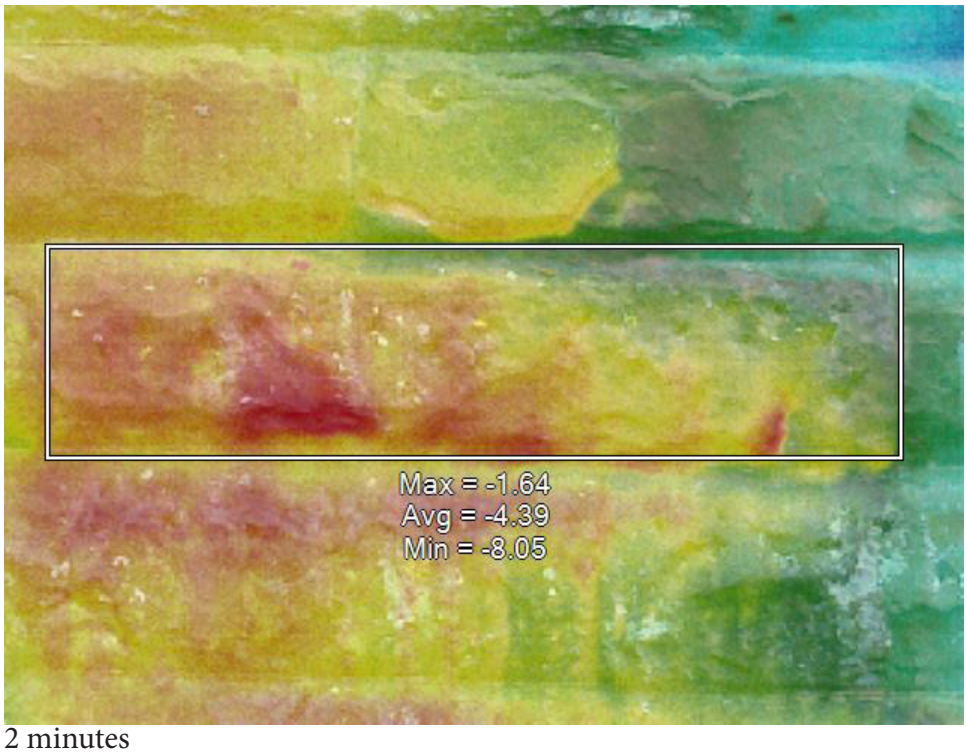


Dry

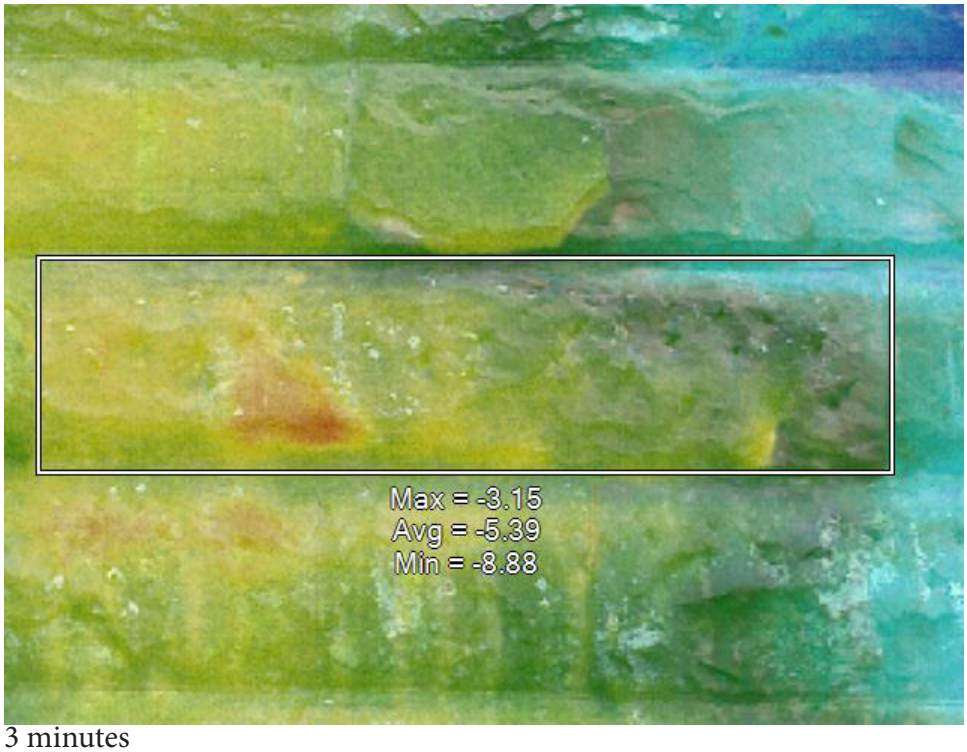


1 minute

Green biocolonization treated with BIoWash
February 7, 2013

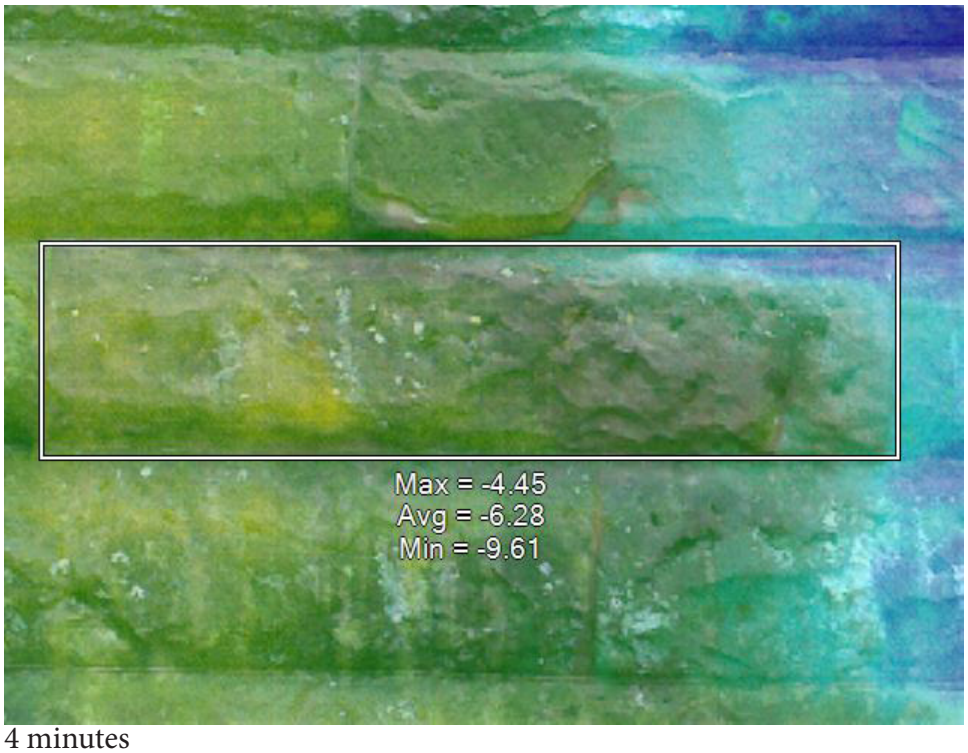


2 minutes

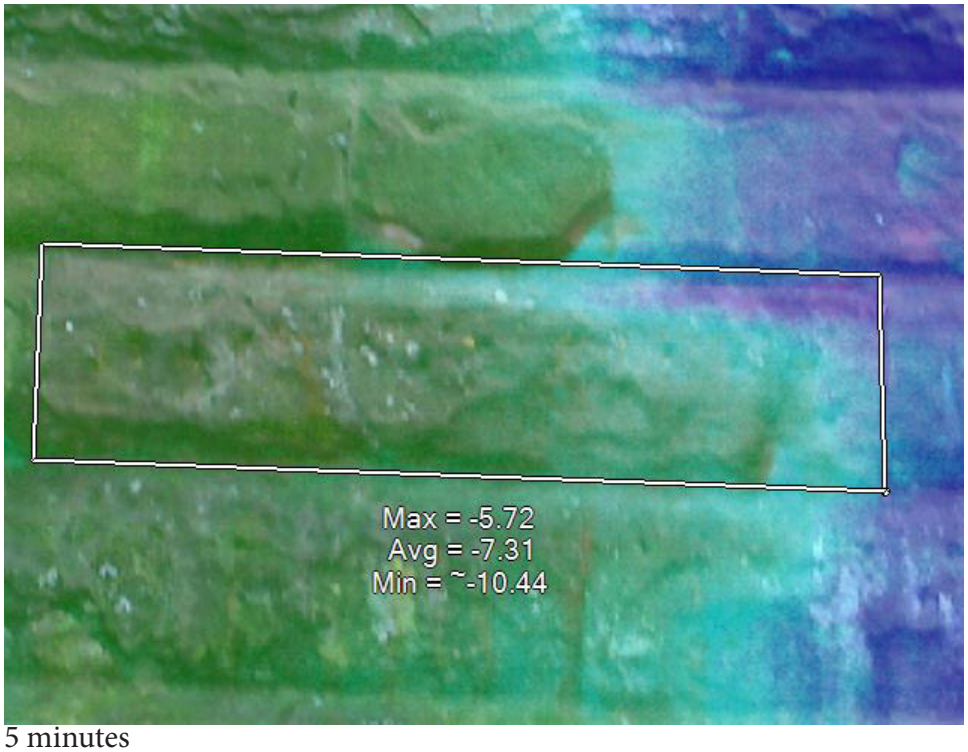


3 minutes

Green biocolonization treated with BioWash
February 7, 2013

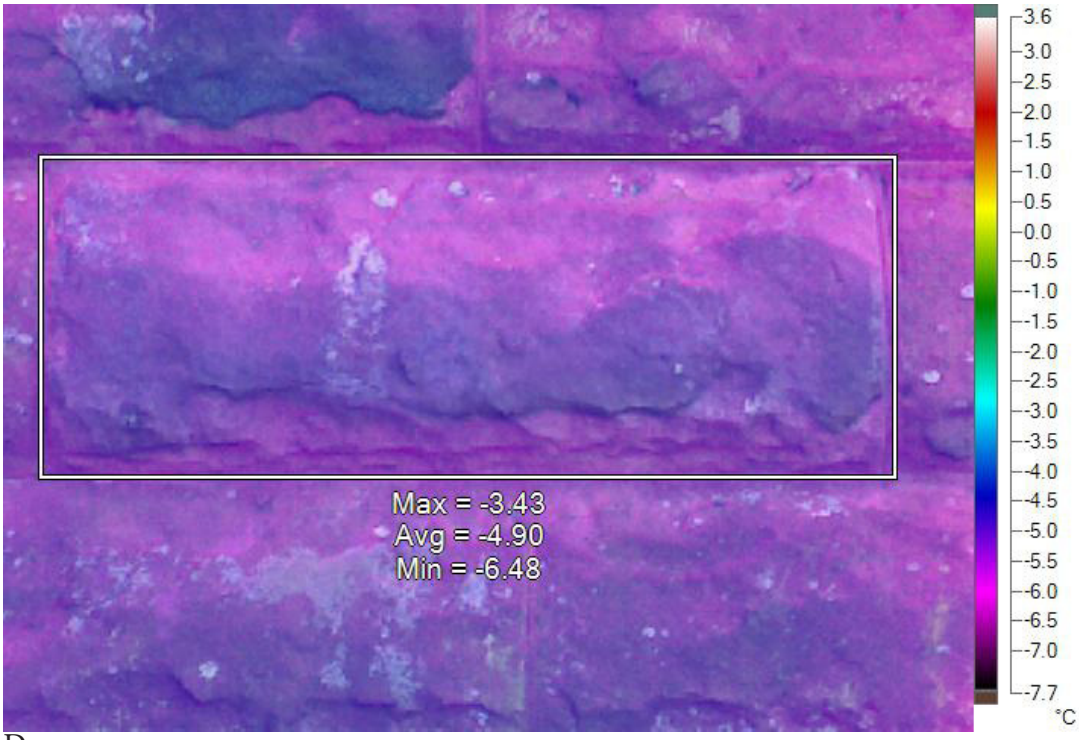


4 minutes



5 minutes

Green biocolonization treated with D/2
March 5, 2013



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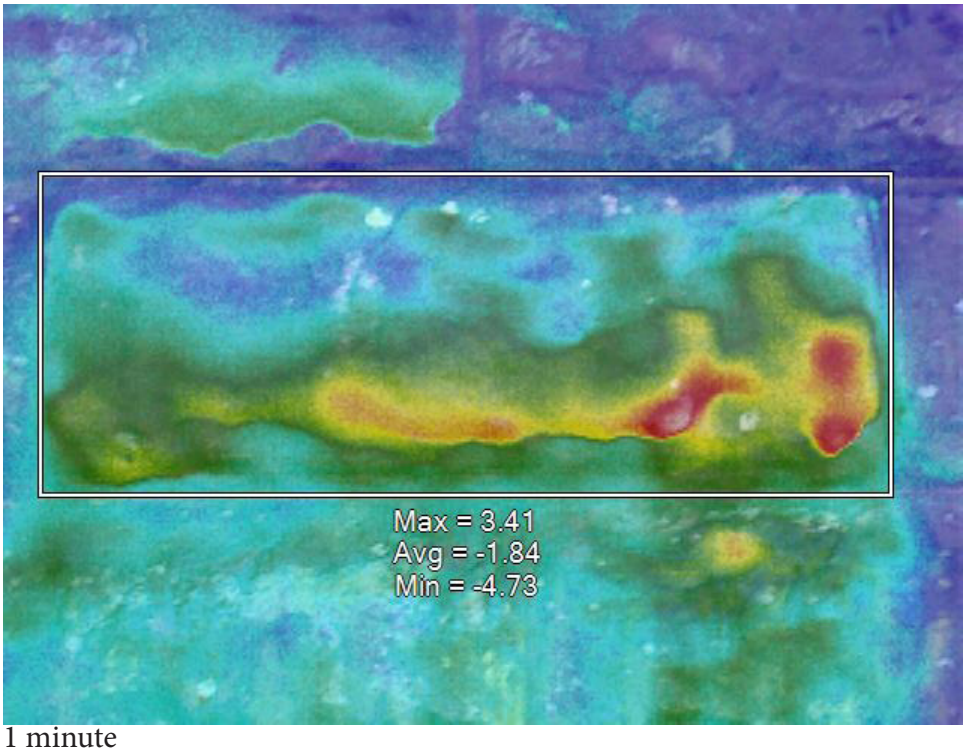
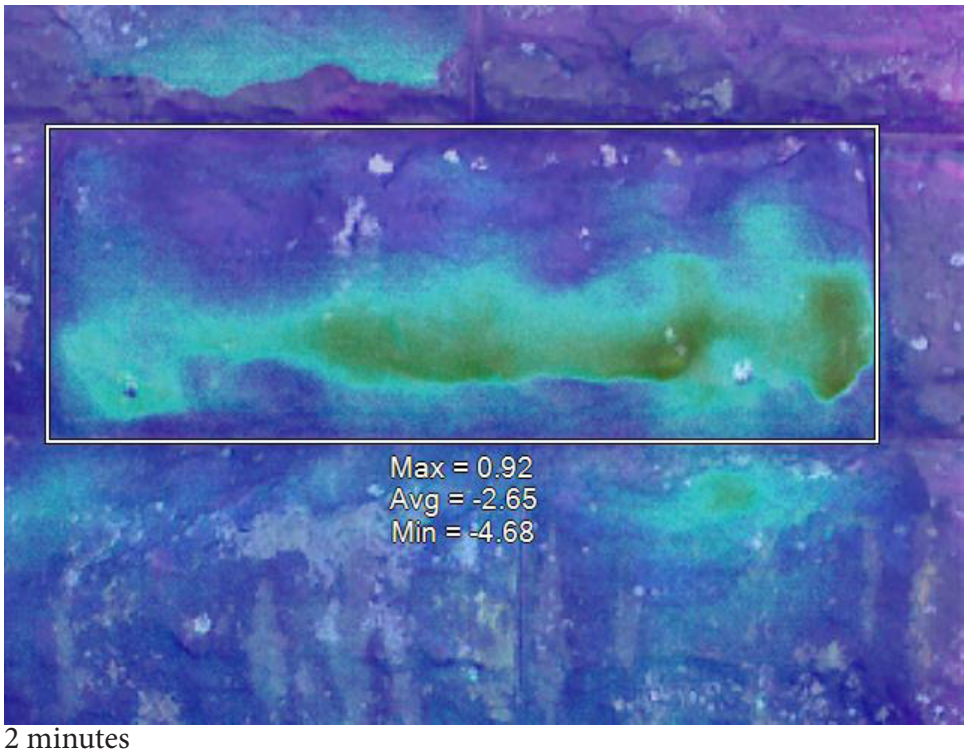
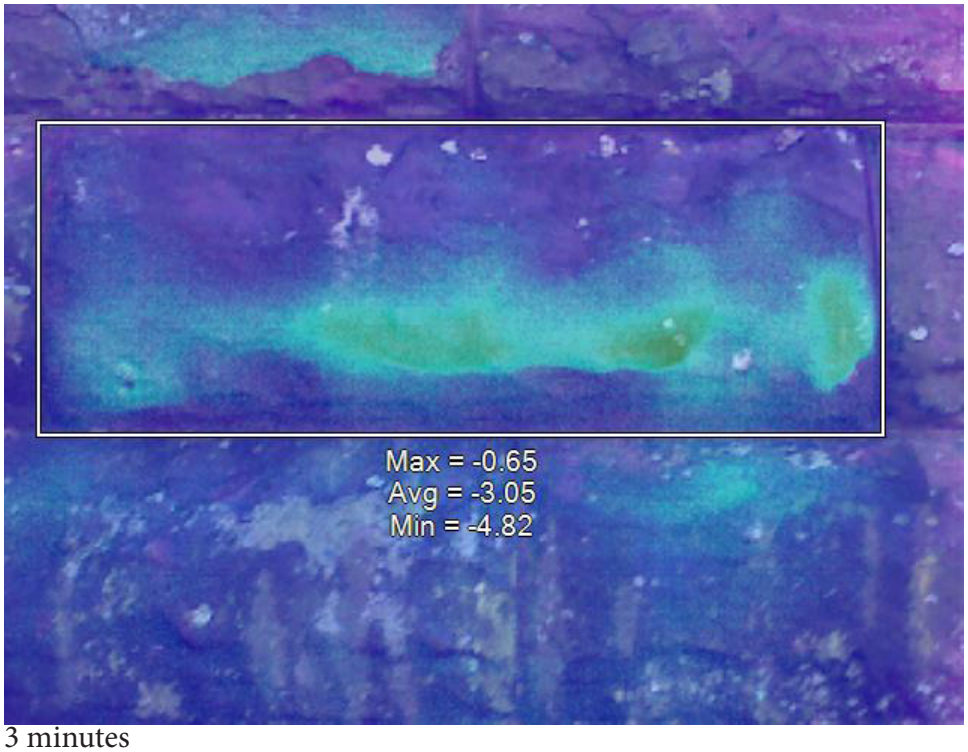


1 minute

Green biocolonization treated with D/2
March 5, 2013

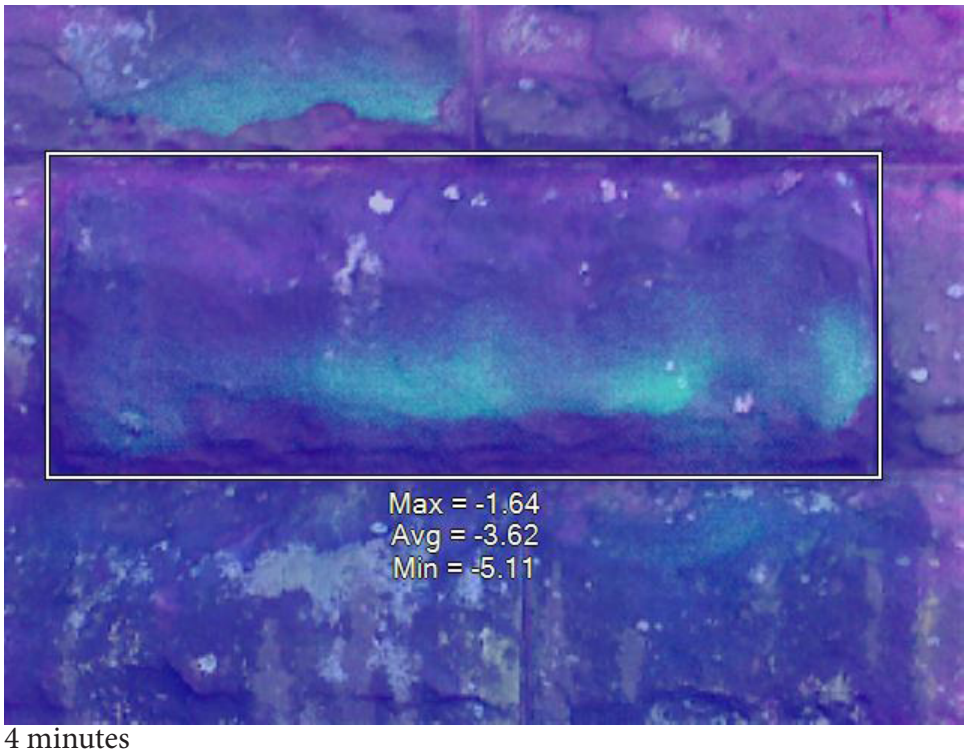


2 minutes

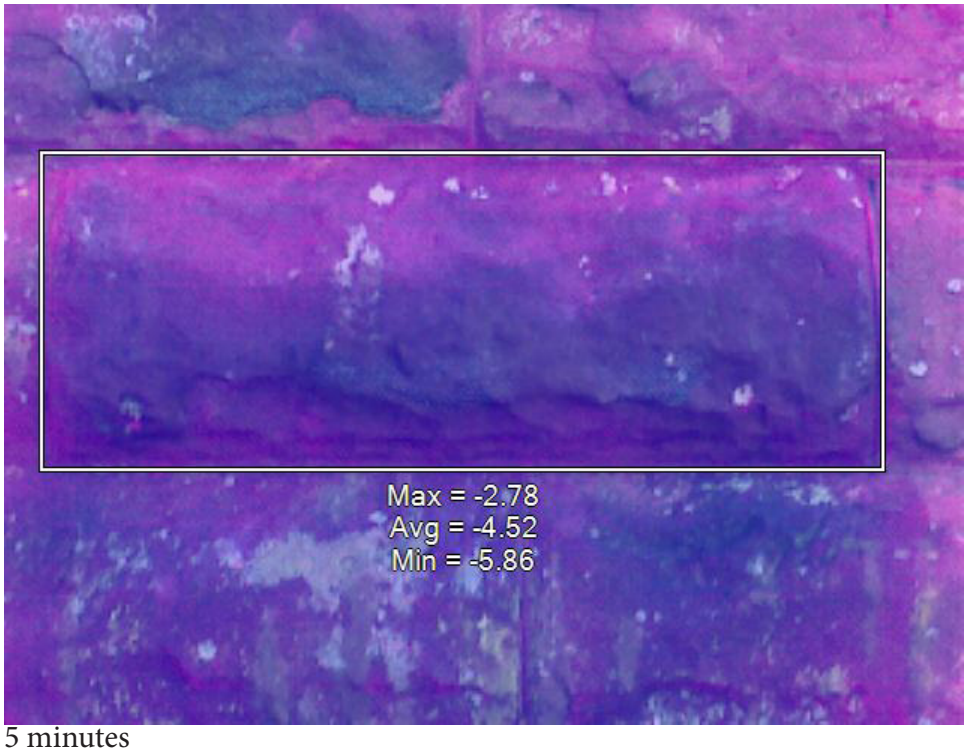


3 minutes

Green biocolonization treated with D/2
March 5, 2013

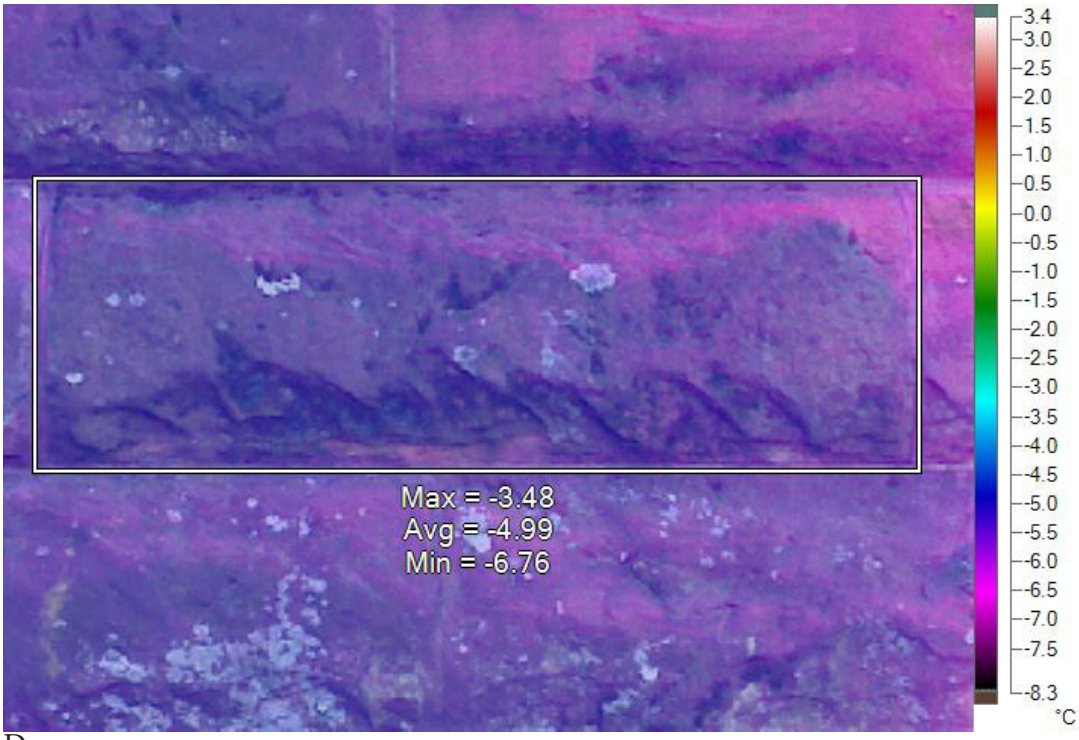


4 minutes

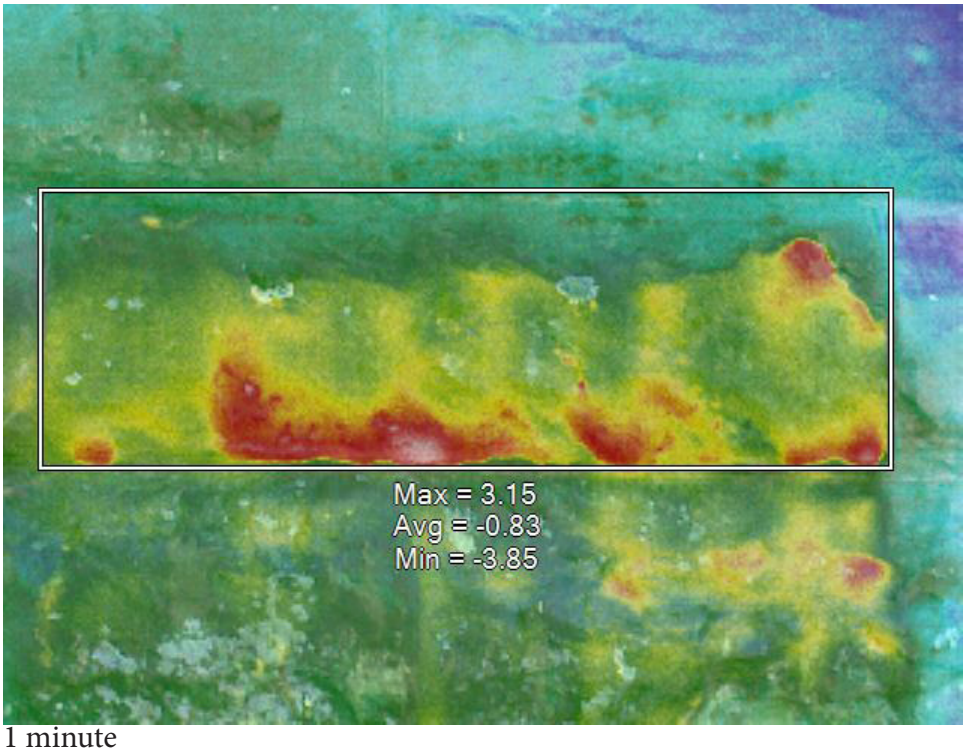


5 minutes

Green biocolonization control
March 5, 2013

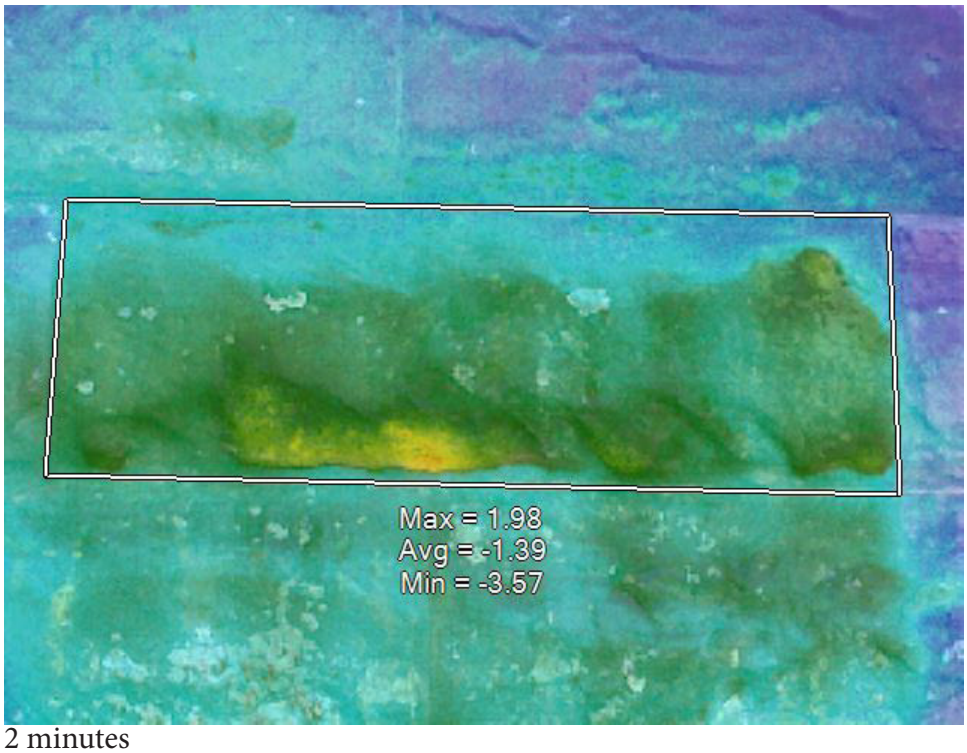


Dry

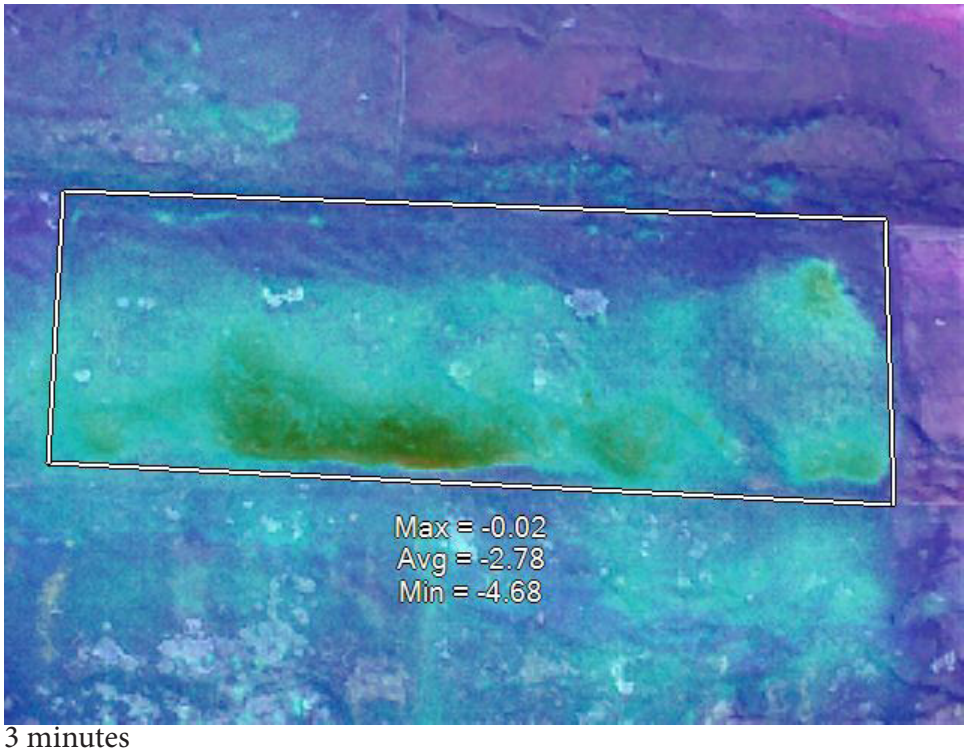


1 minute

Green biocolonization control
March 5, 2013

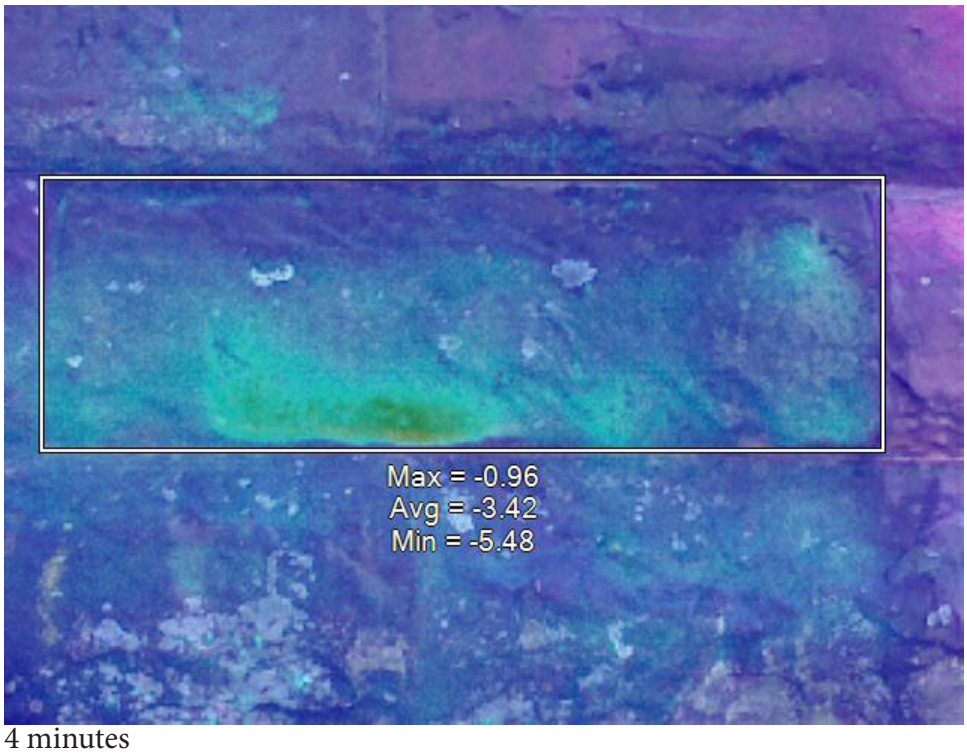


2 minutes

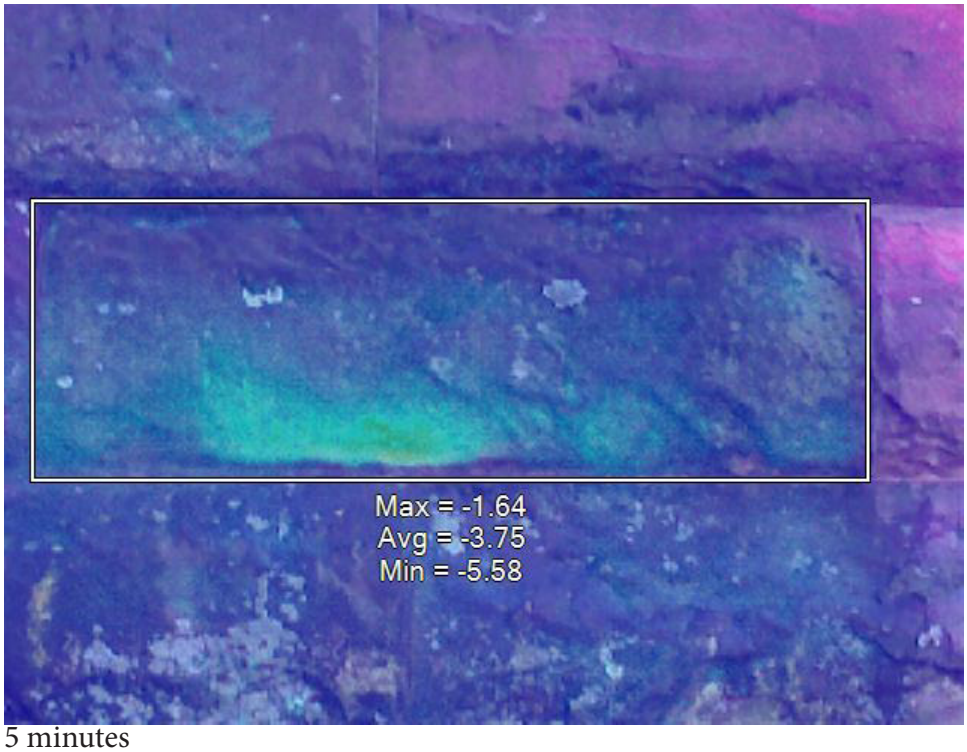


3 minutes

Green biocolonization control
March 5, 2013

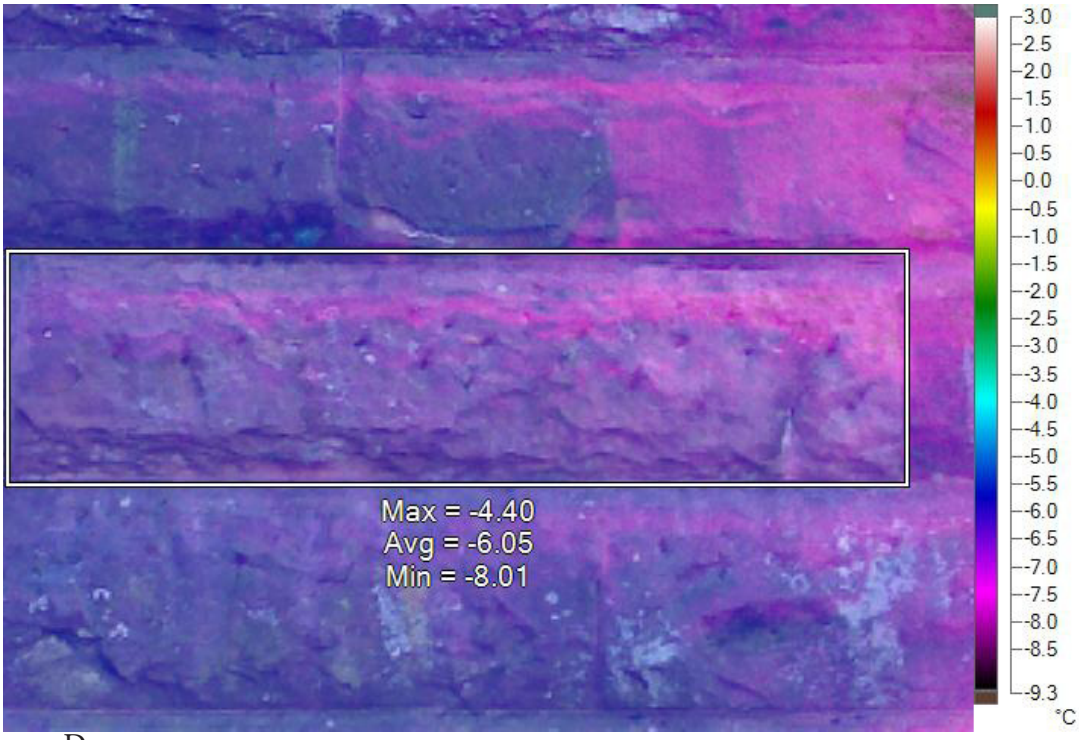


4 minutes

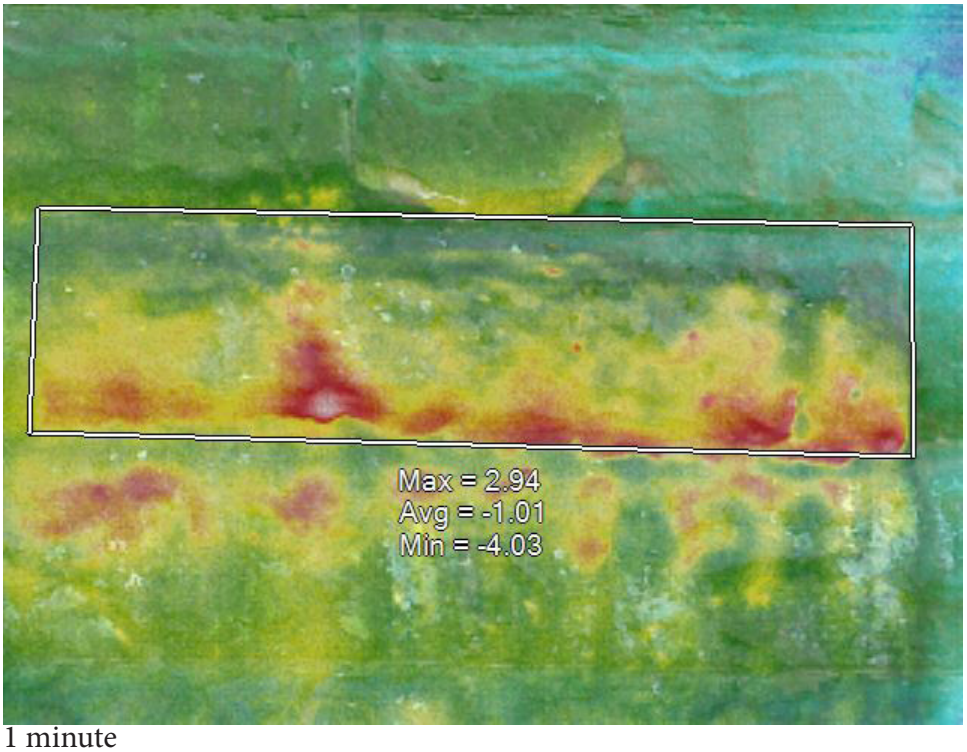


5 minutes

Green biocolonization treated with BioWash
March 5, 2013

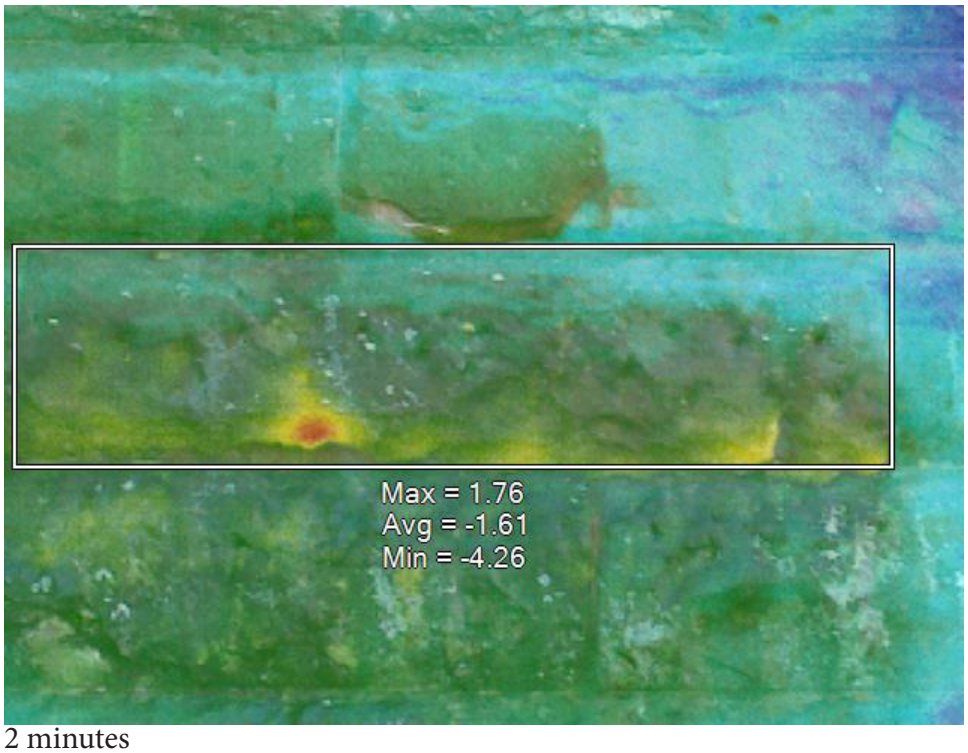


Dry

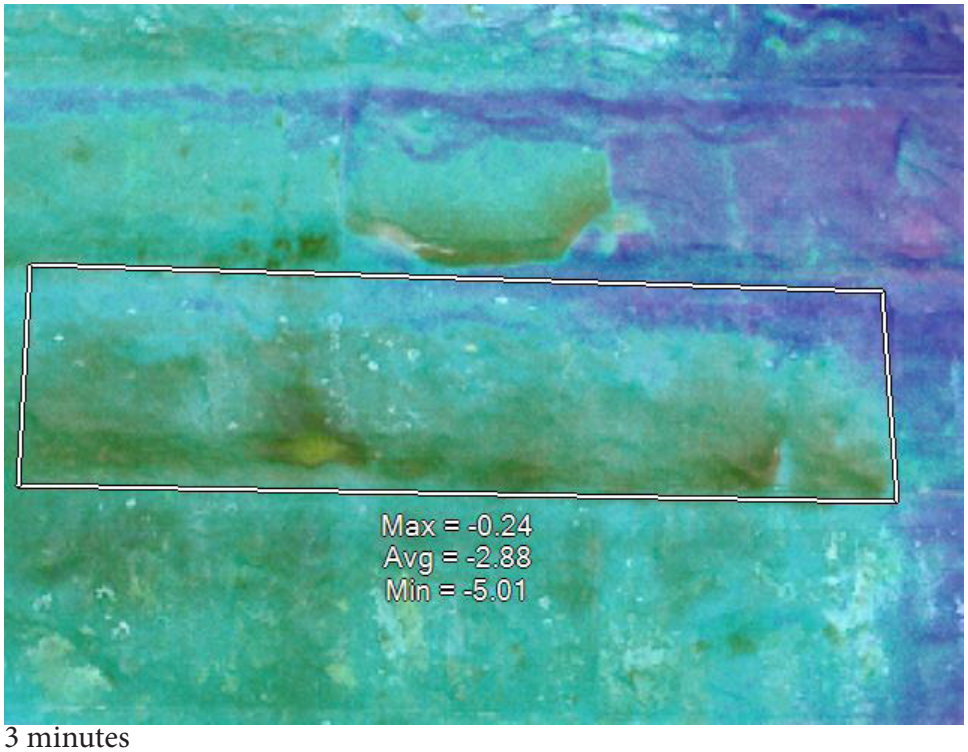


1 minute

Green biocolonization treated with BIoWash
March 5, 2013

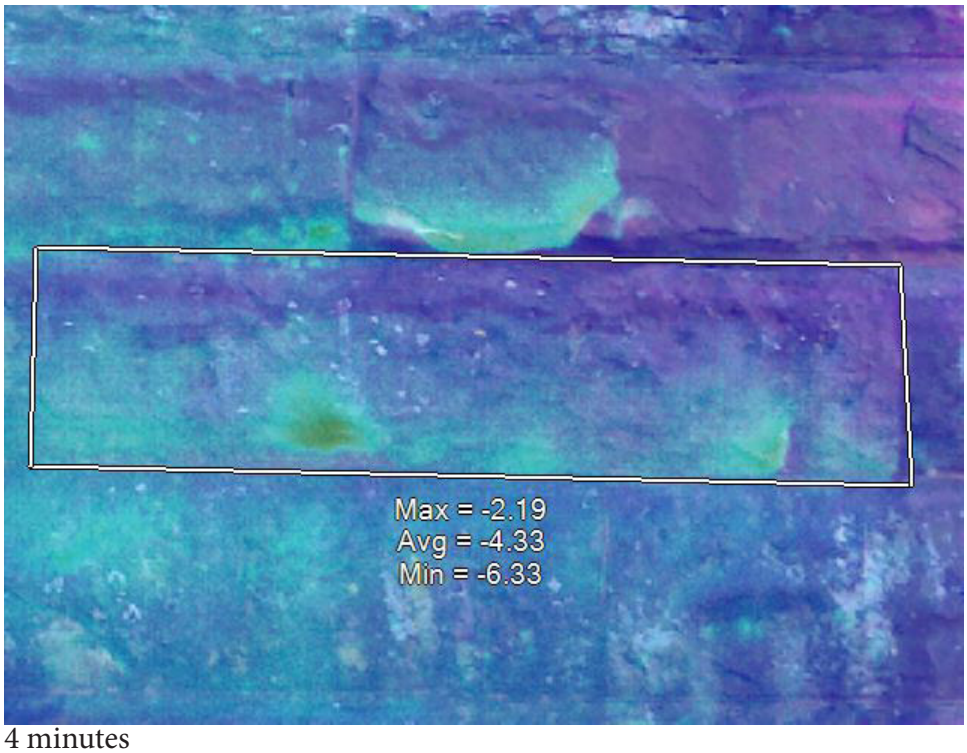


2 minutes

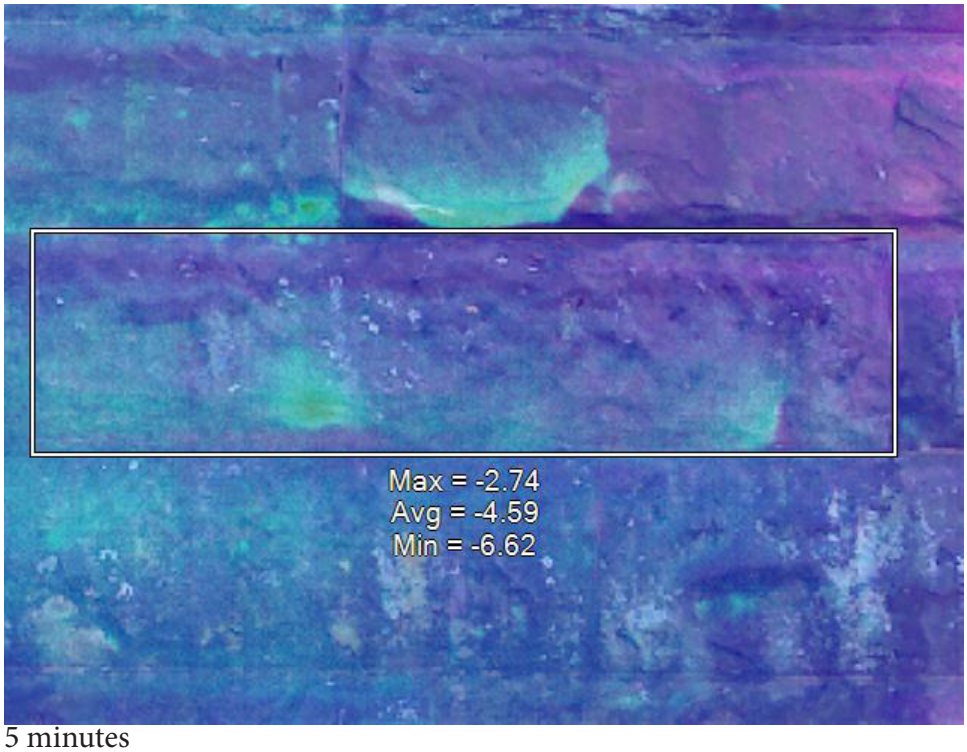


3 minutes

Green biocolonization treated with BioWash
March 5, 2013



4 minutes

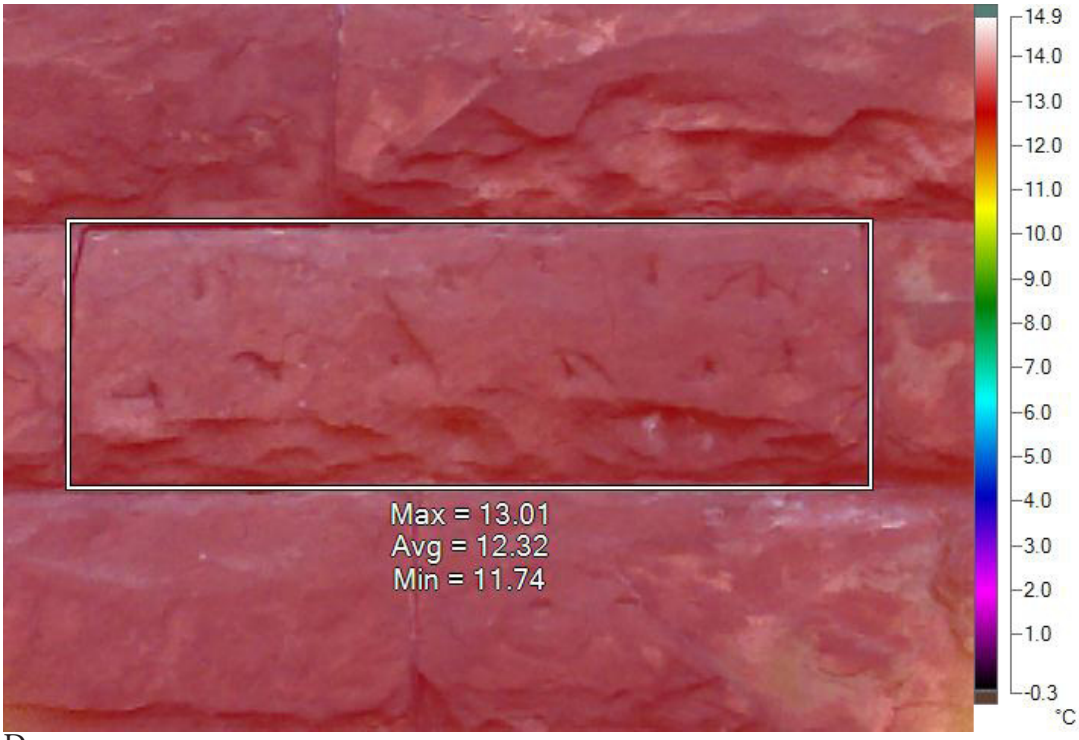


5 minutes

B.2 Black Biocolonization

Average Stone Temperature (°C)										
	Time (min)	**DATE**	**Nov.19**	**Nov. 26**	**Nov. 28**	**Dec. 4**	**Jan. 10**	**Feb. 7**	**Mar. 5**
Biocide: D/2	**0**	Dry	12.32	-	6.97	23.87	4.51	0.32	2.28
	1		1.79	9.23	1.68	17.49	3.89	0.08	1.59
	2		2.63	8.36	-1.65	16.72	2.34	-1.55	-0.09
	3		2.54	6.32	-3.97	16.27	0.25	-3.07	-3.06
	4		2.36	4.66	-4.60	15.89	-0.38	-6.10	-5.01
	5		2.23	3.67	-5.85	15.66	-1.40	-7.21	-6.31
Biocide: none (control)	**0**	Dry	11.62	3.05	-	24.37	3.76	1.67	2.80
	1		8.84	1.58	-	18.49	3.14	-4.48	-3.45
	2		7.95	0.84	-	18.18	2.72	-4.17	-3.89
	3		7.44	0.03	-	17.67	2.27	-4.88	-4.30
	4		6.21	-0.08	-	17.13	1.71	-6.53	-4.77
	5		5.45	0.13	-	16.74	1.19	-7.58	-5.24
Biocide: BioWash	**0**	Dry	10.92	-1.03	-	23.27	4.11	0.81	2.05
	1		9.16	-0.85	-	19.72	3.32	-4.10	-0.68
	2		7.78	-1.97	-	18.70	2.26	-3.60	-1.60
	3		7.11	-1.52	-	17.93	2.15	-4.64	-2.42
	4		5.32	-1.29	-	16.87	1.42	-5.16	-3.33
	5		4.25	-1.28	-	15.94	1.21	-6.75	-3.81
Environmental Conditions	**Temp (°C)**		11.6	8.8	10.0	18.6	8.6	4.0	4.7
	RH (%)		51	31	28	59	37	30	43
	Wind		+	+	++	+	++	+	+
	Sunny (s); partly cloudy (pc); cloudy (c)		s	s	pc	pc	s	c	s
Block Conditions	**Sunny (O); partly shaded (ps); shaded (s)**		s	s	s	s	s	s	s
Temperature (°C)	**Water**		20	21	24	21	21	22	22
	D/2		21	-	-	-	-	-	-
	BioWash		21	-	-	-	-	-	-

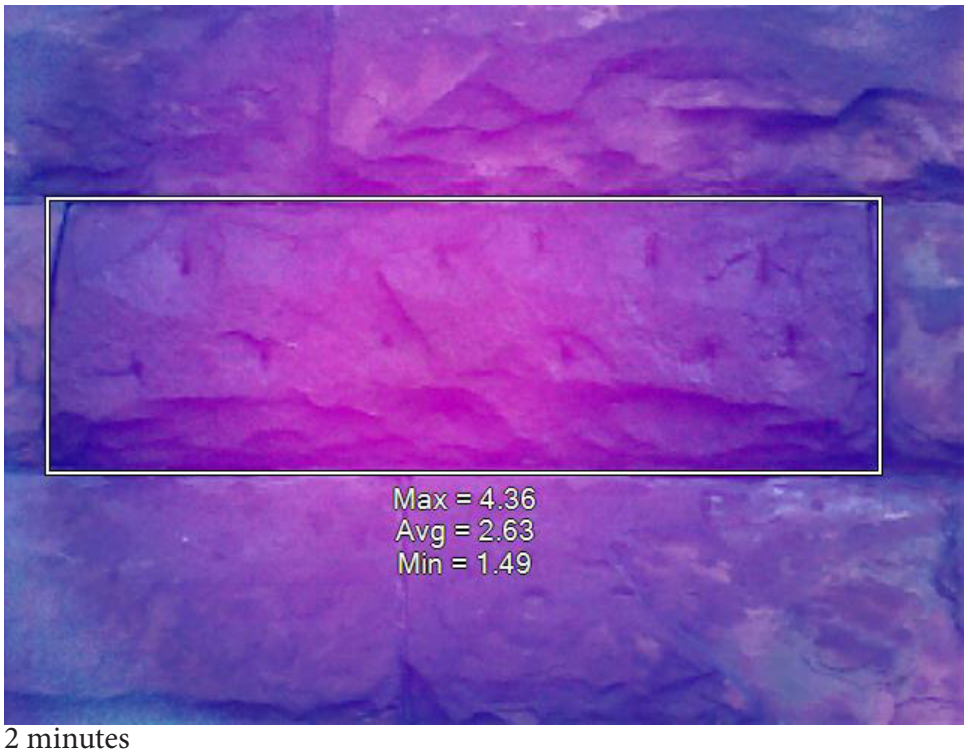
Black biocolonization treated with D/2
November 19, 2012



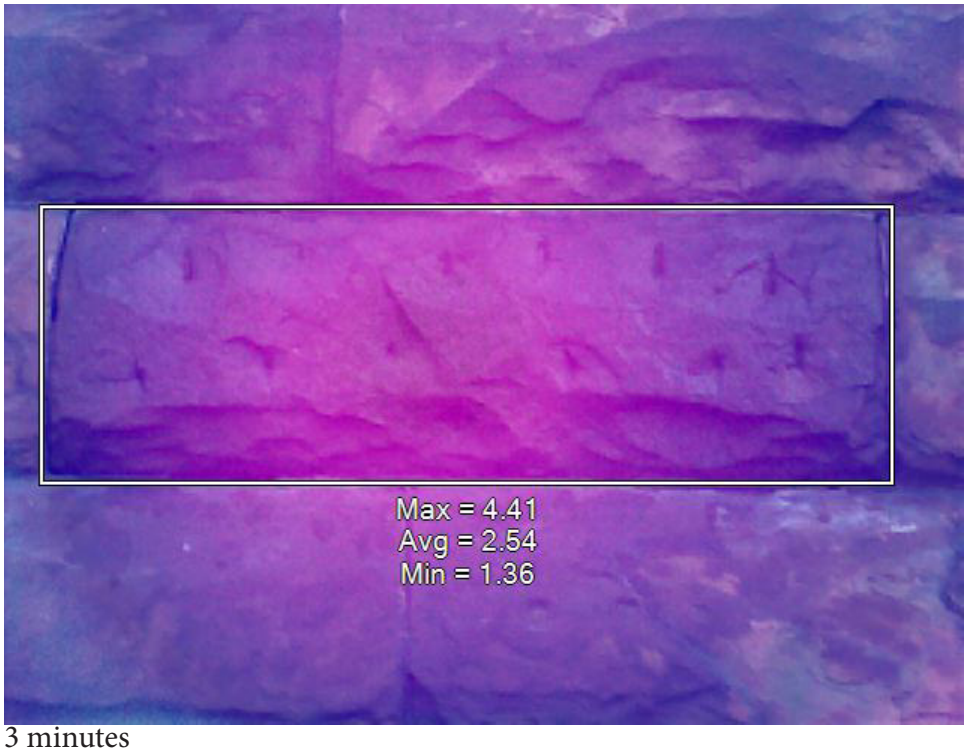
Dry

1 minute

Black biocolonization treated with D/2
November 19, 2012

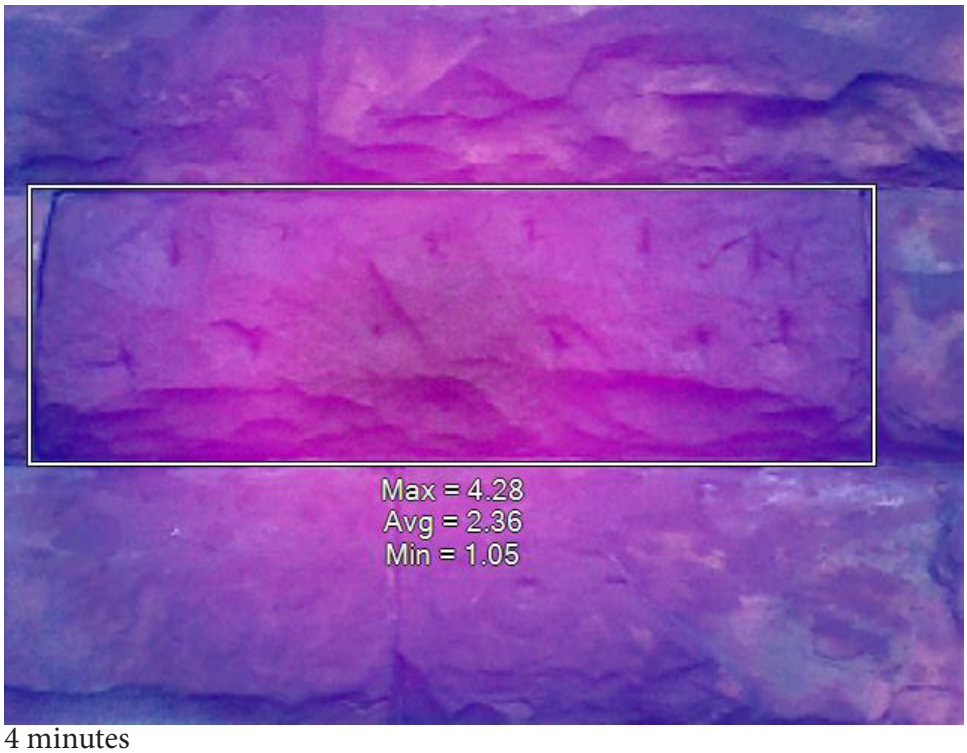


2 minutes

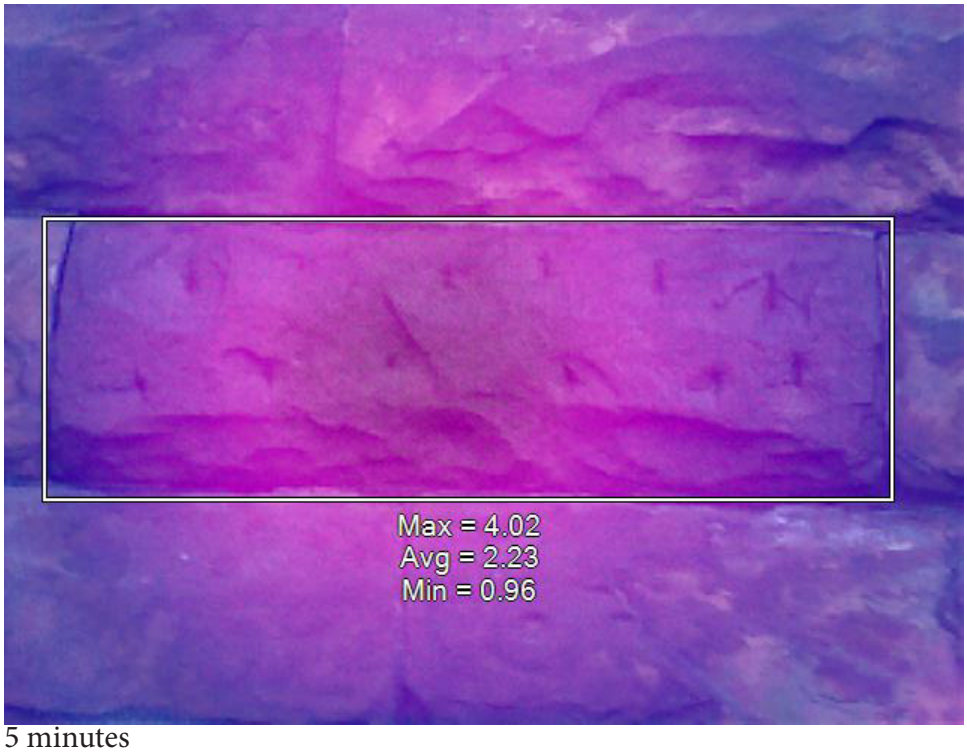


3 minutes

Black biocolonization treated with D/2
November 19, 2012

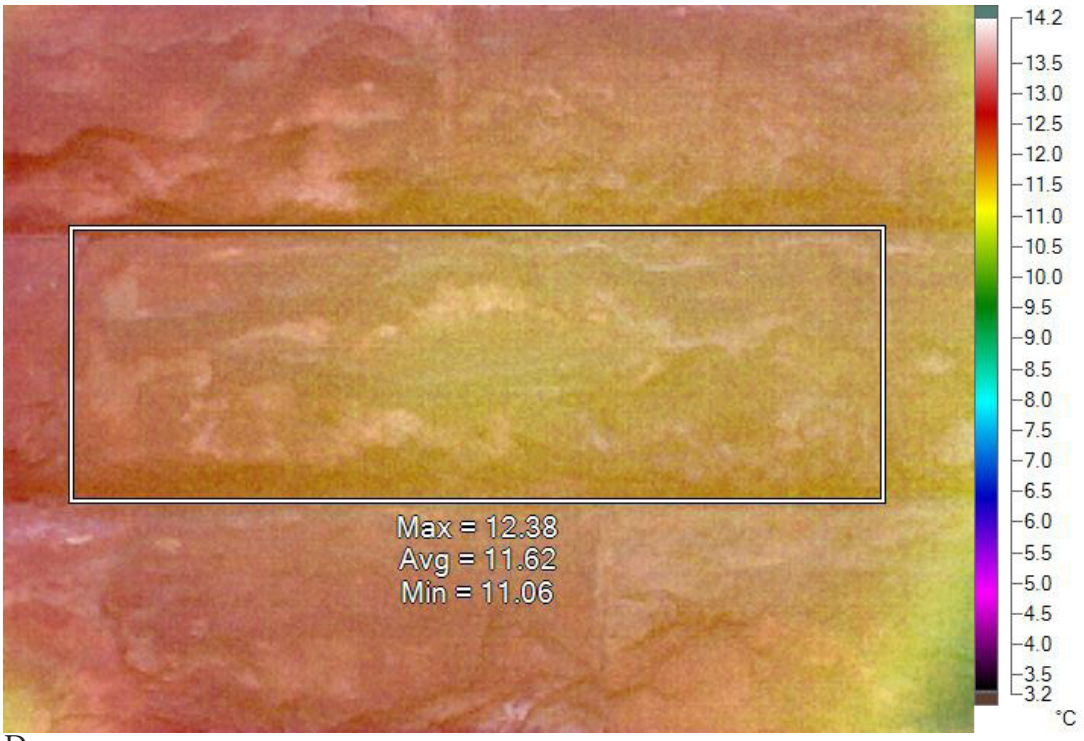


4 minutes

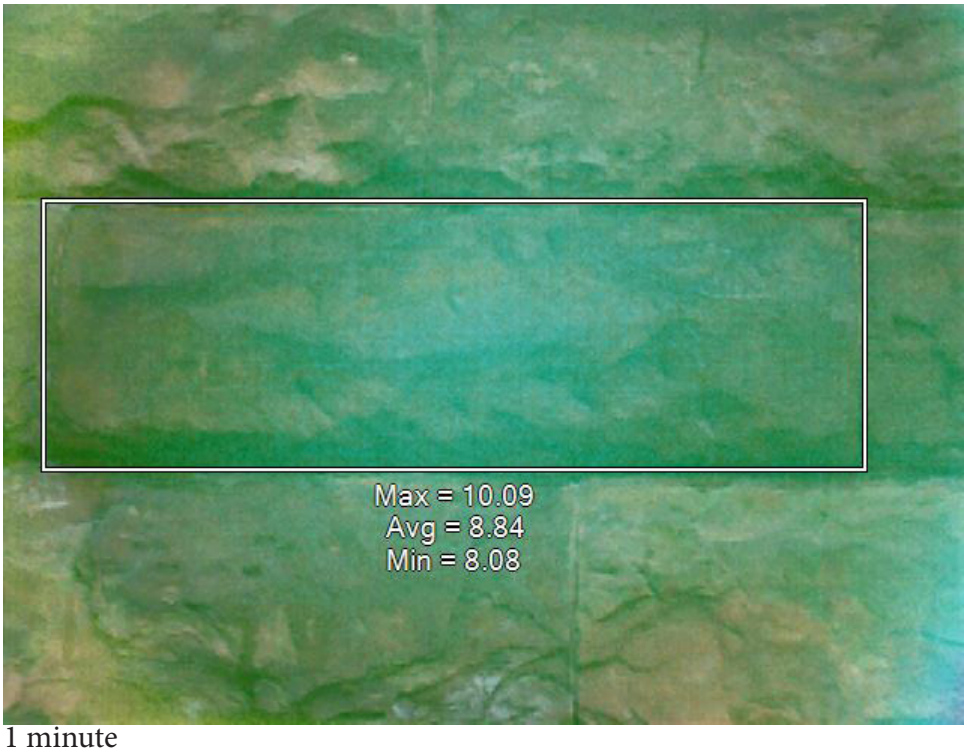


5 minutes

Black biocolonization control
November 19, 2012

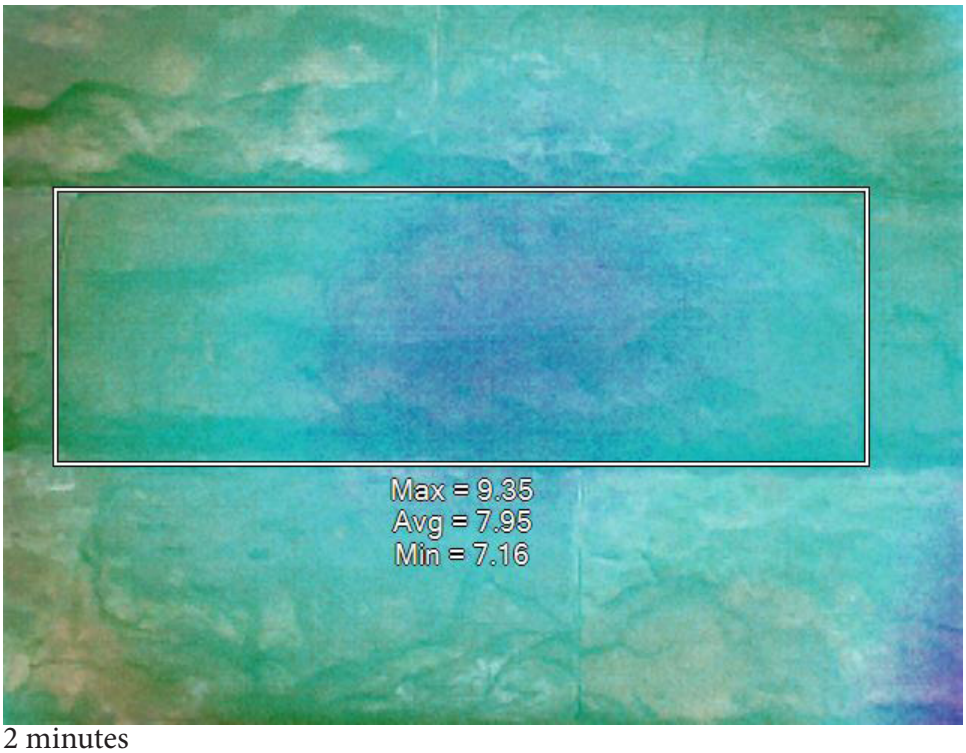


Dry

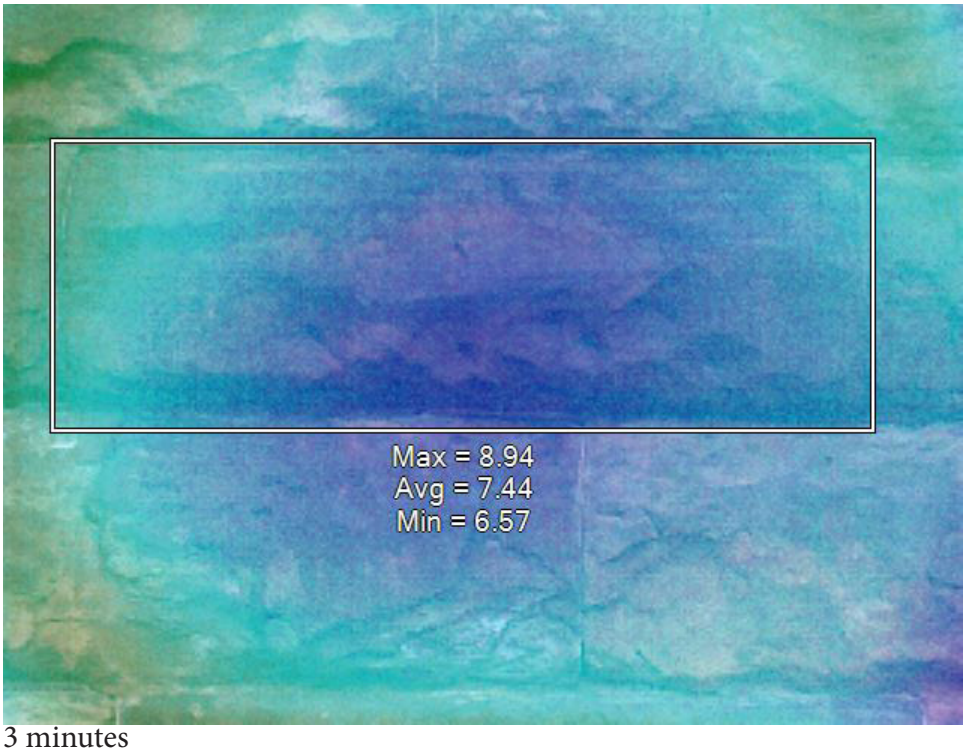


1 minute

Black biocolonization control
November 19, 2012

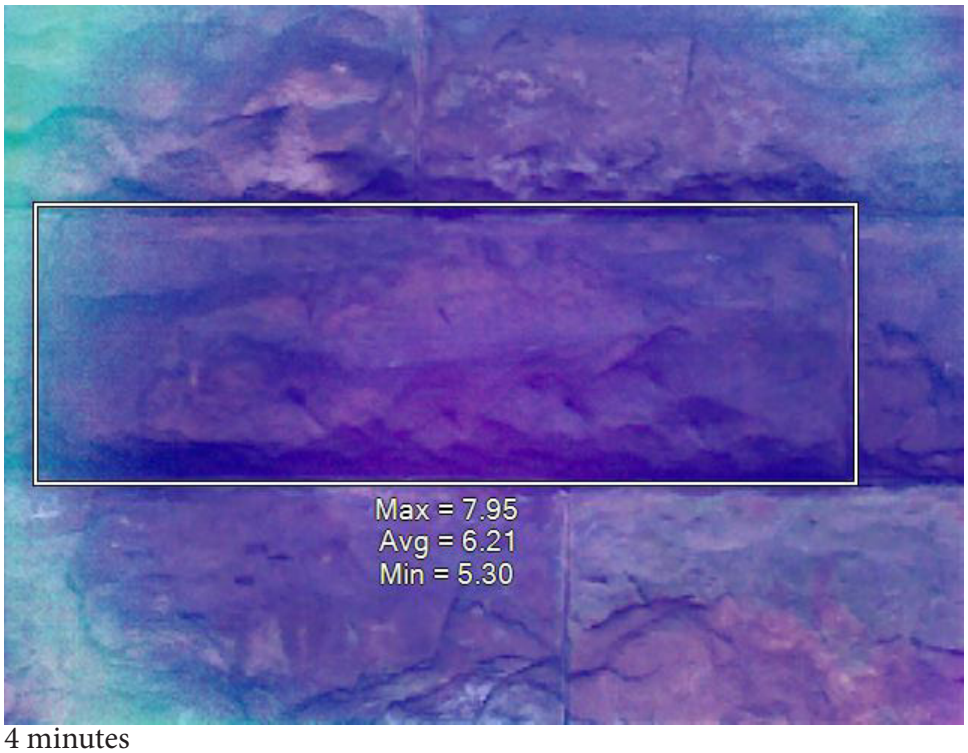


2 minutes



3 minutes

Black biocolonization control
November 19, 2012



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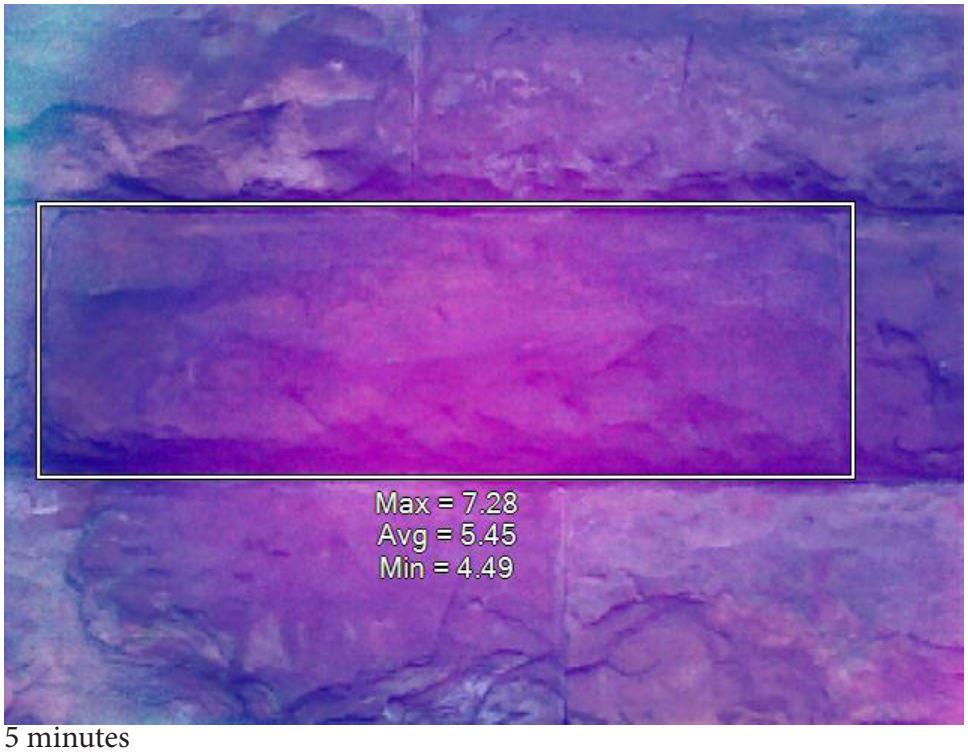
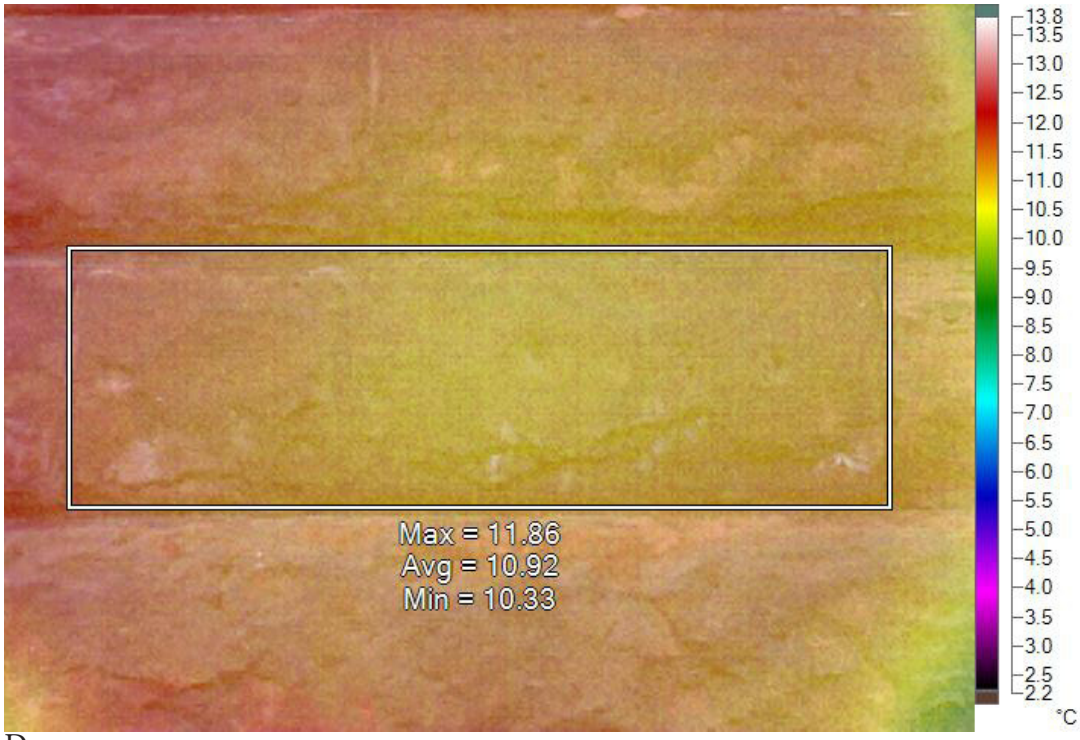


5 minutes

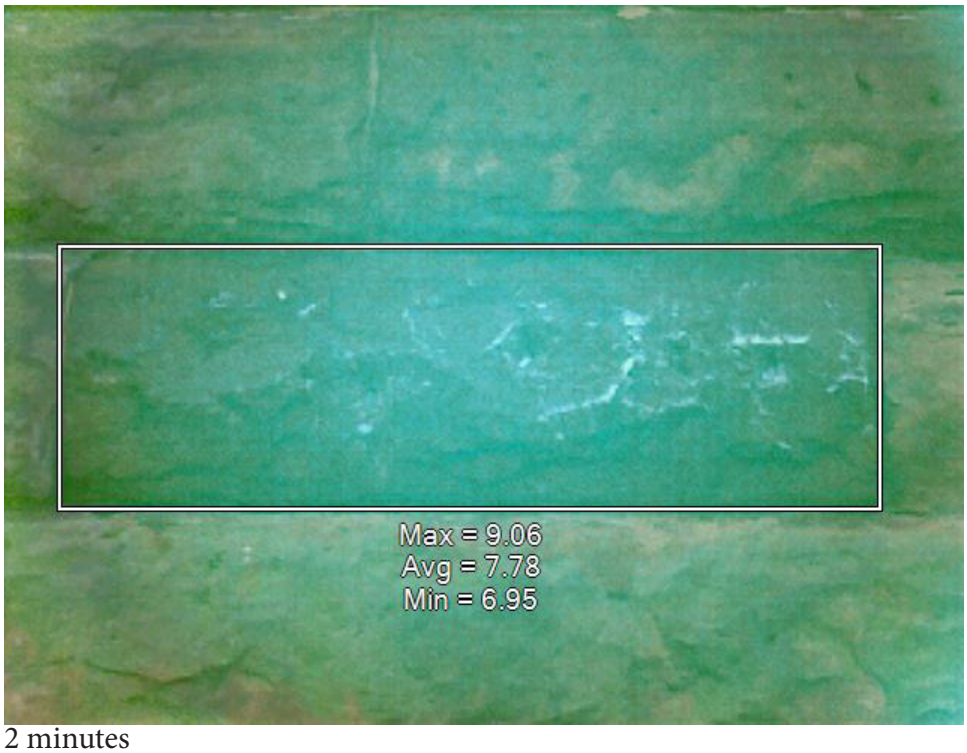
Black biocolonization treated with BioWash
November 19, 2012



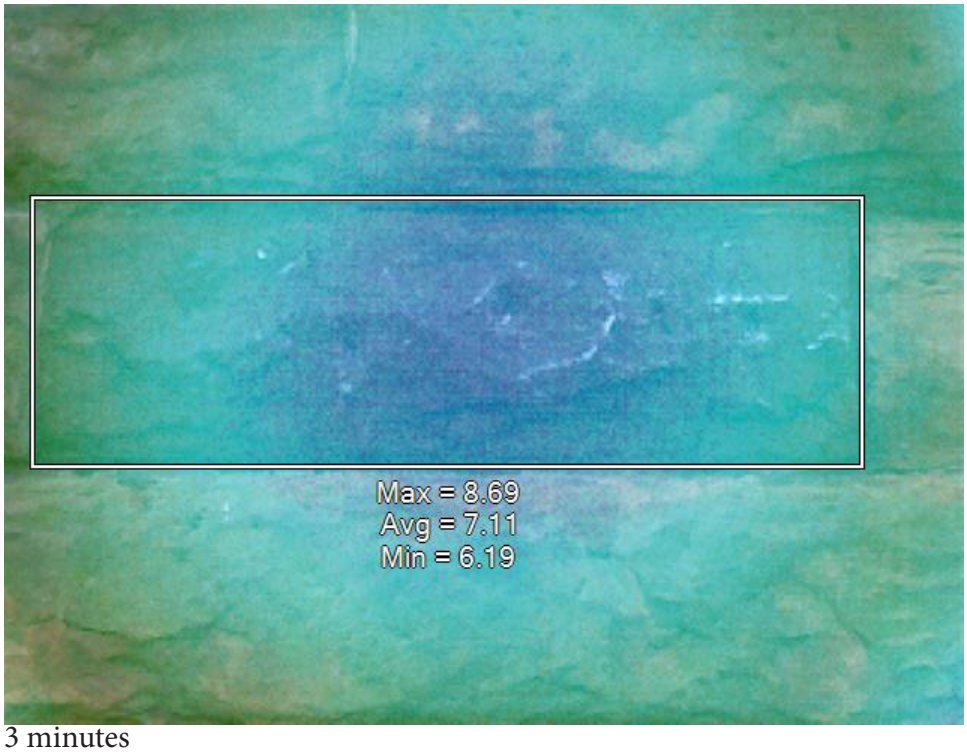
Dry

1 minute

Black biocolonization treated with BioWash
November 19, 2012



2 minutes



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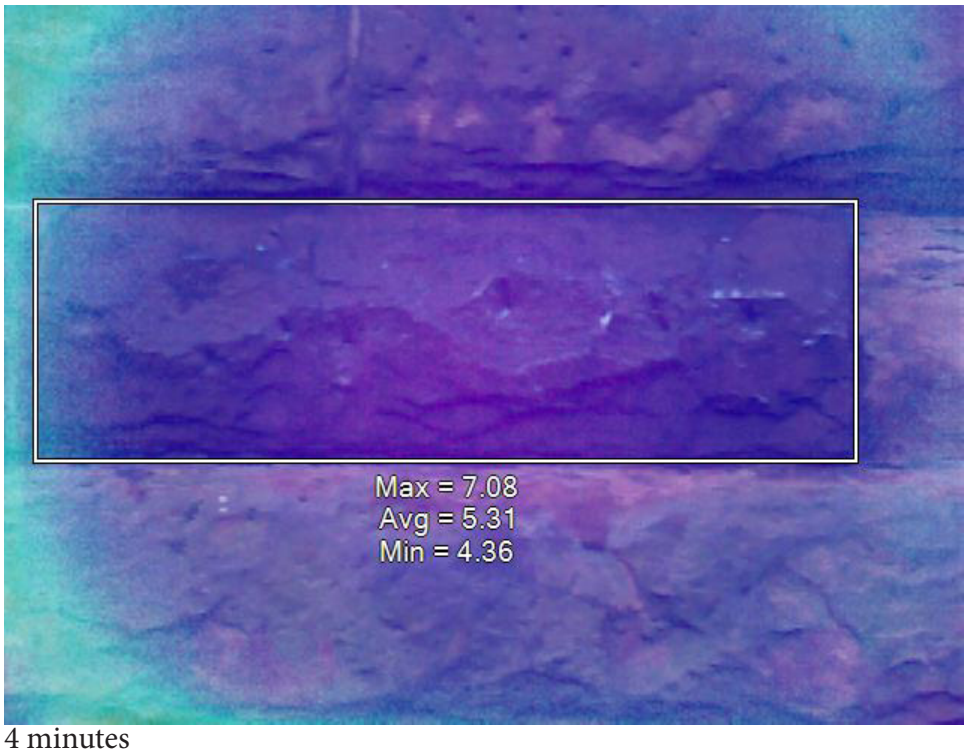
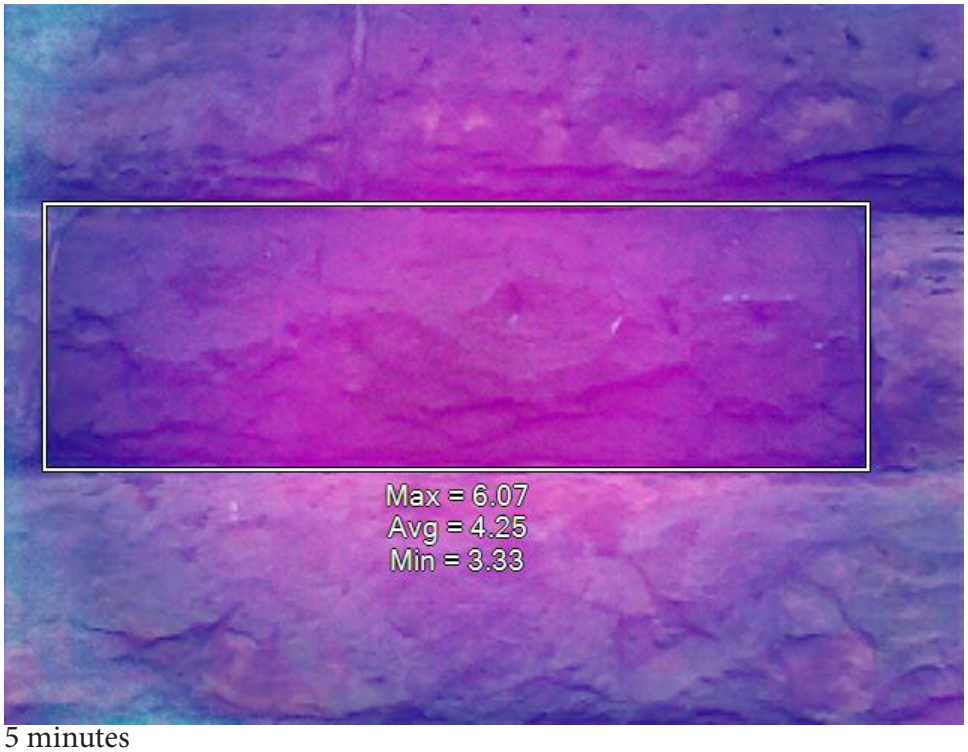
Black biocolonization treated with BioWash
November 19, 2012

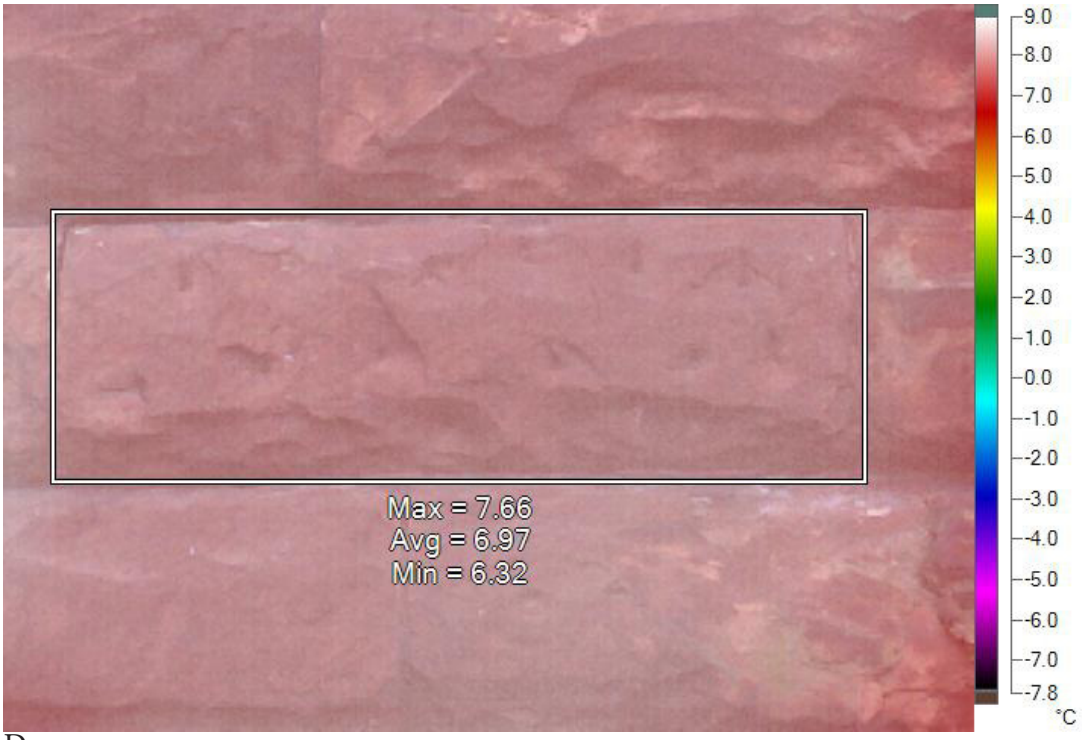


4 minutes

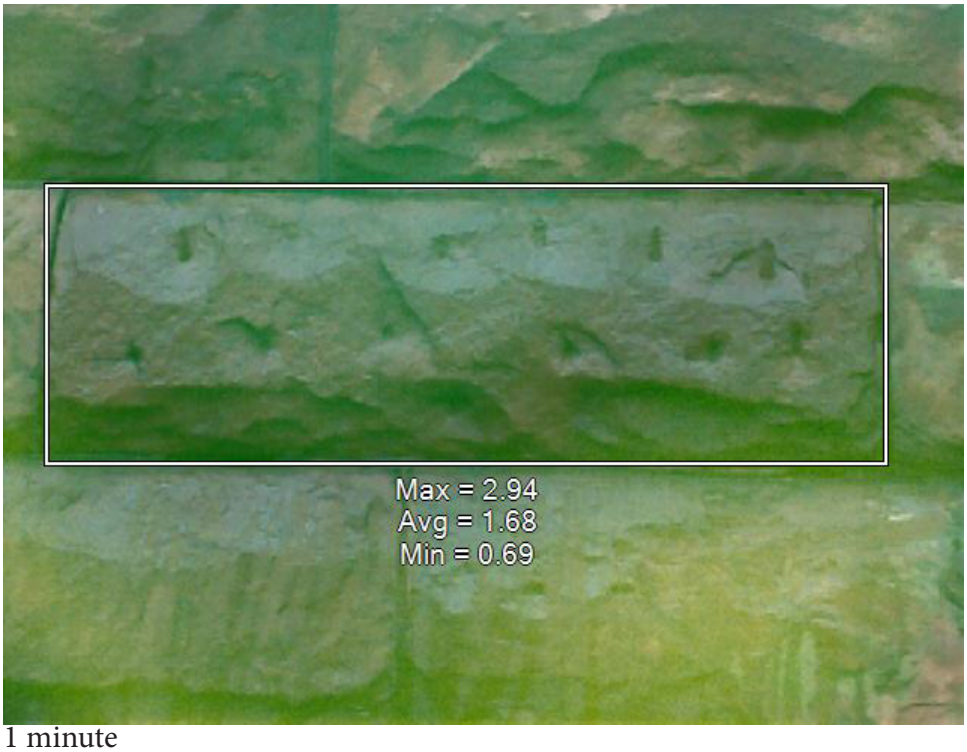


5 minutes

Black biocolonization treated with D/2
November 28, 2012



Dry



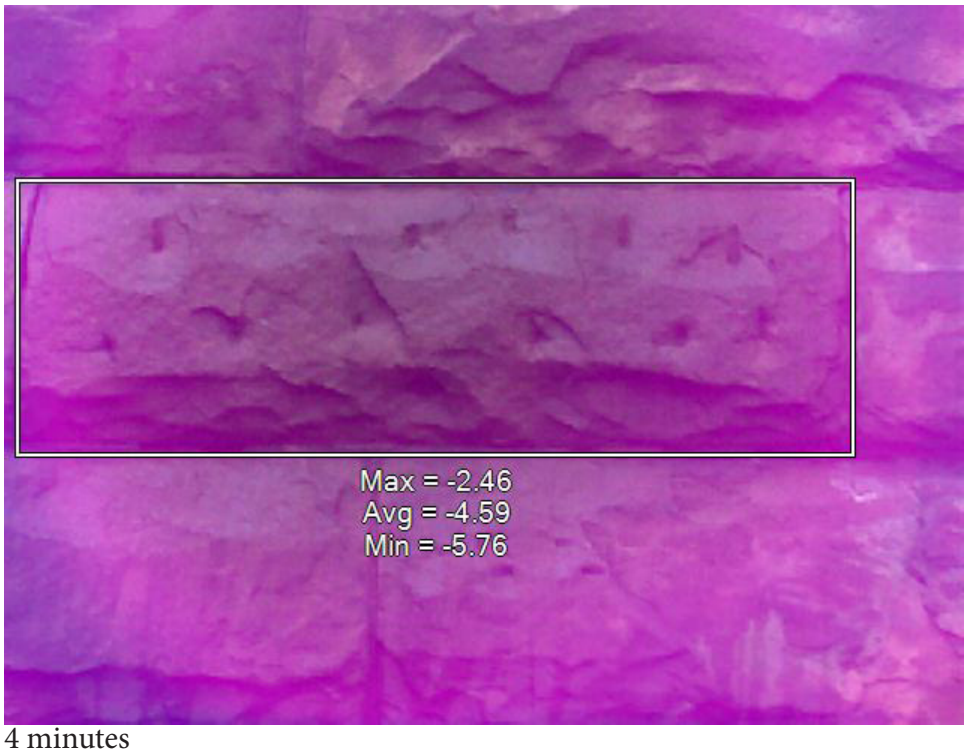
1 minute

Black biocolonization treated with D/2
November 28, 2012

2 minutes

3 minutes

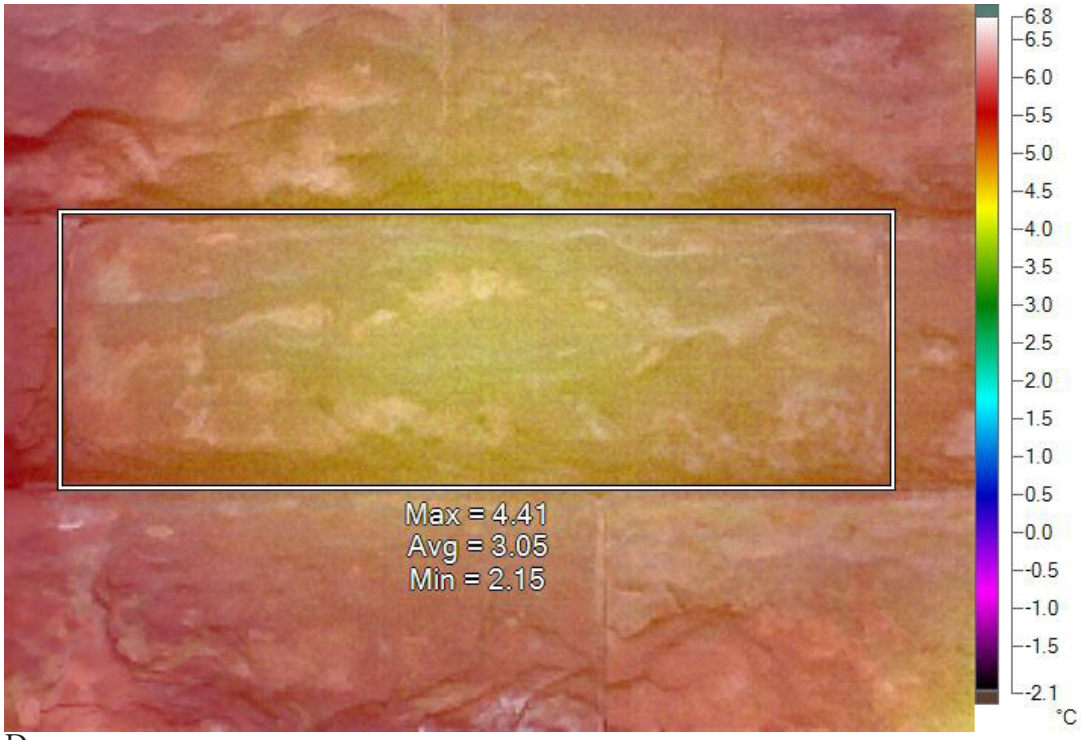
Black biocolonization treated with D/2
November 28, 2012



4 minutes

5 minutes

Black biocolonization control
November 26, 2012



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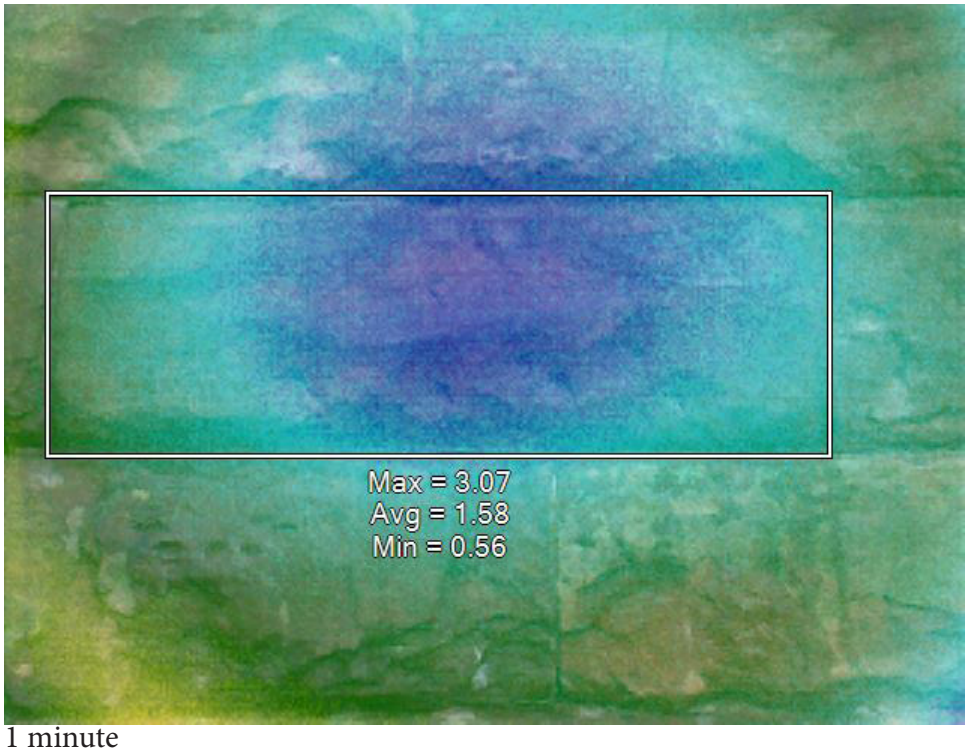
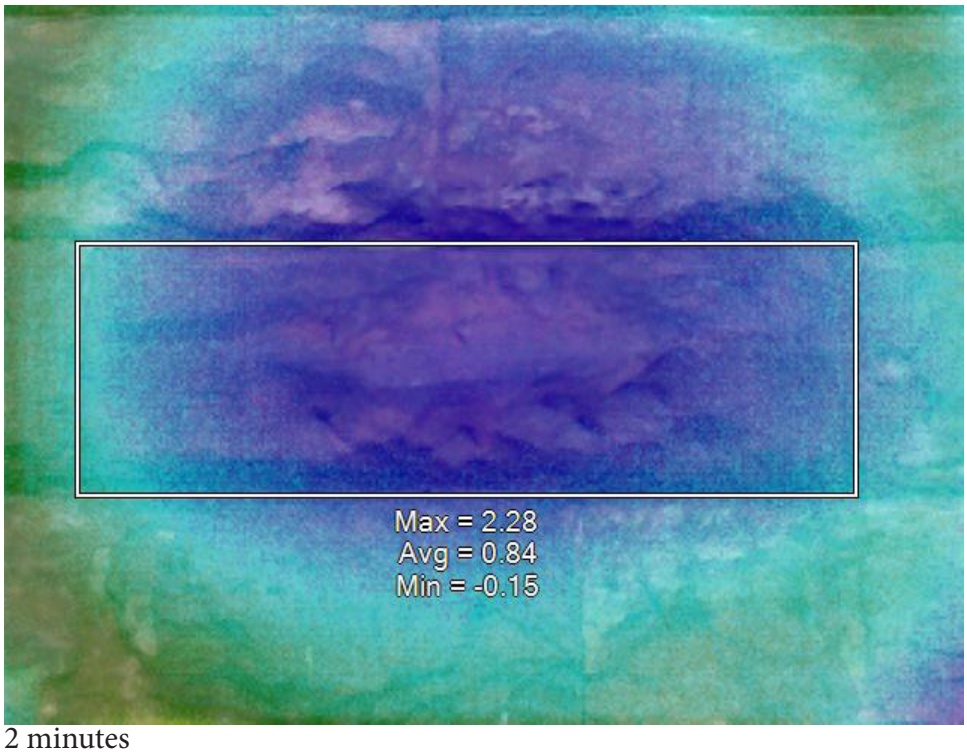
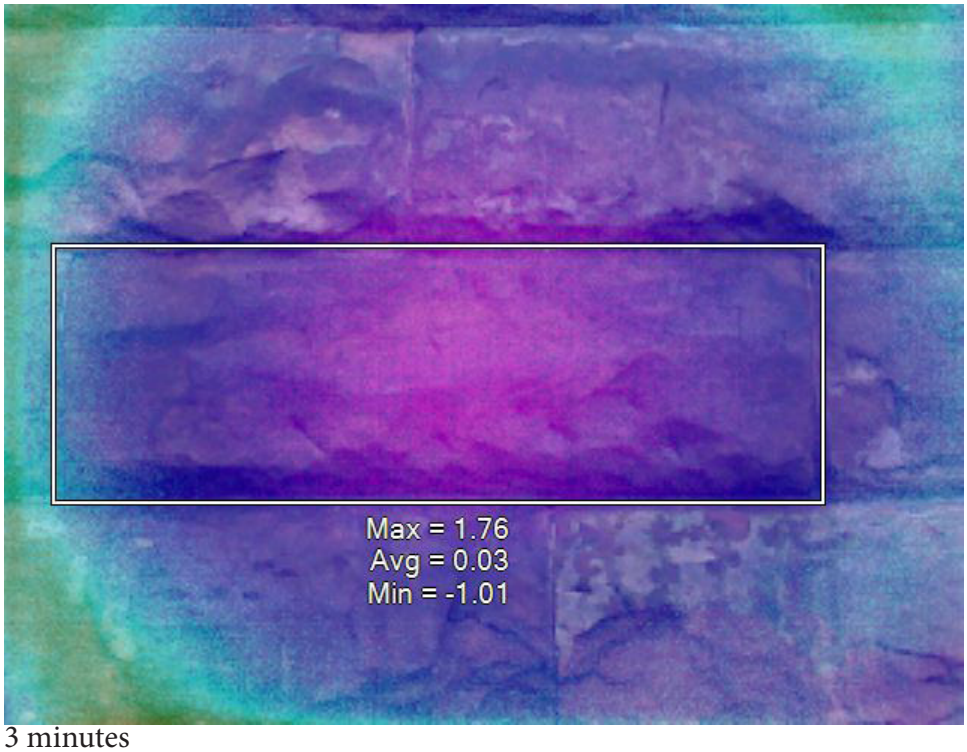


1 minute

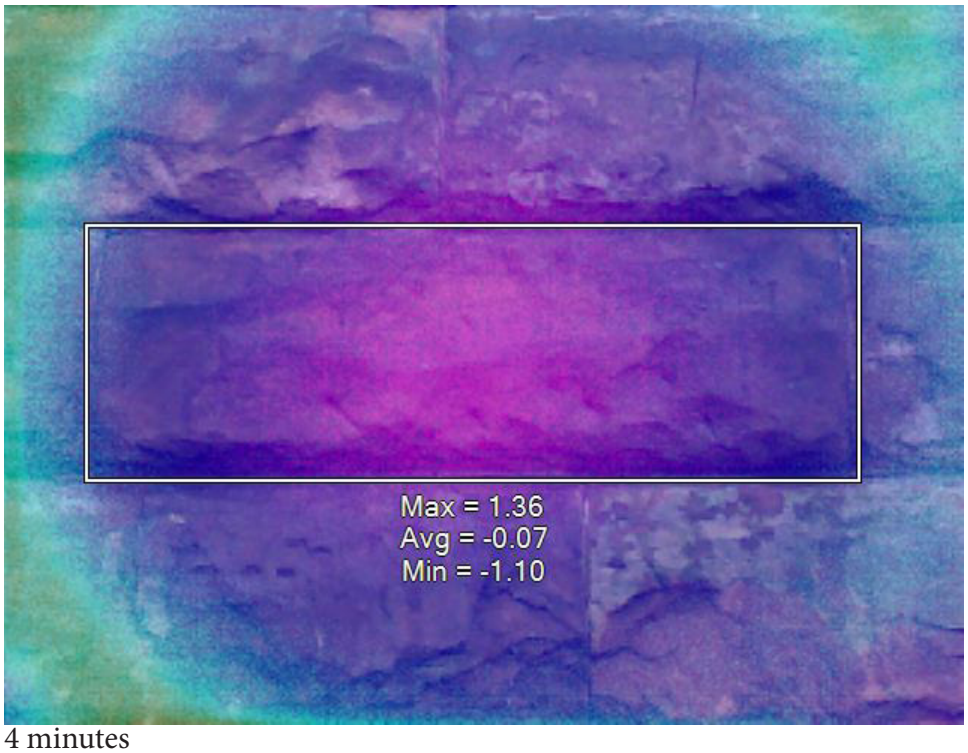


2 minutes



3 minutes

Black biocolonization control
November 26, 2012



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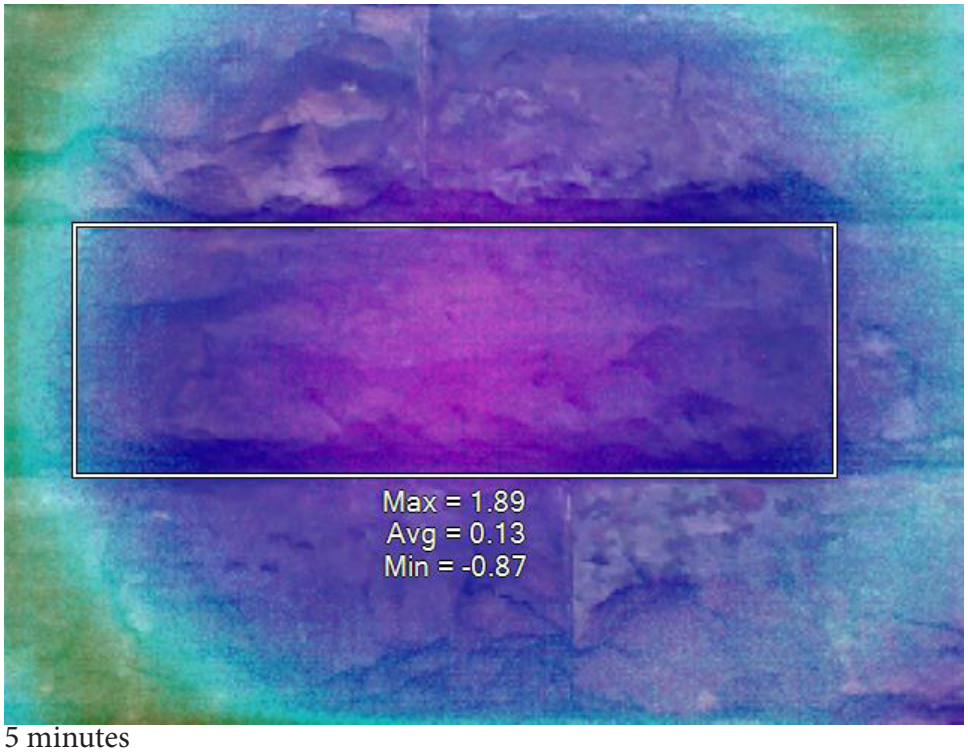
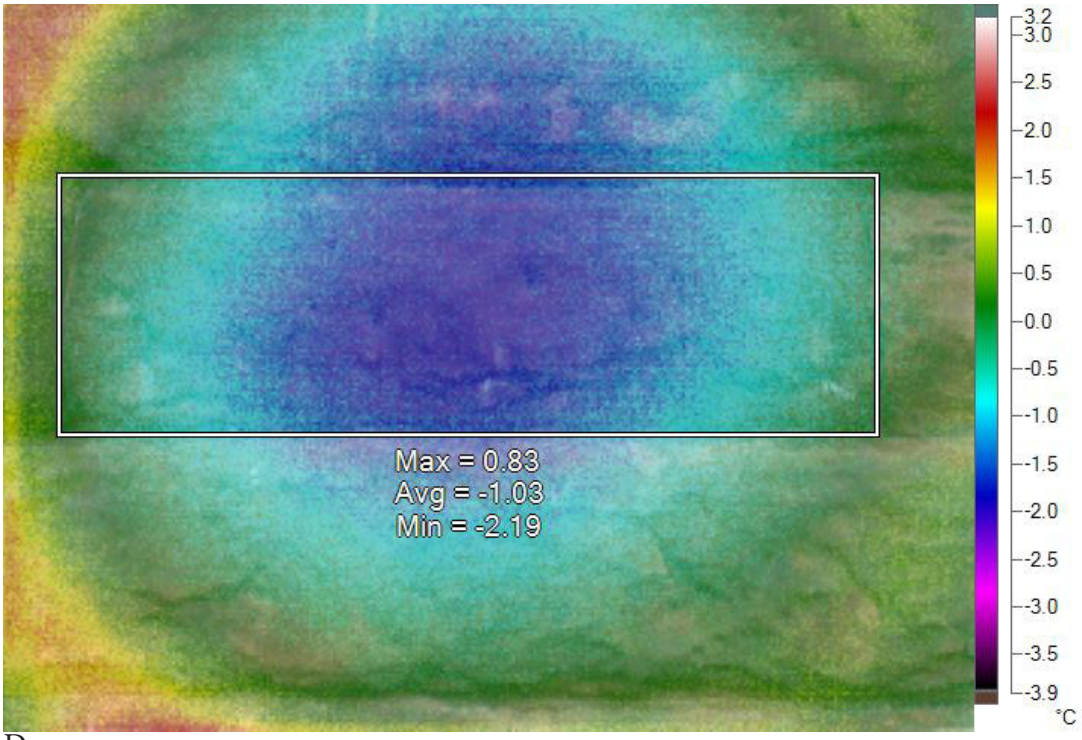


5 minutes

Black biocolonization treated with BioWash
November 26, 2012



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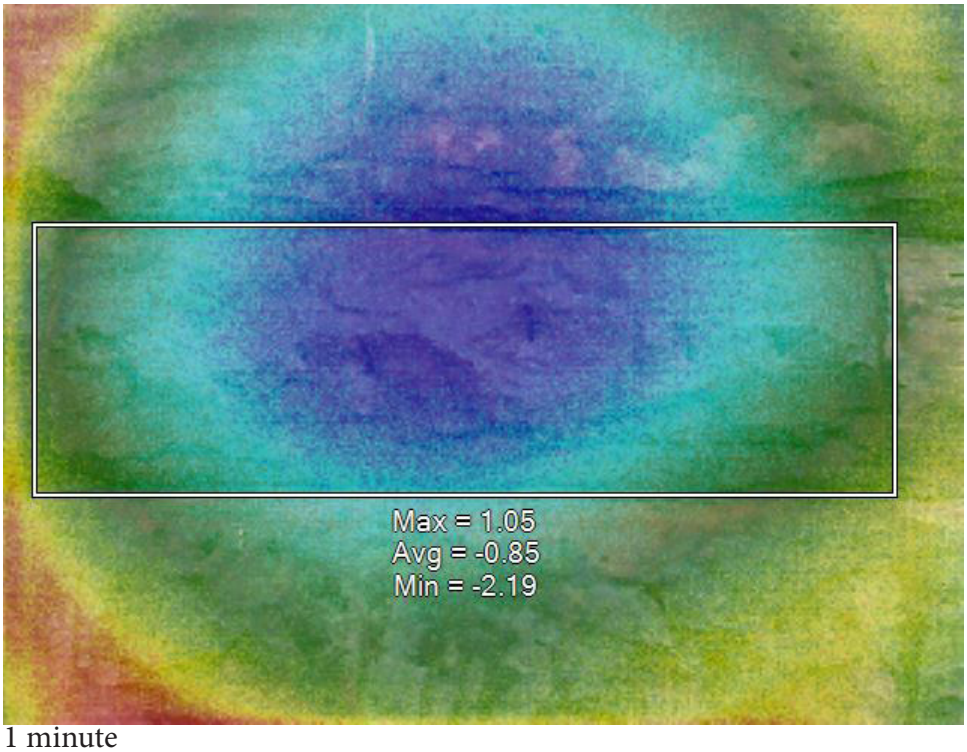
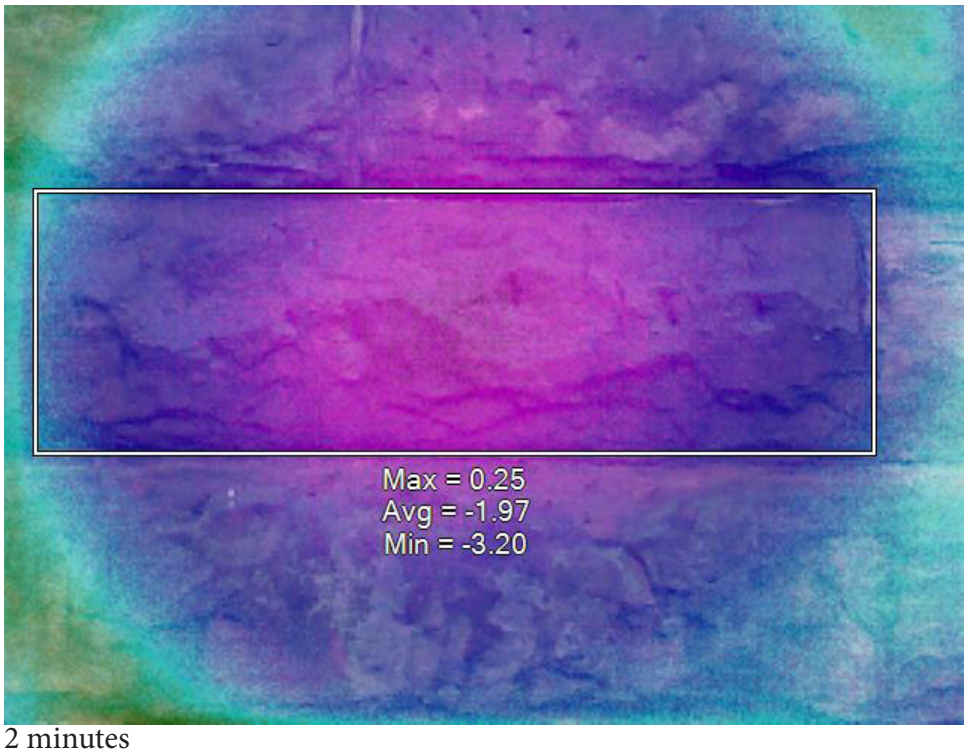
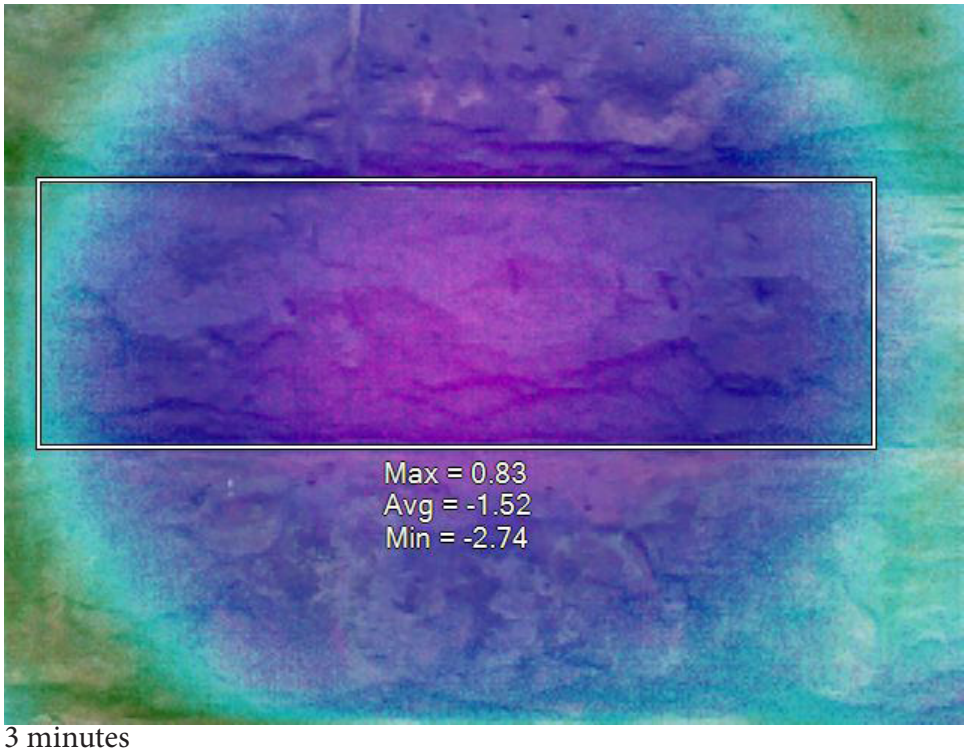


1 minute

Black biocolonization treated with BioWash
November 26, 2012

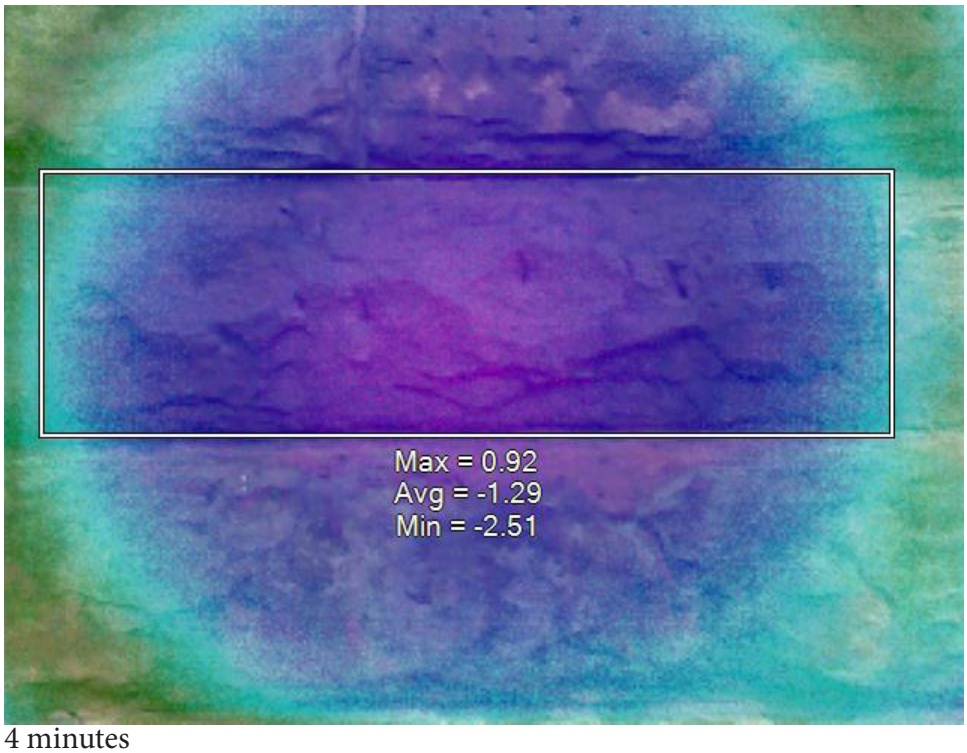


2 minutes

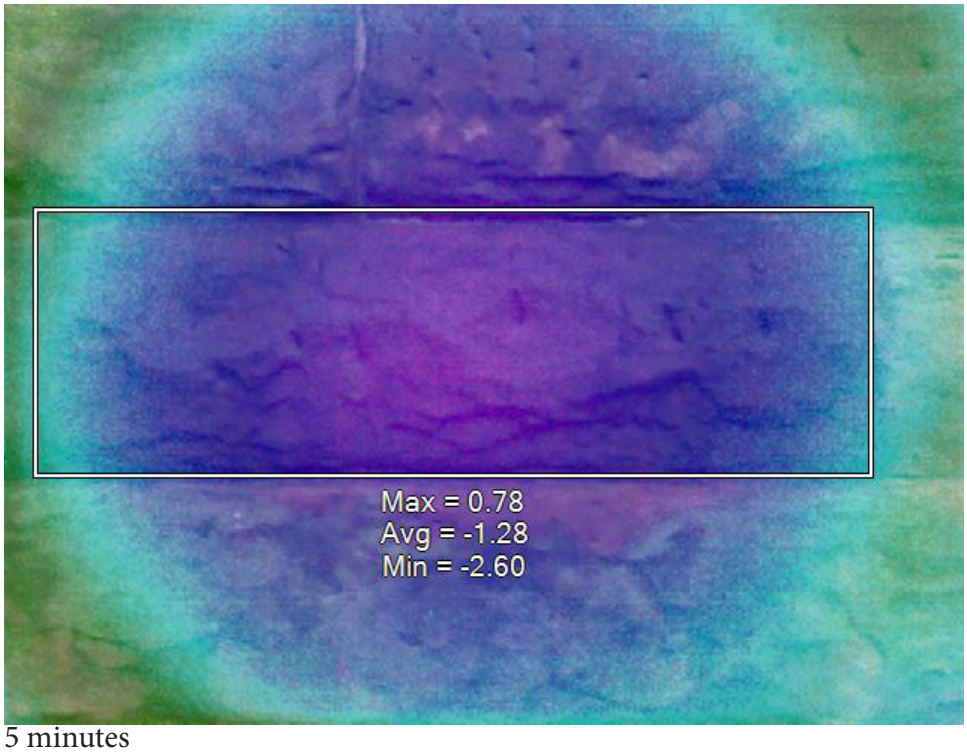


3 minutes

Black biocolonization treated with BioWash
November 26, 2012

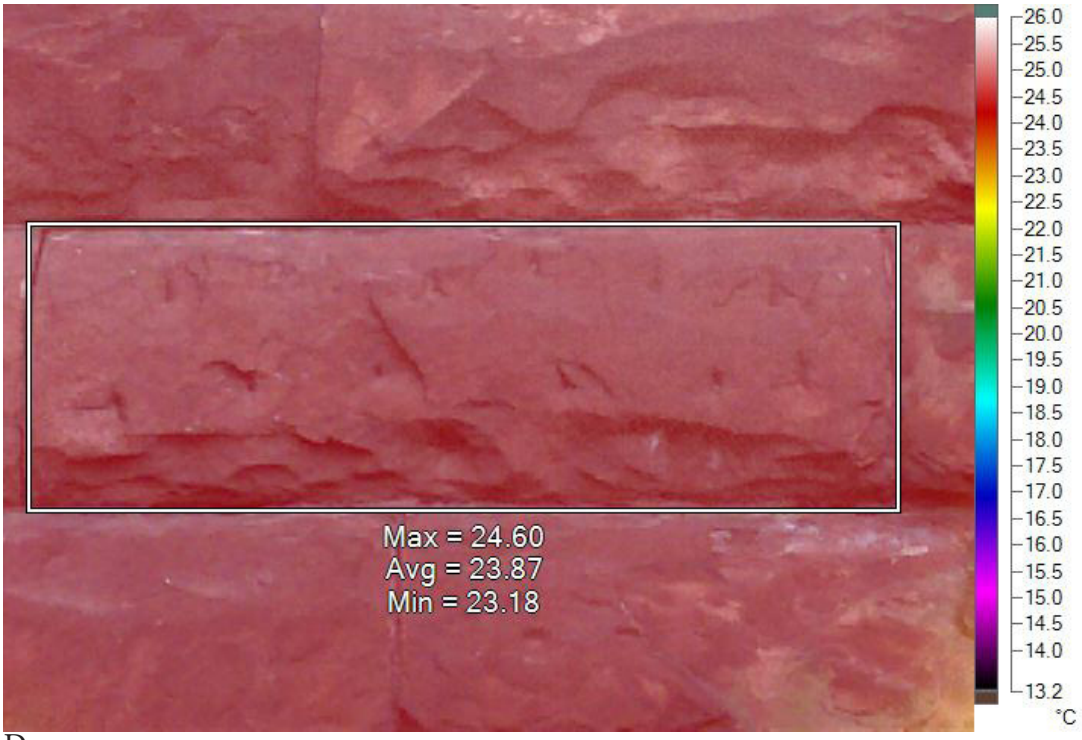


4 minutes



5 minutes

Black biocolonization treated with D/2
December 4, 2012



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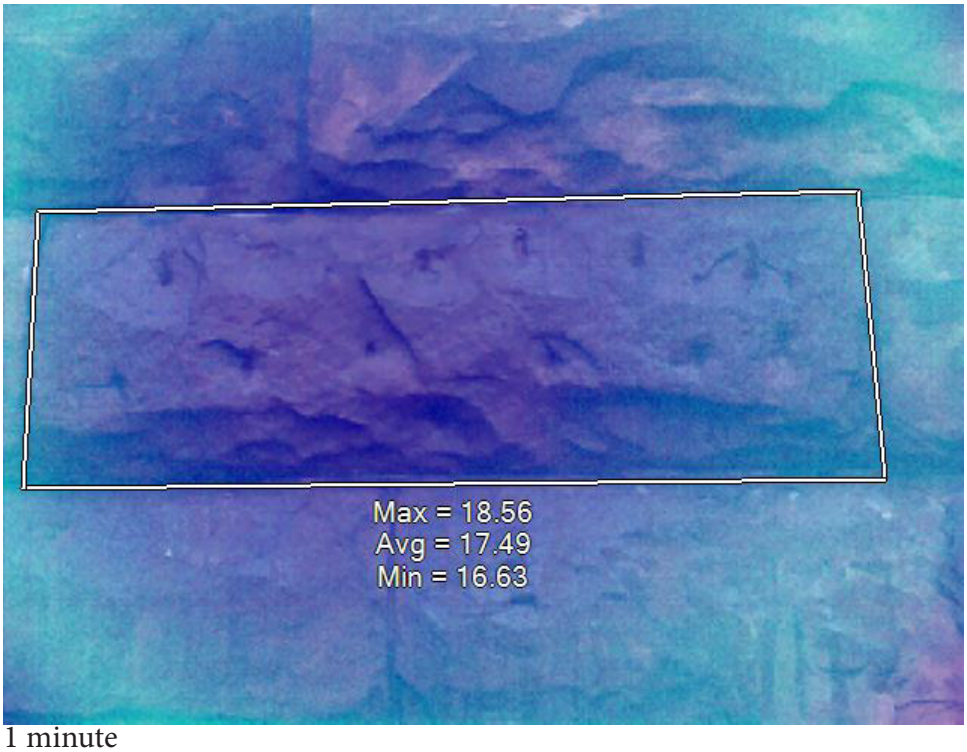
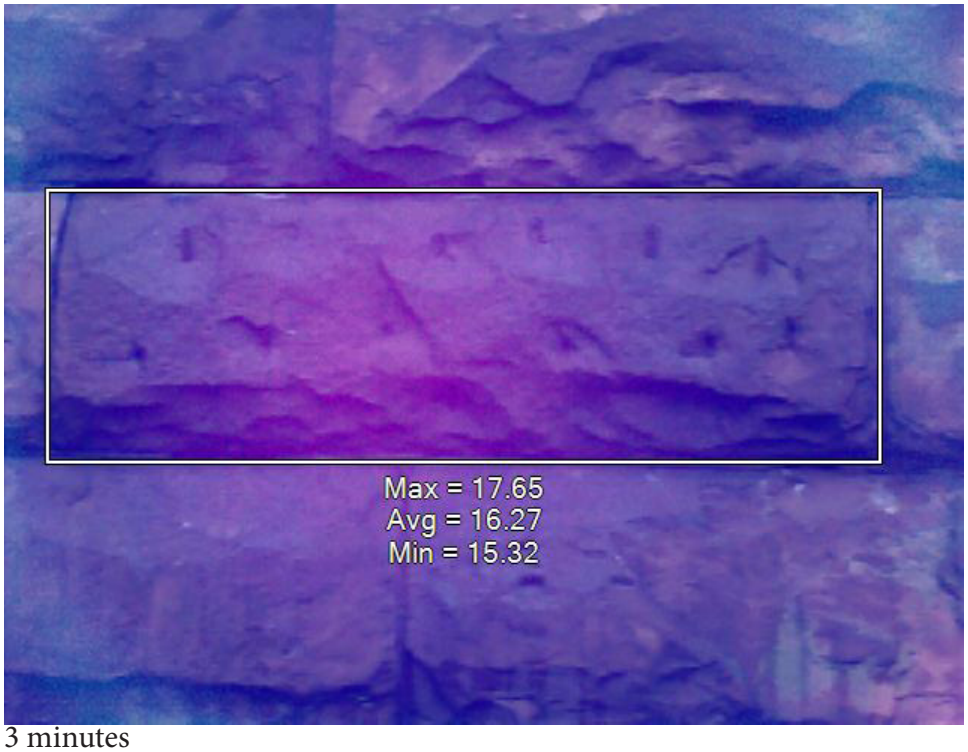


1 minute

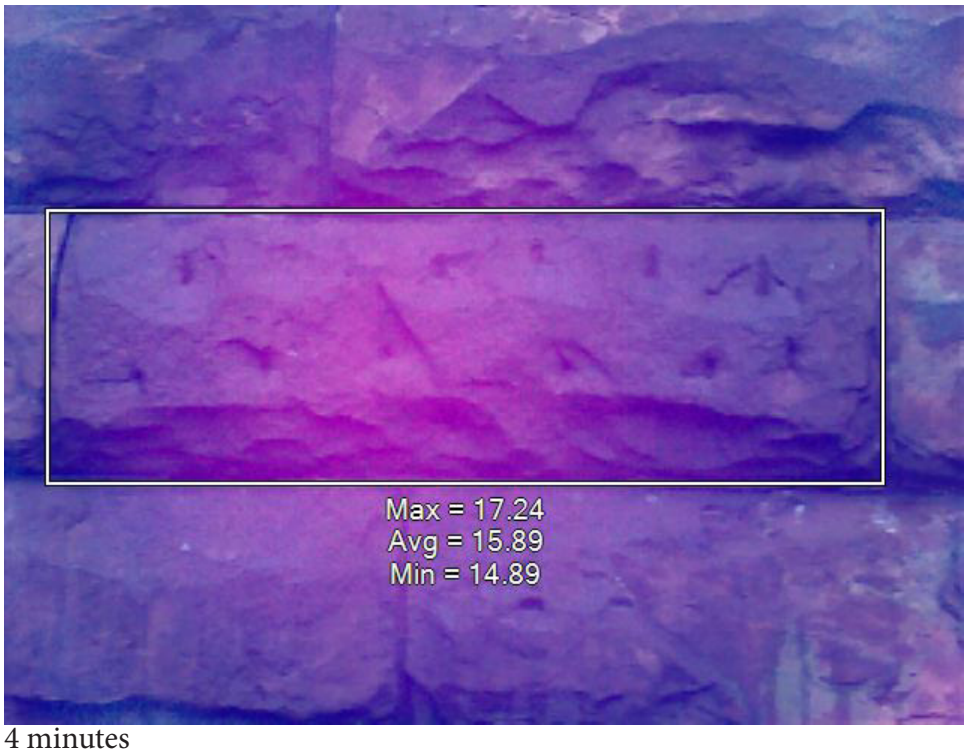
Black biocolonization treated with D/2
December 4, 2012

2 minutes



3 minutes

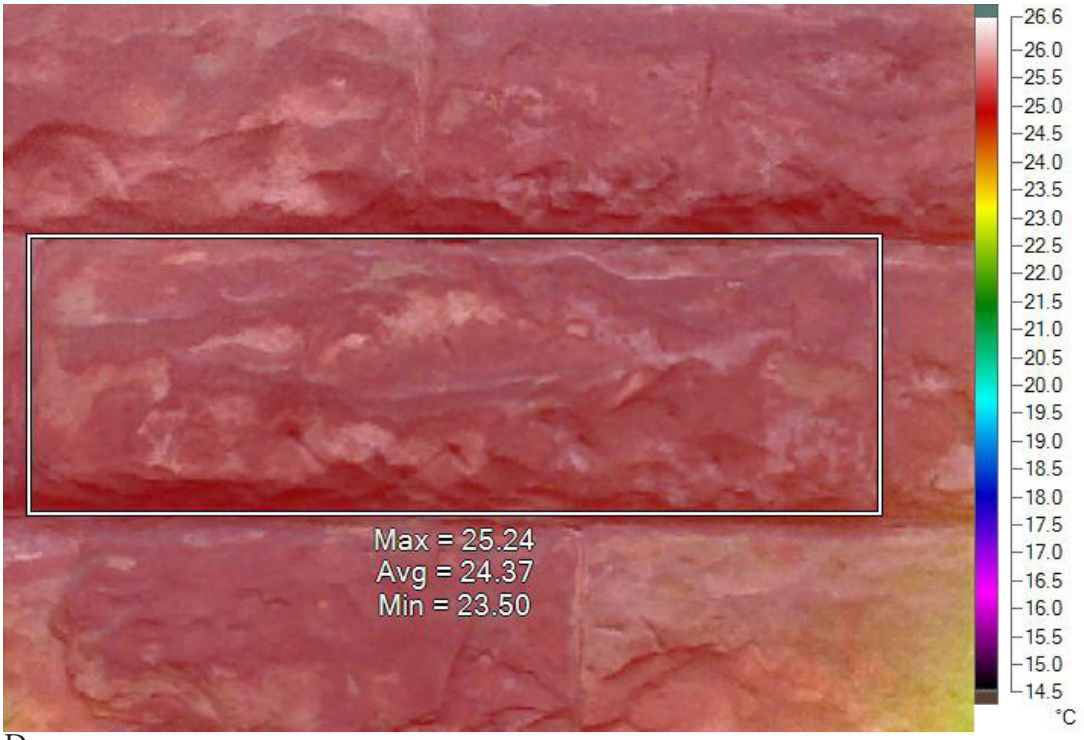
Black biocolonization treated with D/2
December 4, 2012



4 minutes

5 minutes

Black biocolonization control
December 4, 2012



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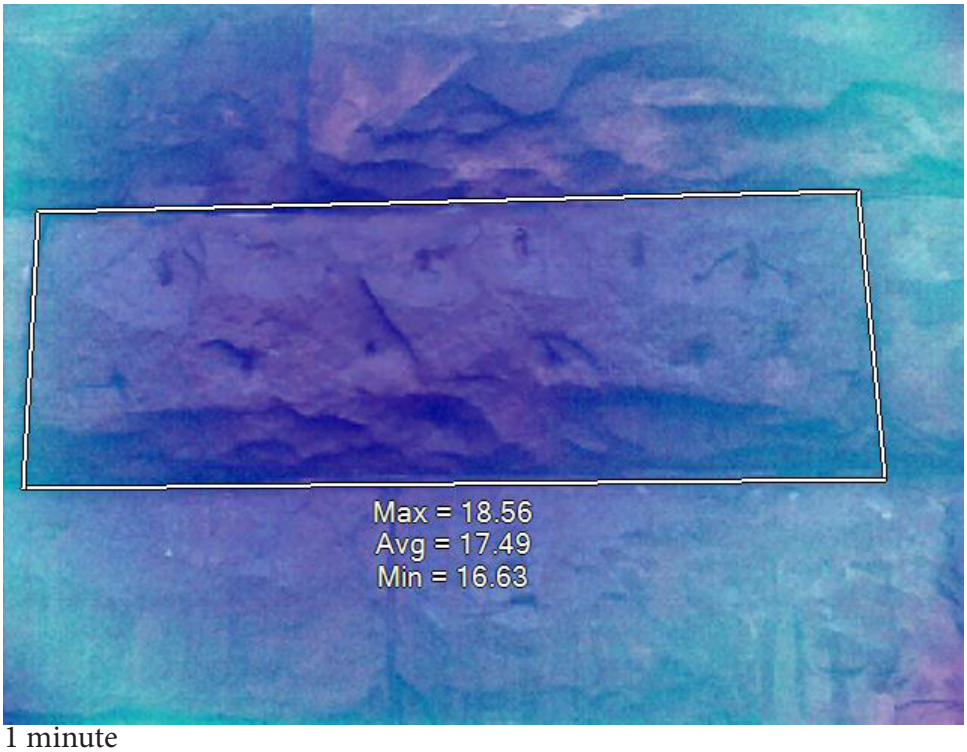
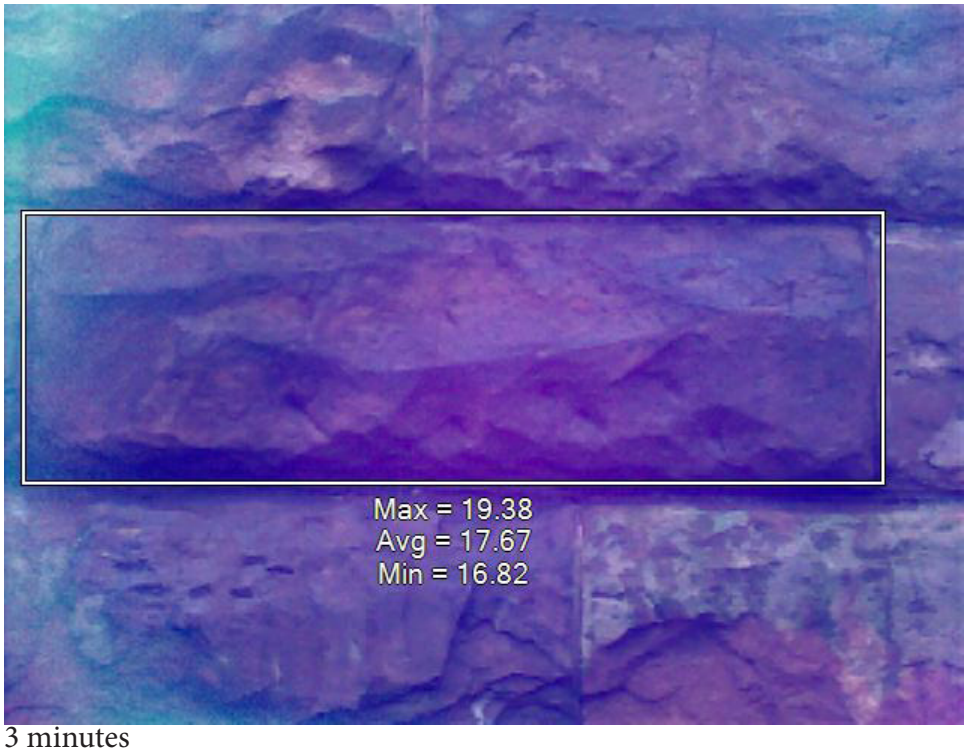


1 minute

Black biocolonization control
December 4, 2012

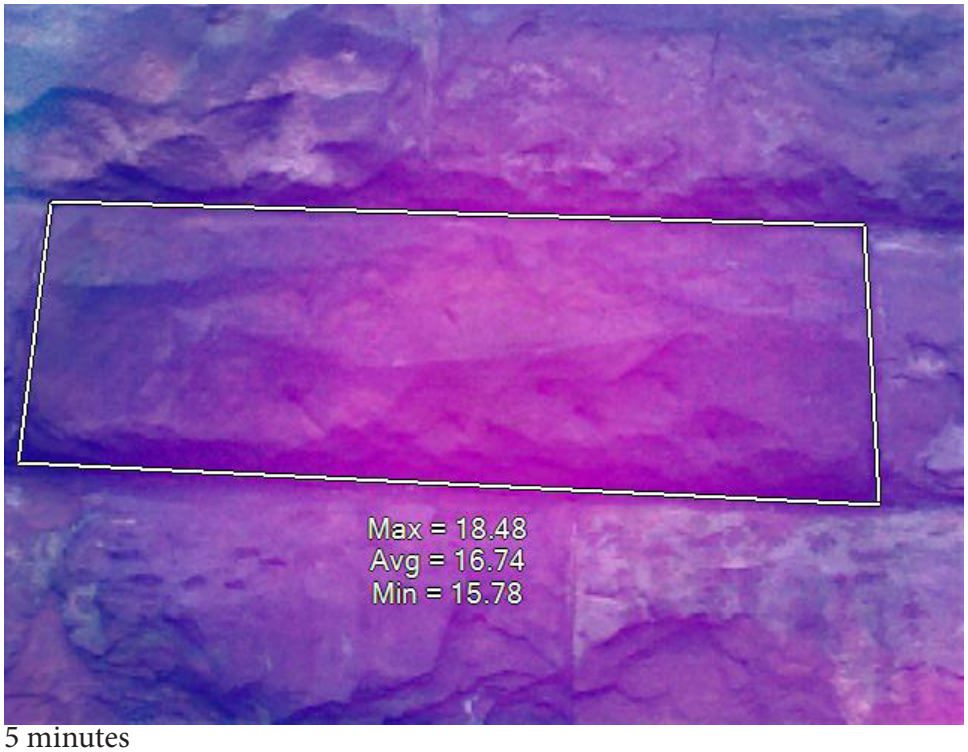
2 minutes



3 minutes

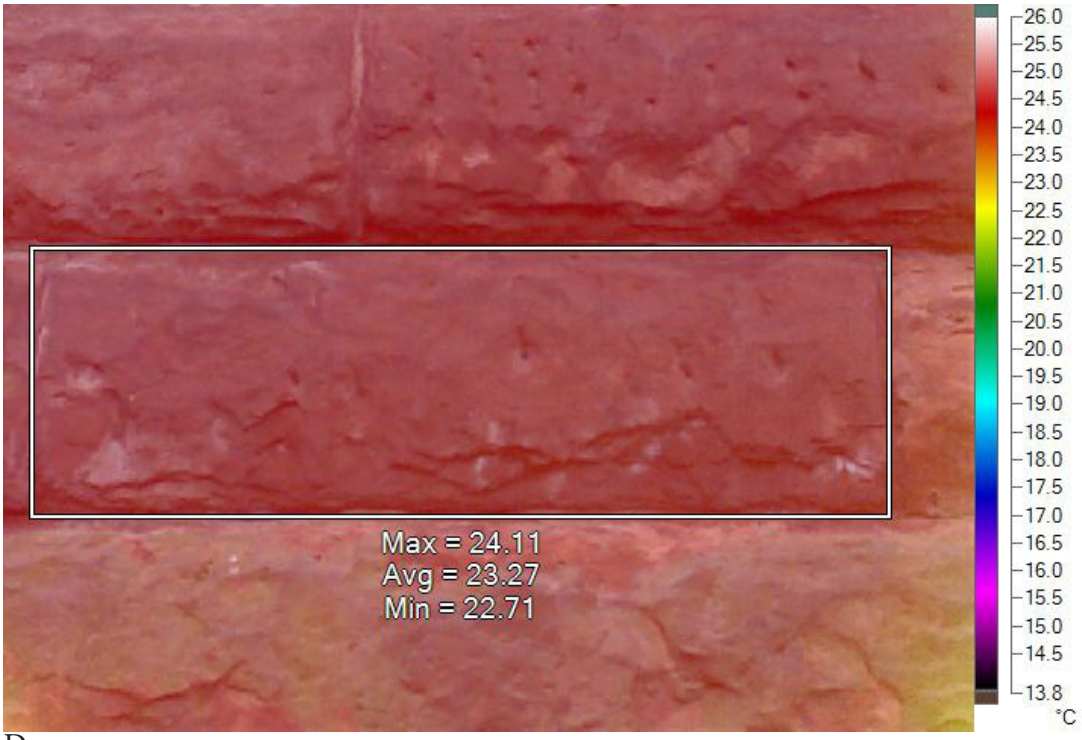
Black biocolonization control
December 4, 2012

4 minutes

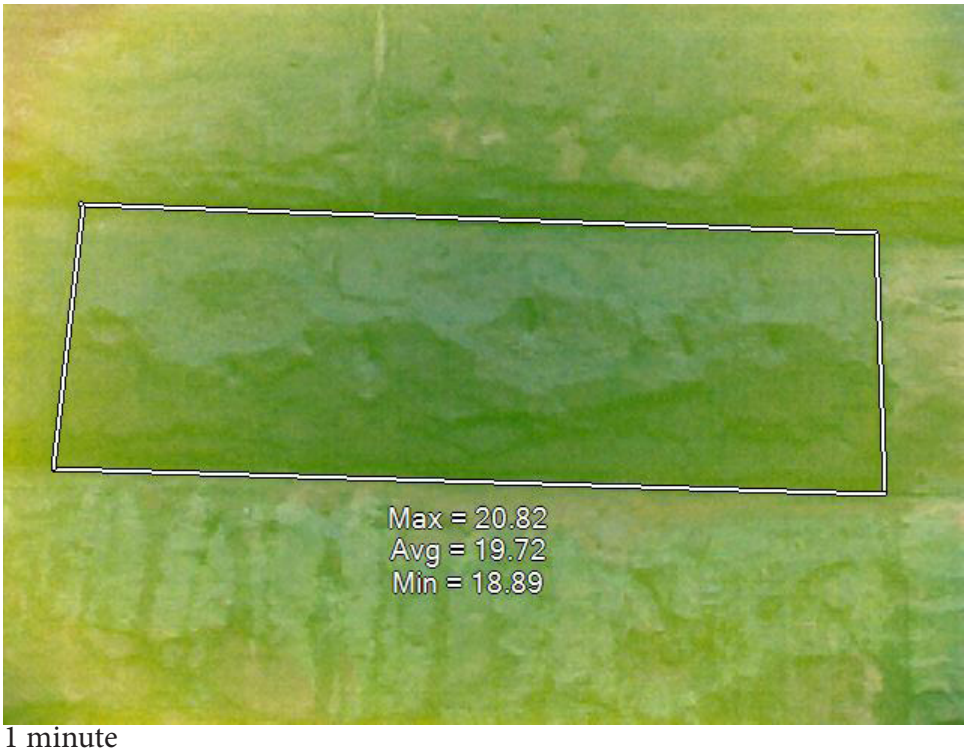


5 minutes

Black biocolonization treated with BioWash
December 4, 2012



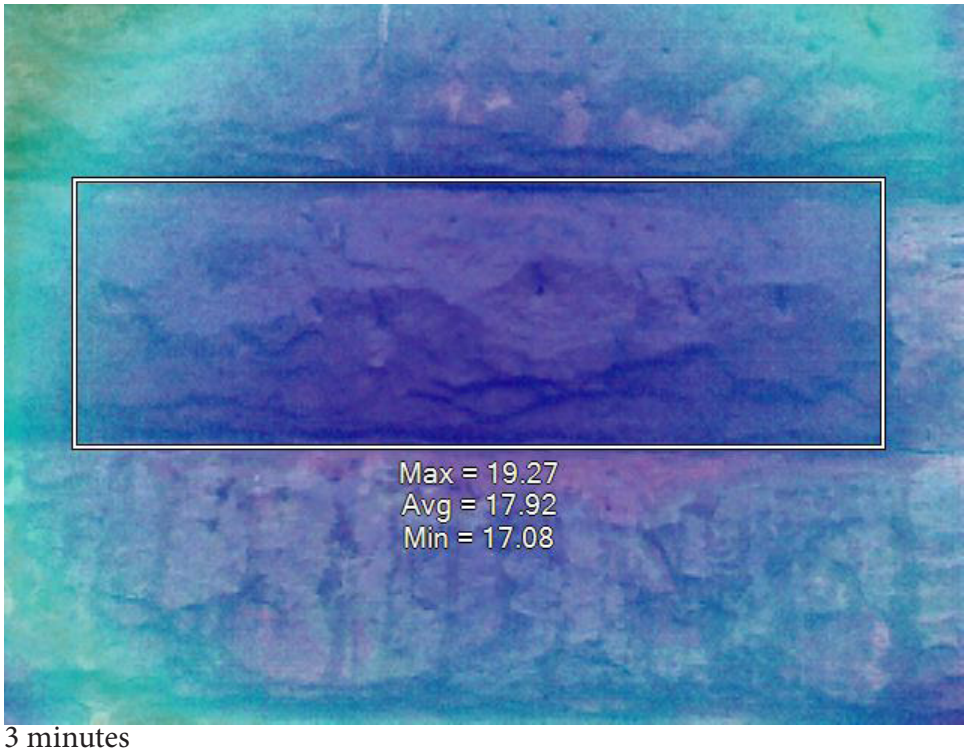
Dry



1 minute

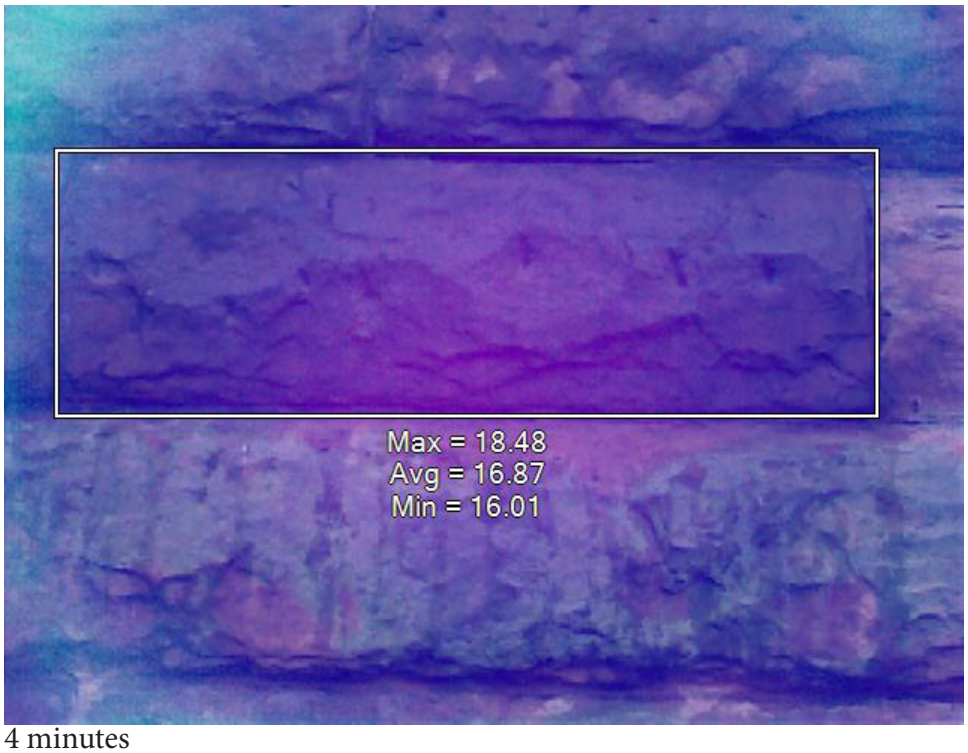
Black biocolonization treated with BioWash
December 4, 2012

2 minutes



3 minutes

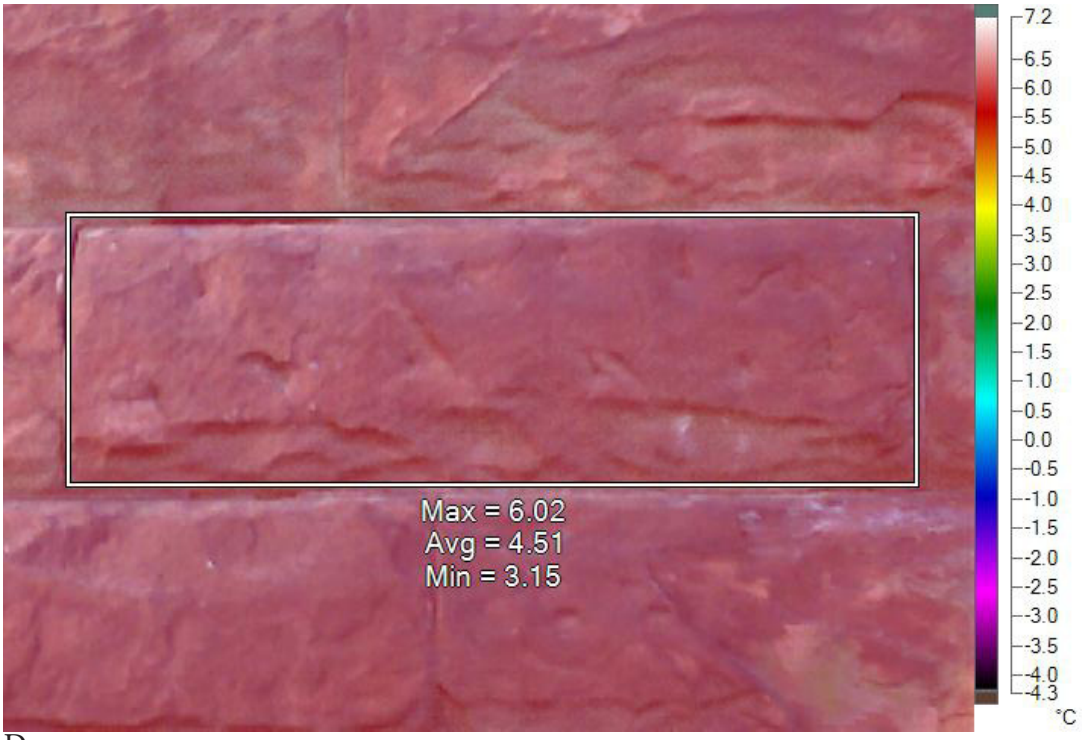
Black biocolonization treated with BioWash
December 4, 2012



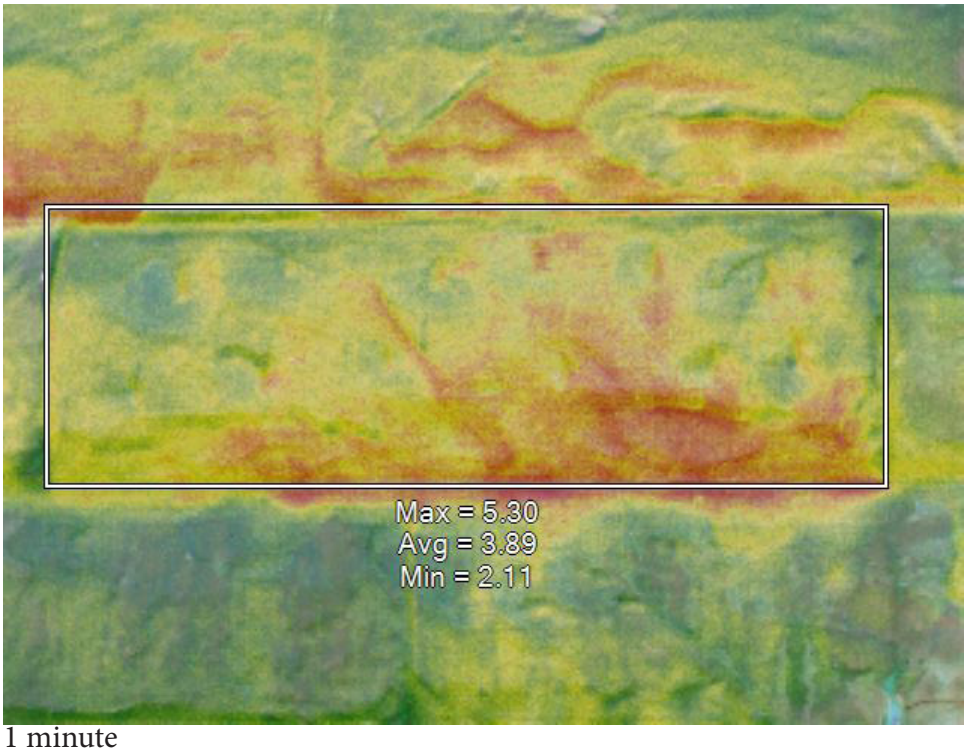
4 minutes

5 minutes

Black biocolonization treated with D/2
January 10, 2013

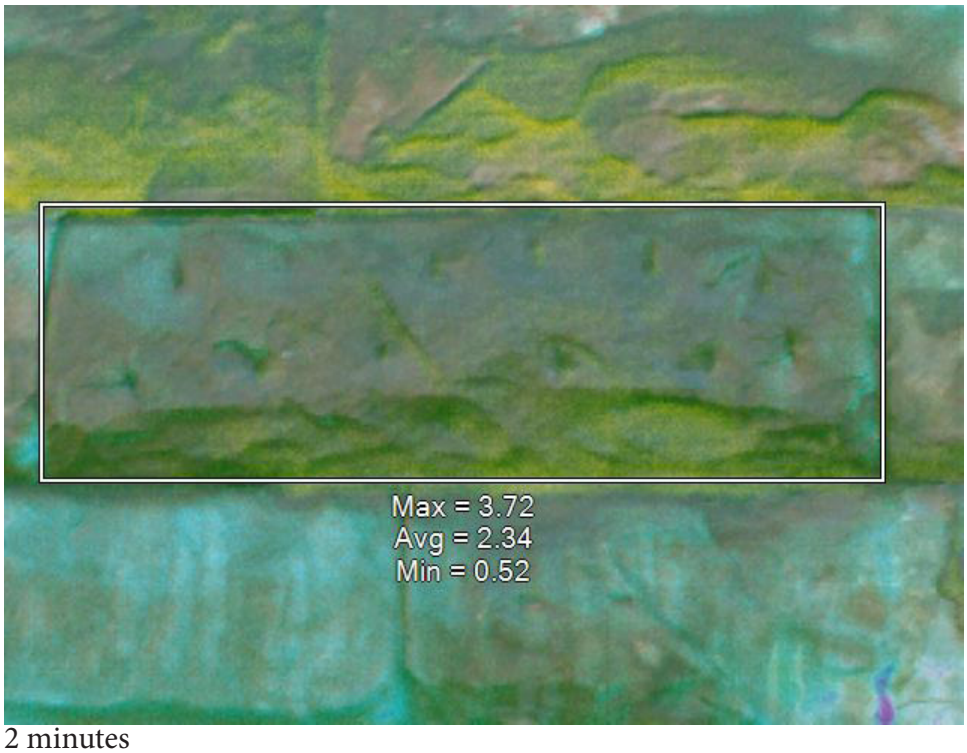


Dry

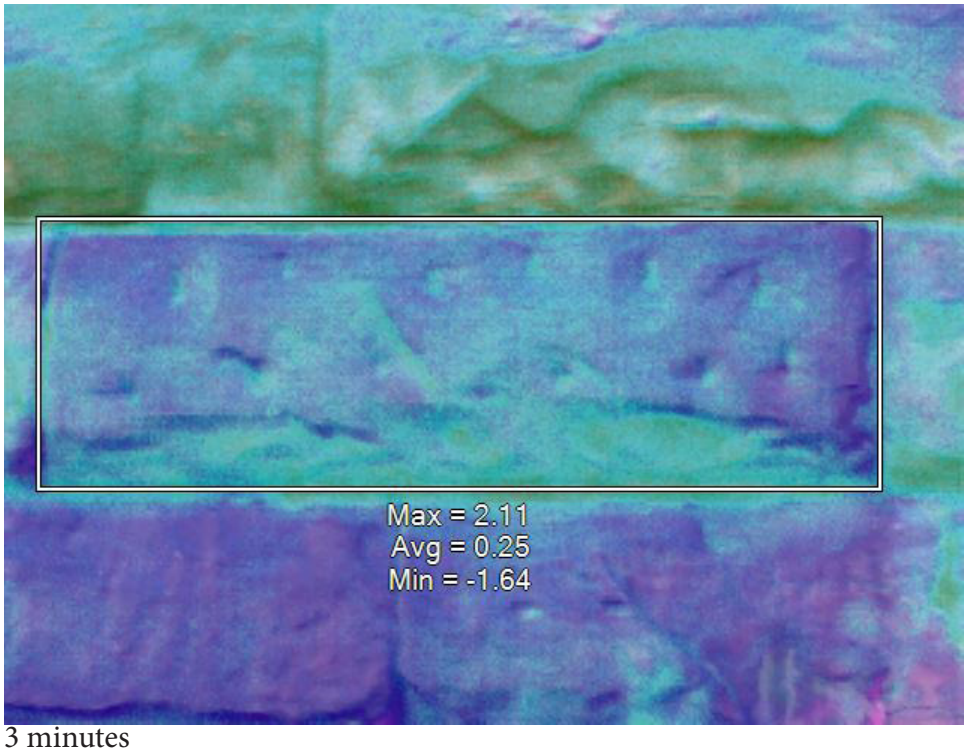


1 minute

Black biocolonization treated with D/2
January 10, 2013

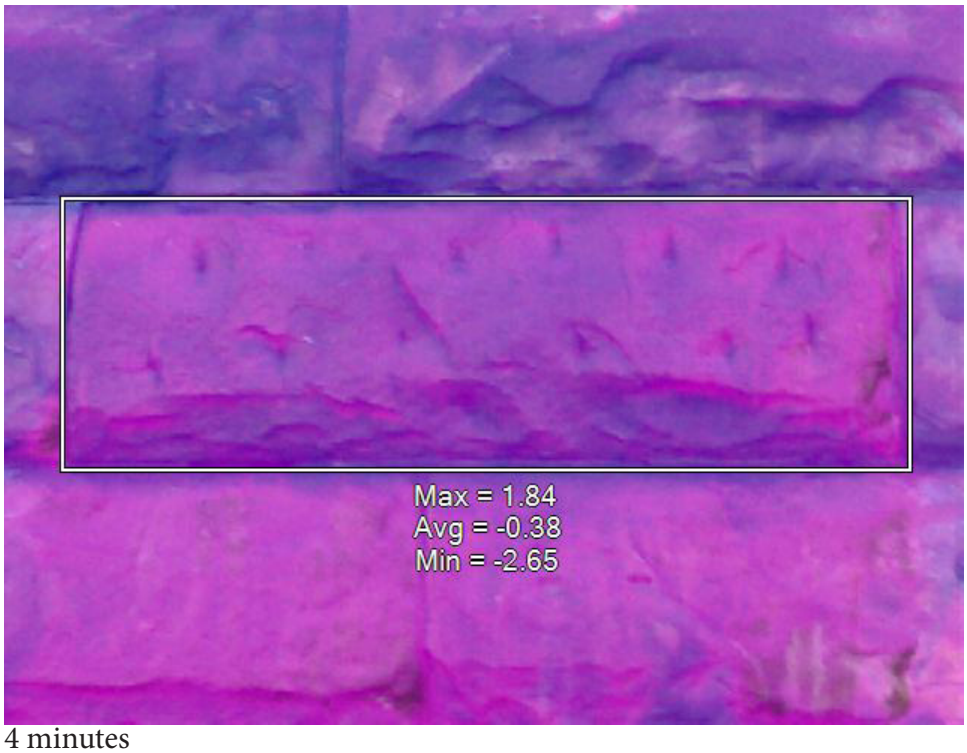


2 minutes

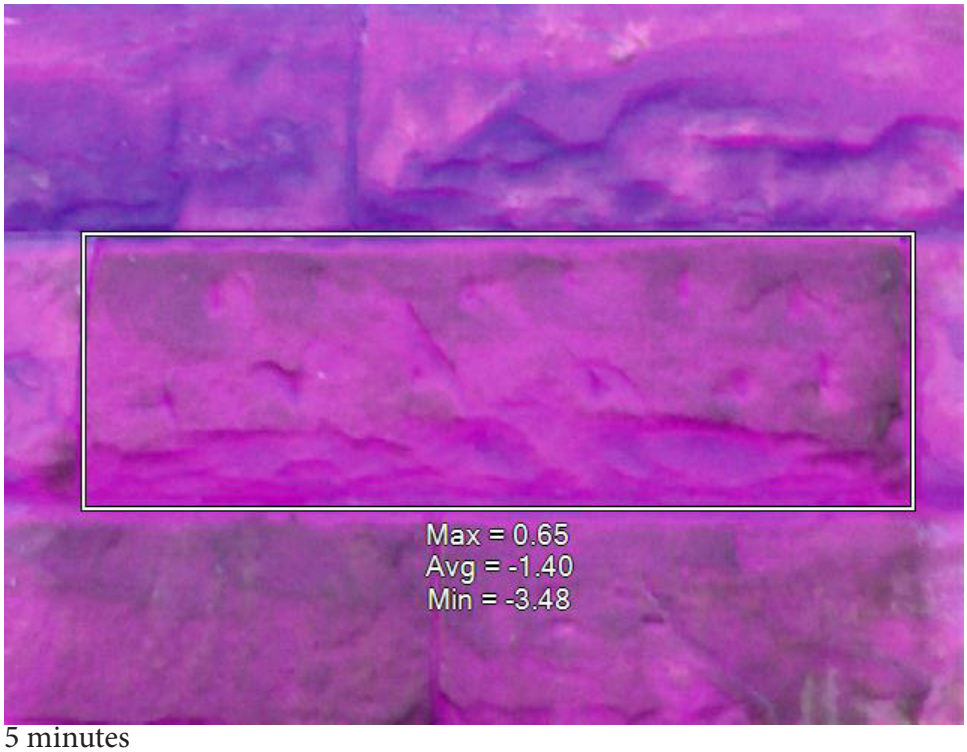


3 minutes

Black biocolonization treated with D/2
January 10, 2013

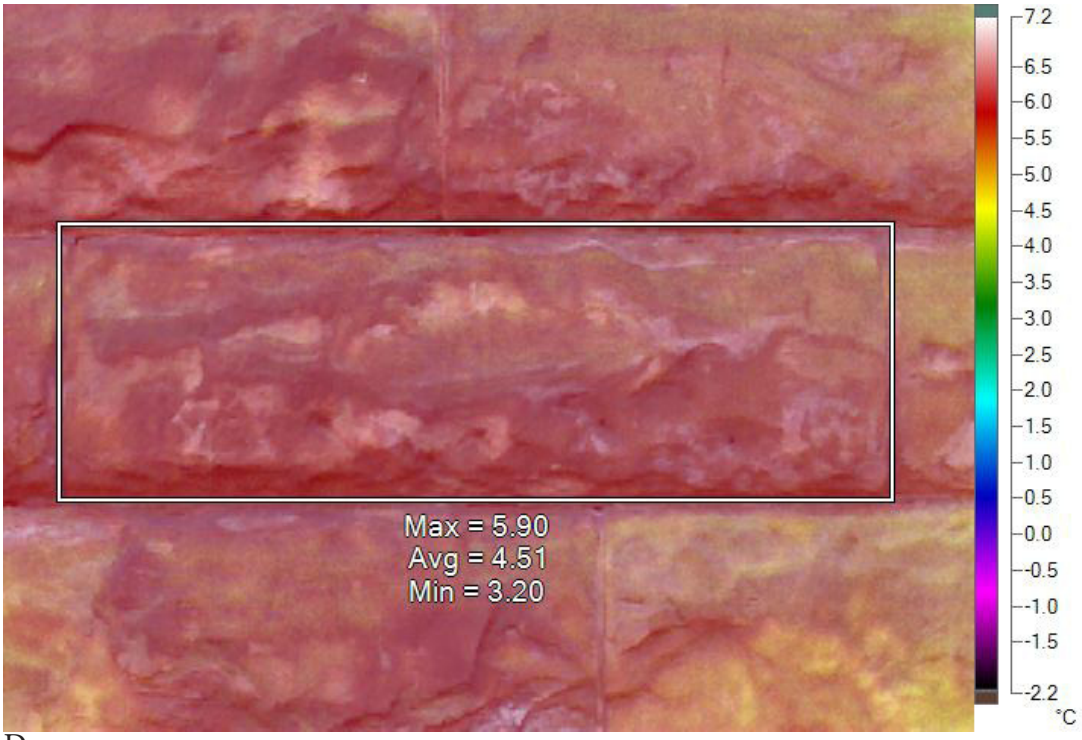


4 minutes

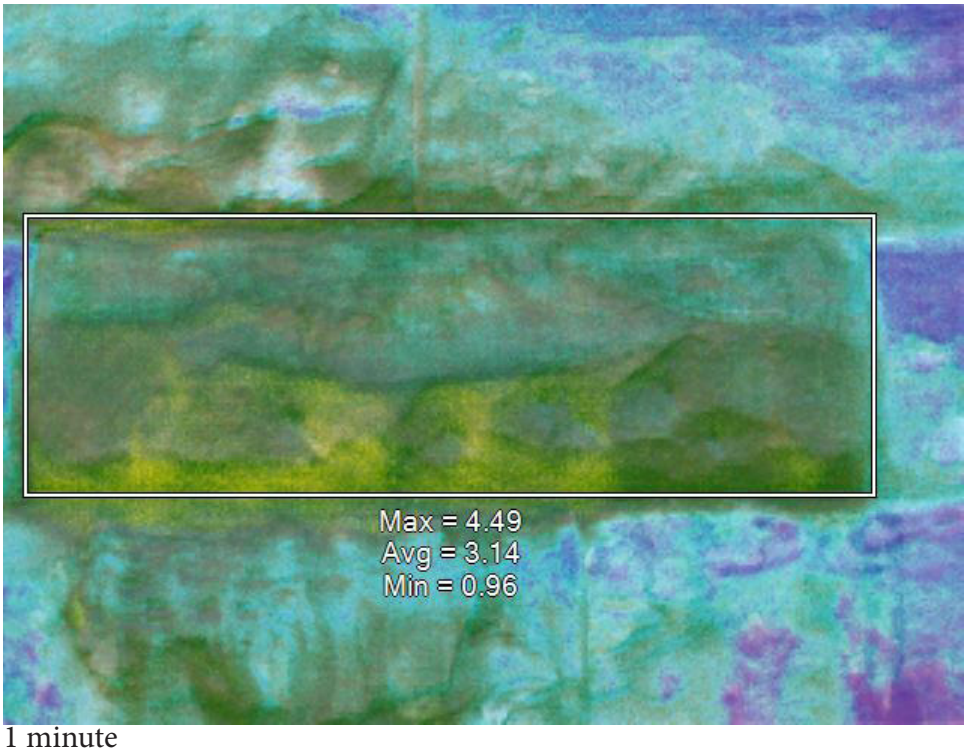


5 minutes

Black biocolonization control
January 10, 2013

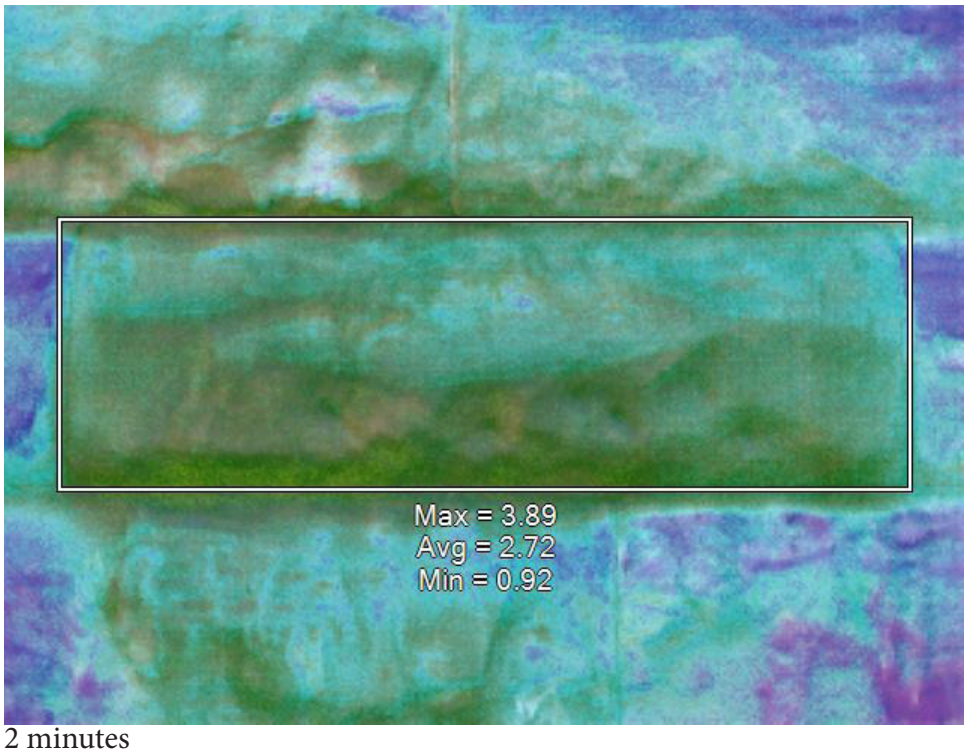


Dry

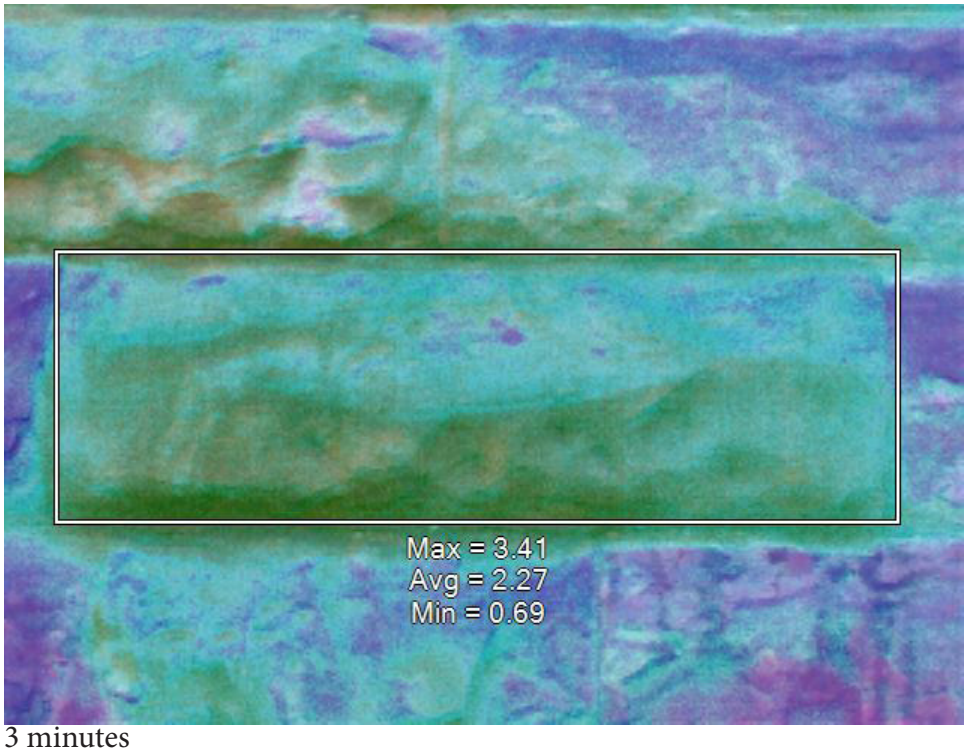


1 minute

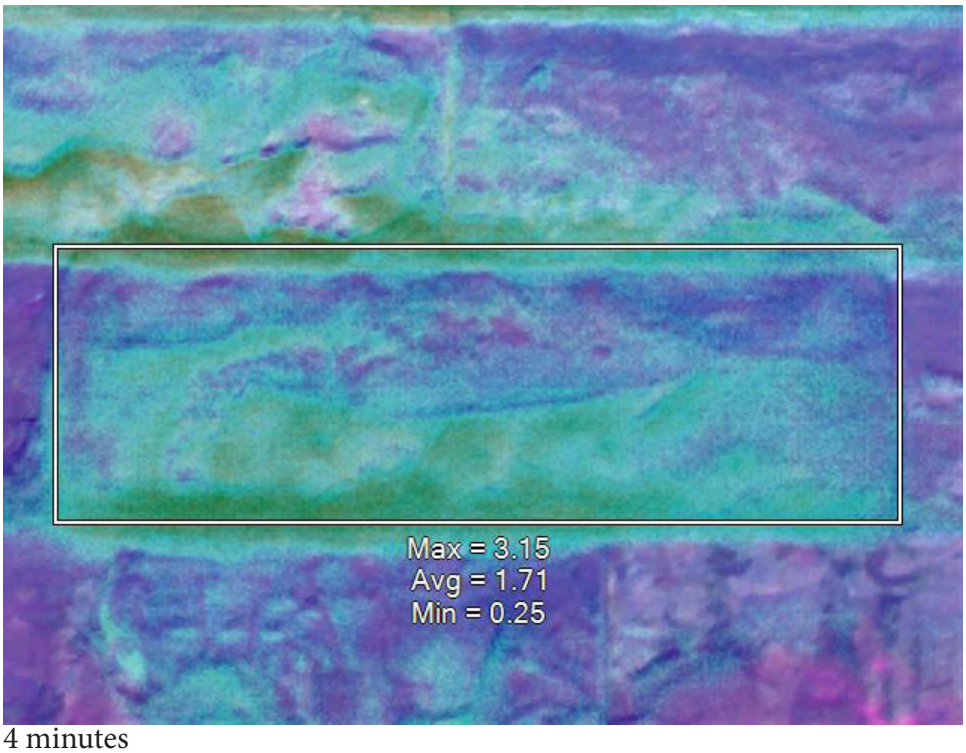
Black biocolonization control
January 10, 2013



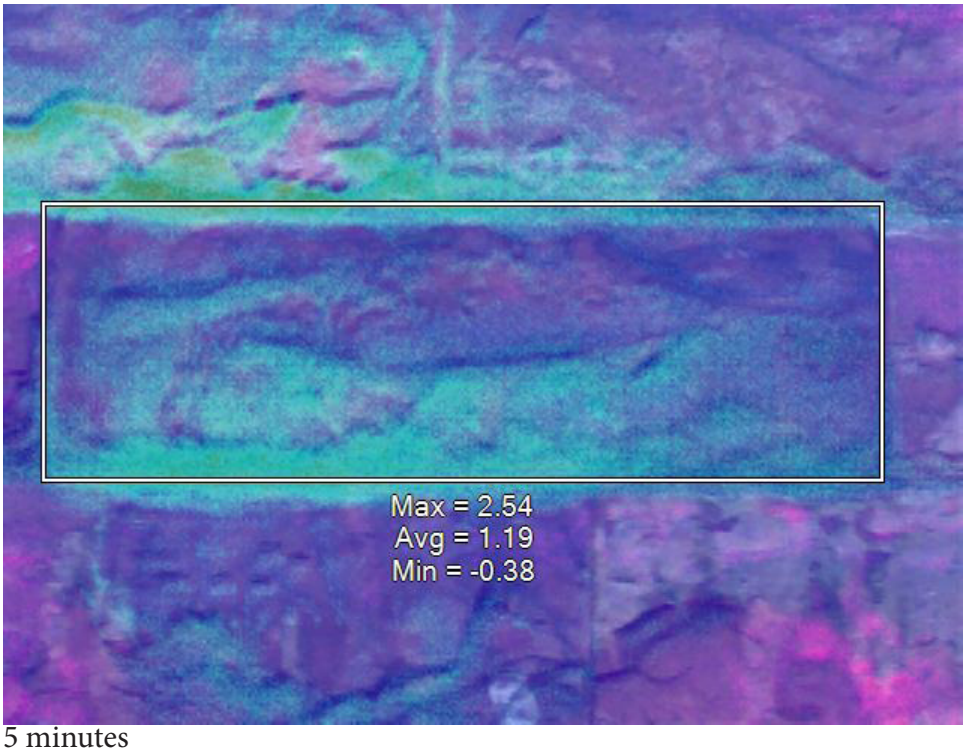
2 minutes



3 minutes

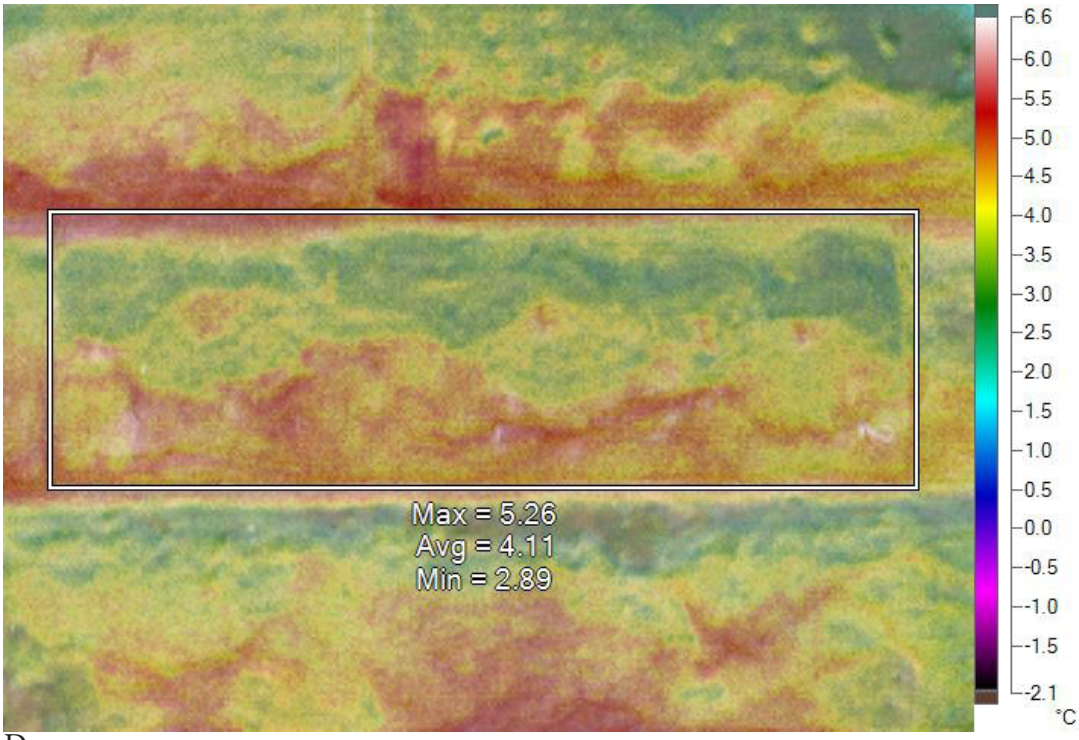


4 minutes

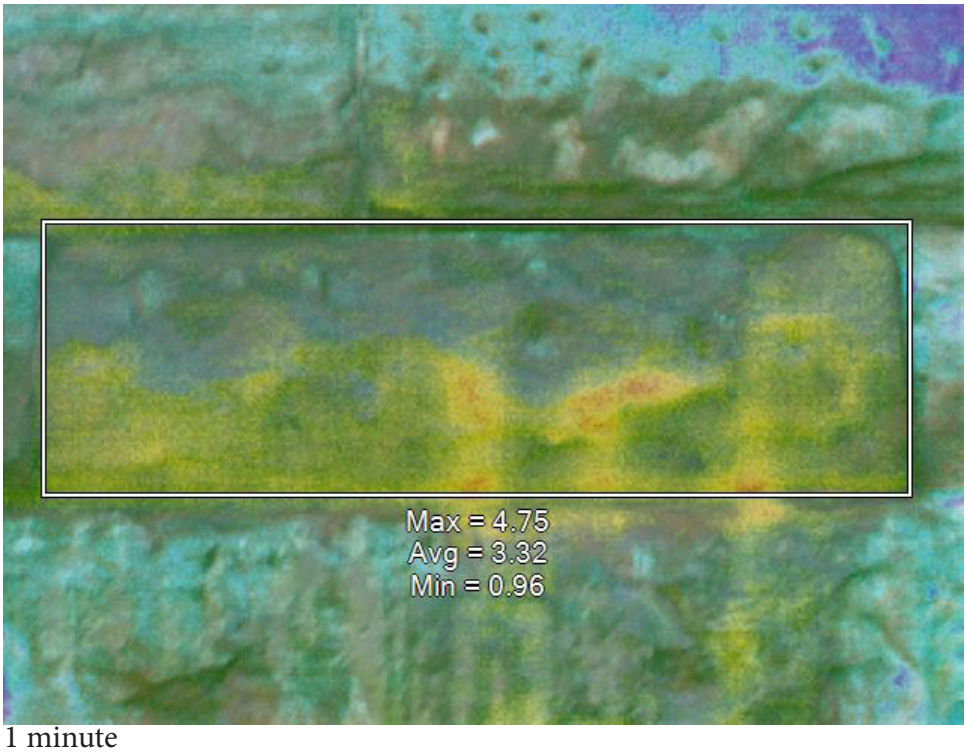


5 minutes

Black biocolonization treated with BioWash
January 10, 2013

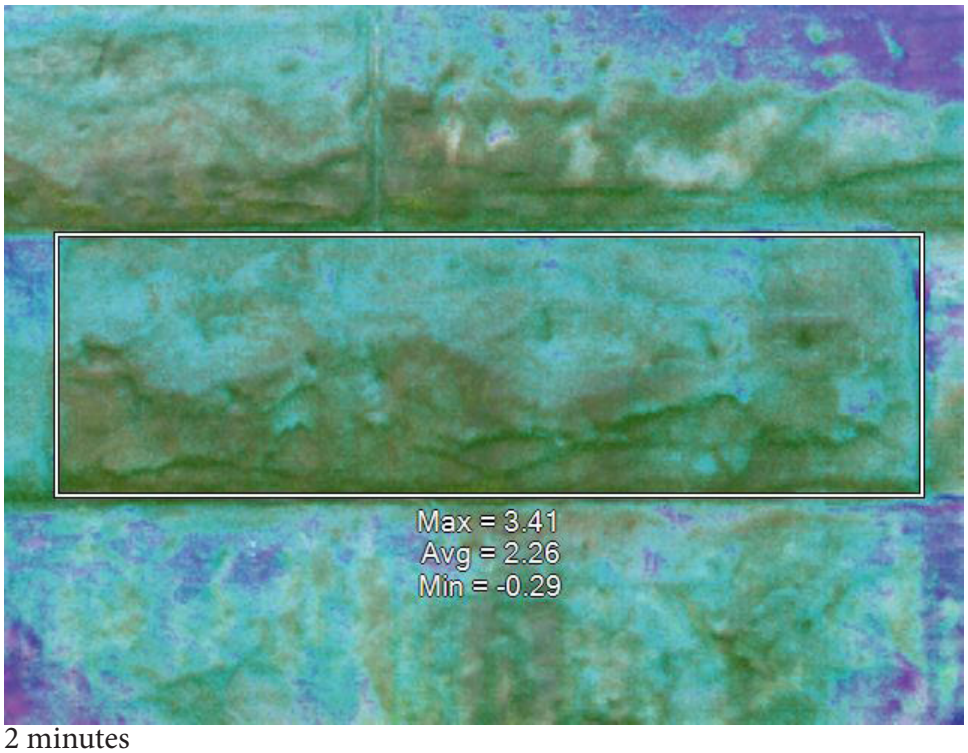


Dry

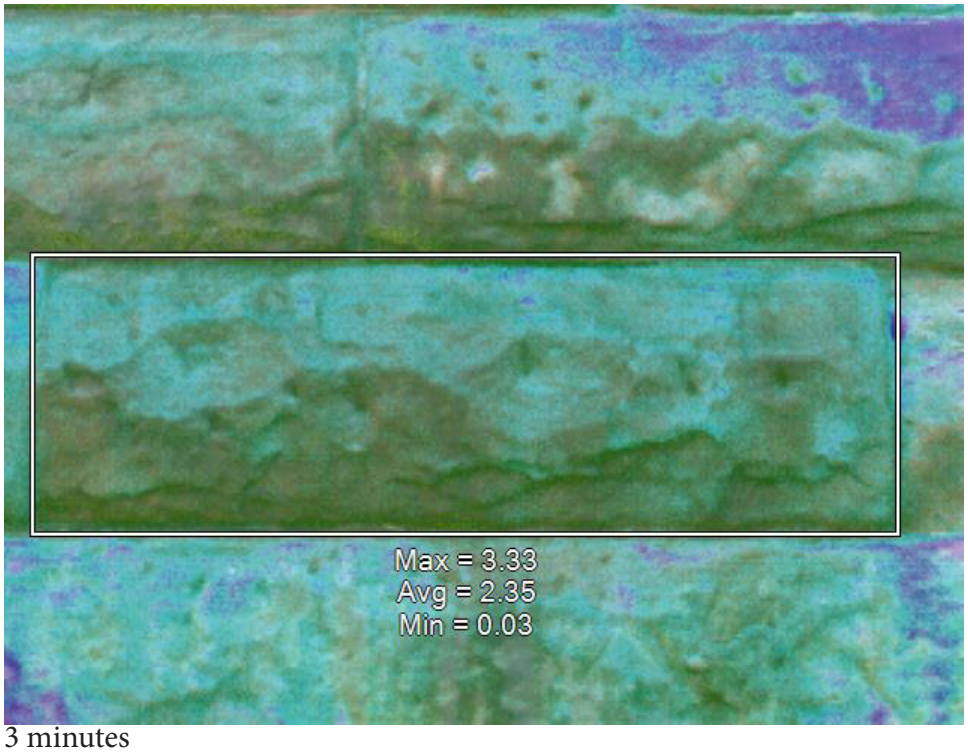


1 minute

Black biocolonization treated with BioWash
January 10, 2013

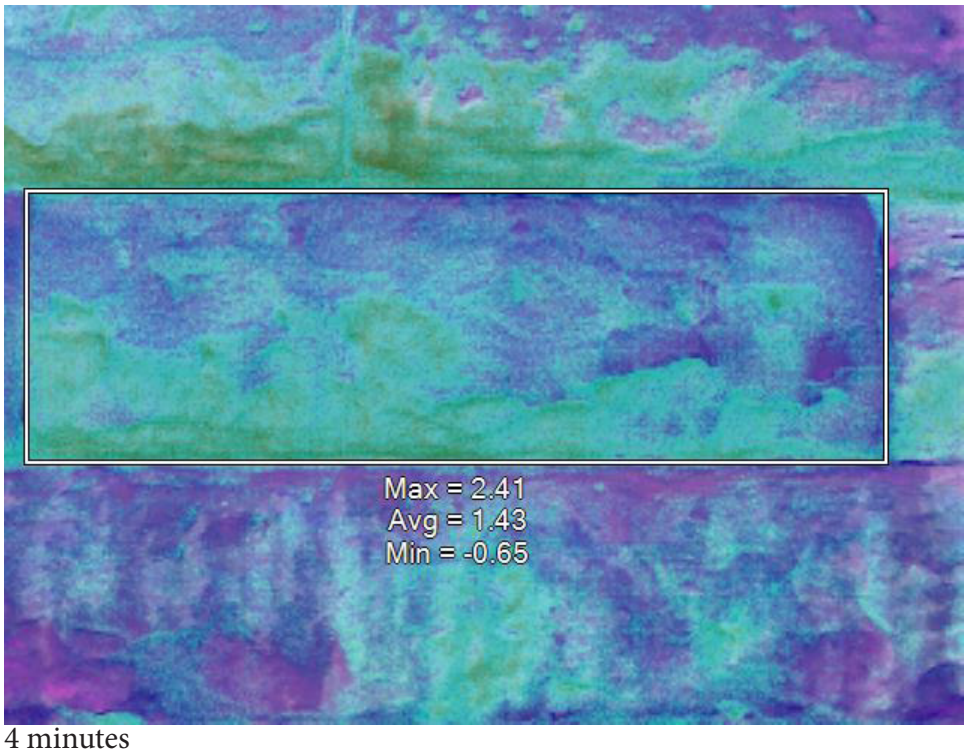


2 minutes

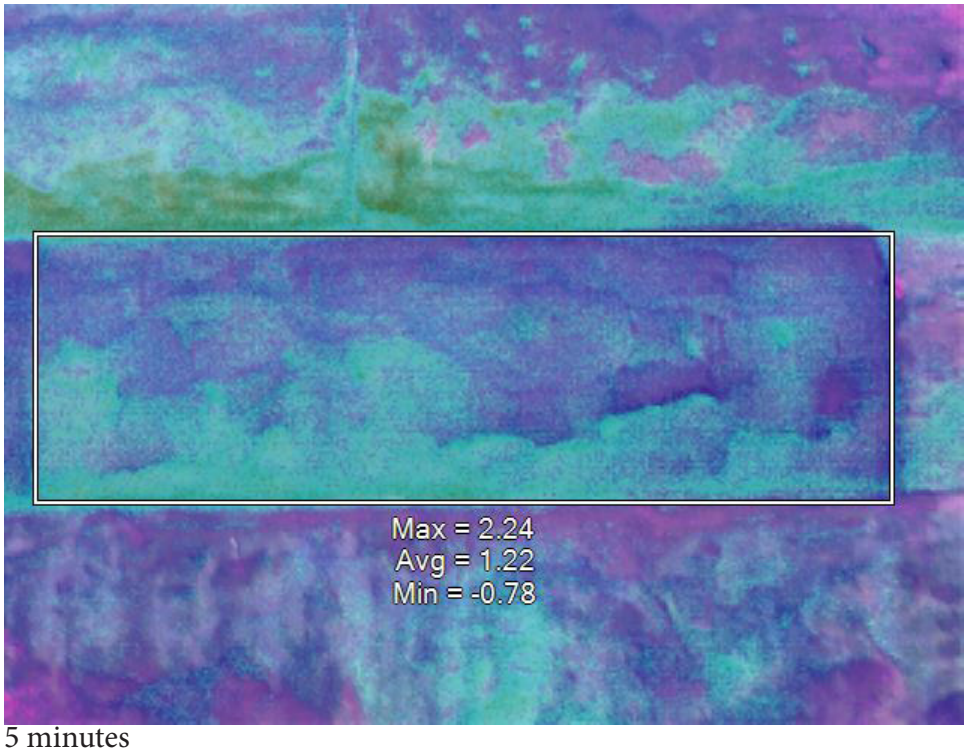


3 minutes

Black biocolonization treated with BioWash
January 10, 2013

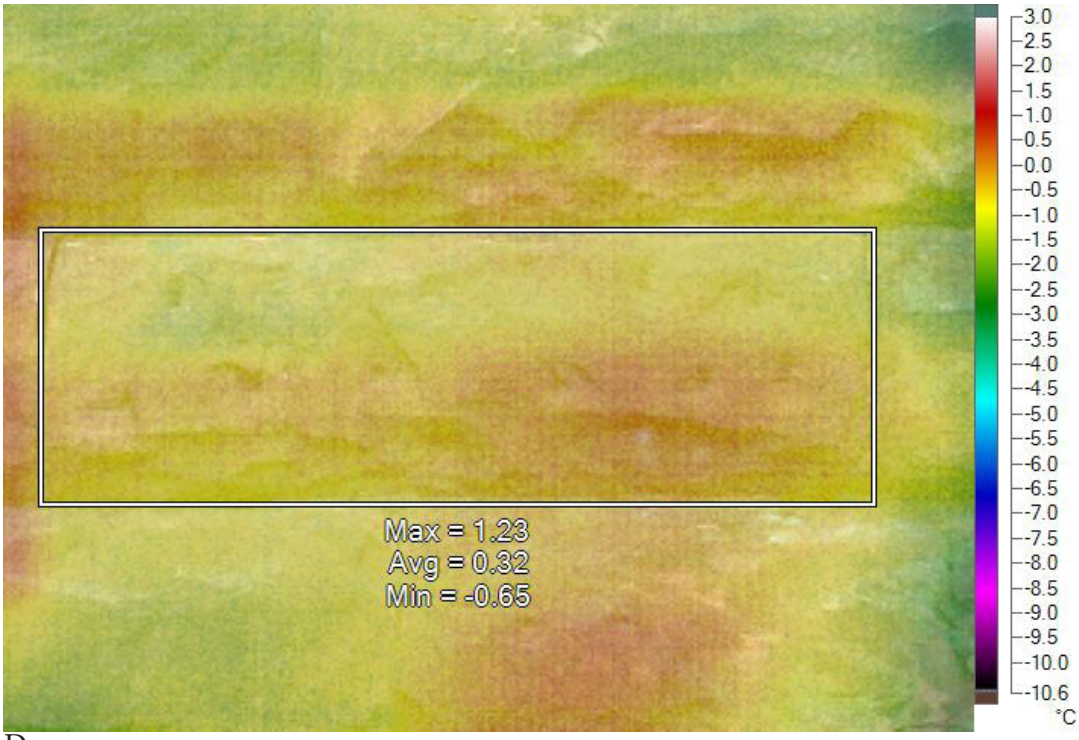


4 minutes



5 minutes

Black biocolonization treated with D/2
February 7, 2013



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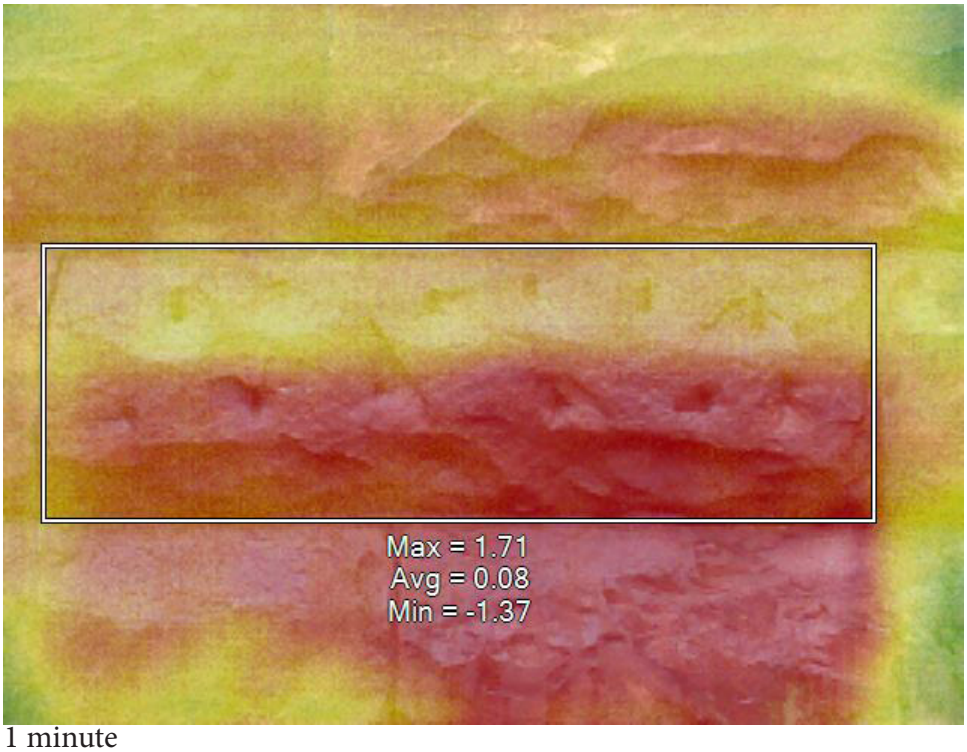
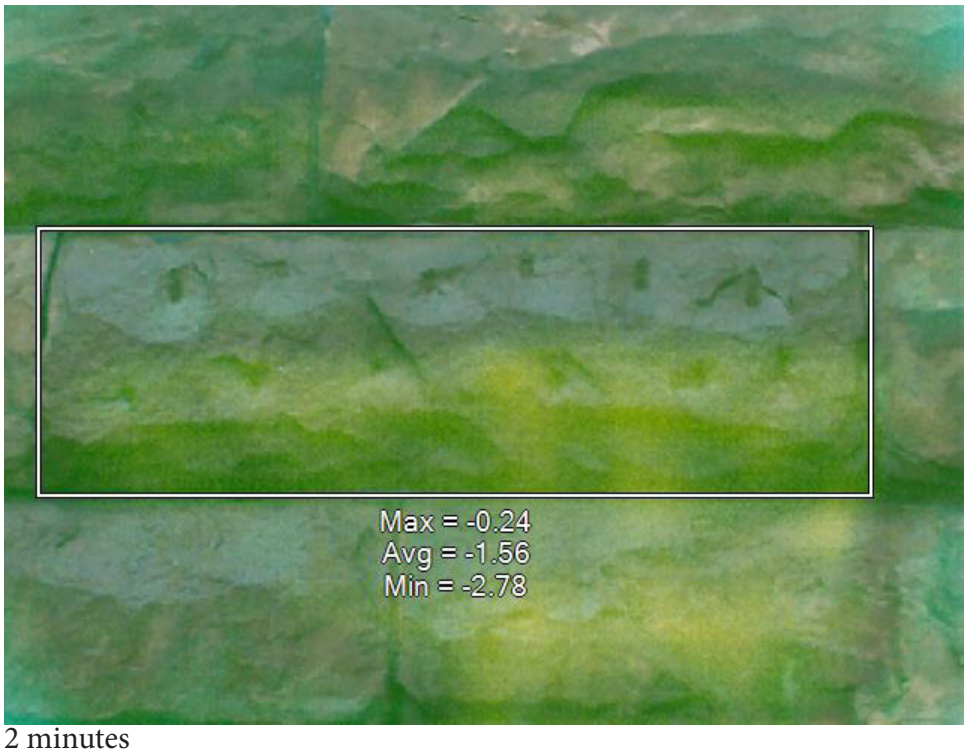
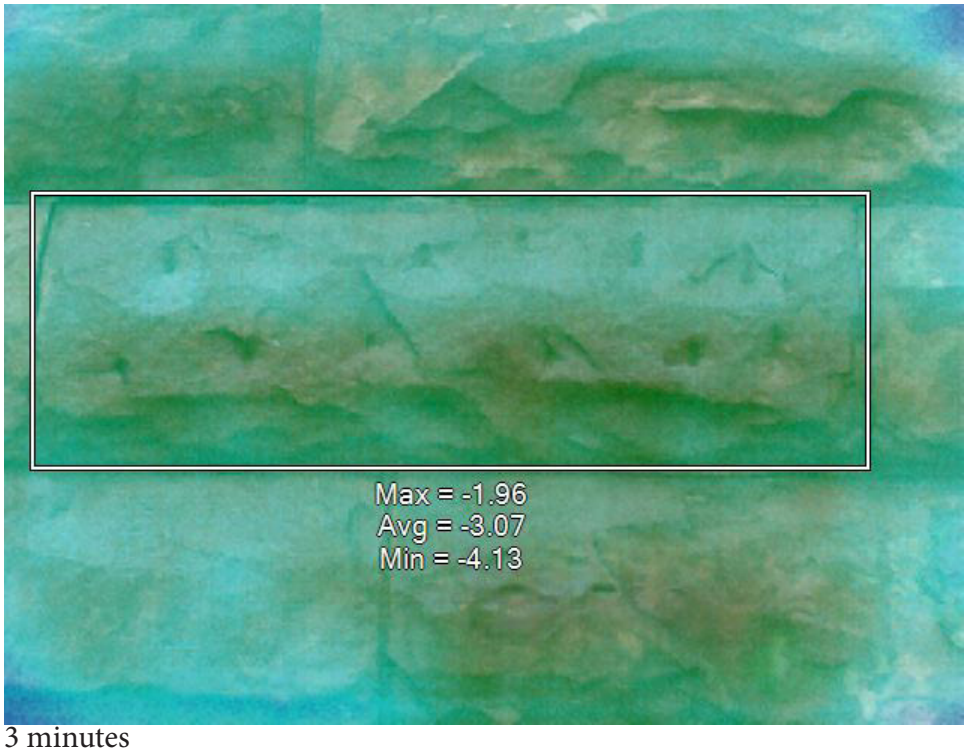


1 minute

Black biocolonization treated with D/2
February 7, 2013

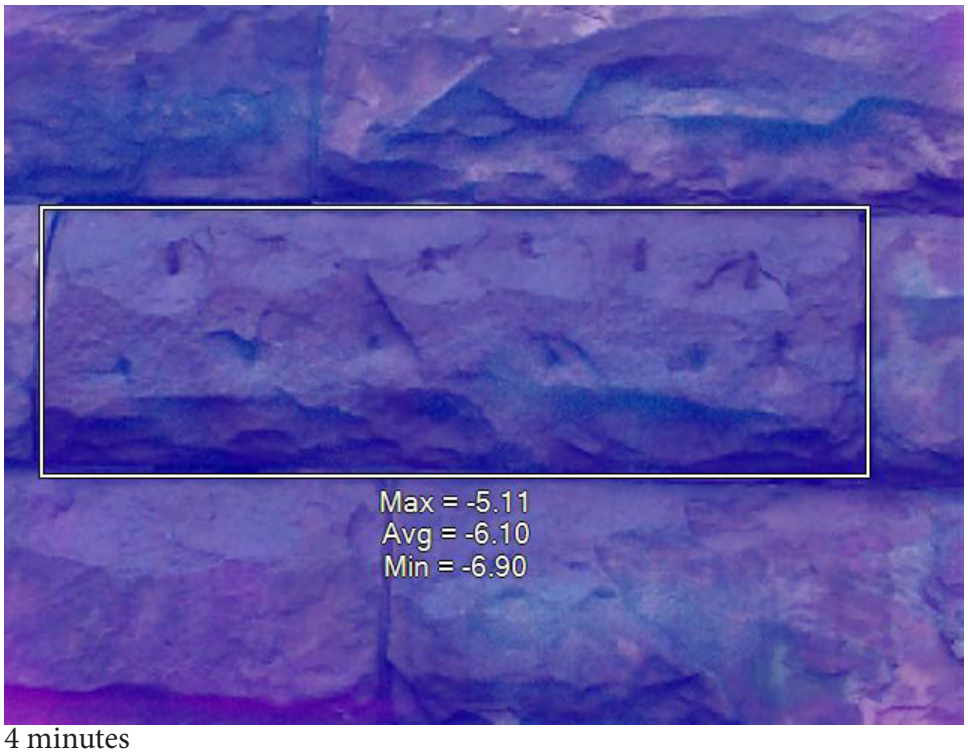


2 minutes



3 minutes

Black biocolonization treated with D/2
February 7, 2013



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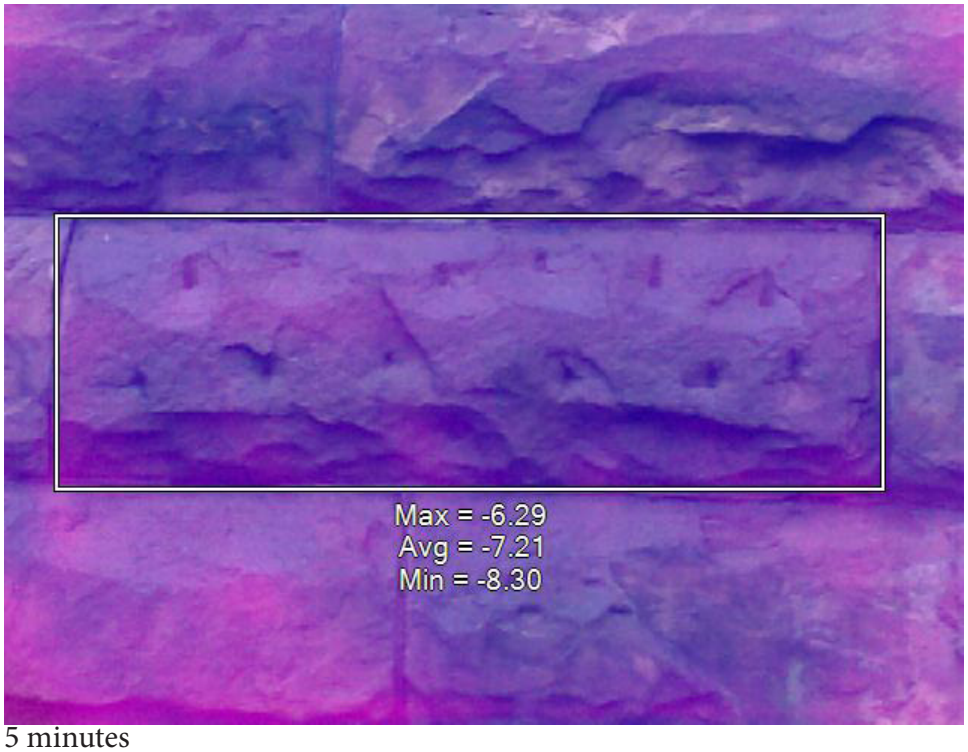
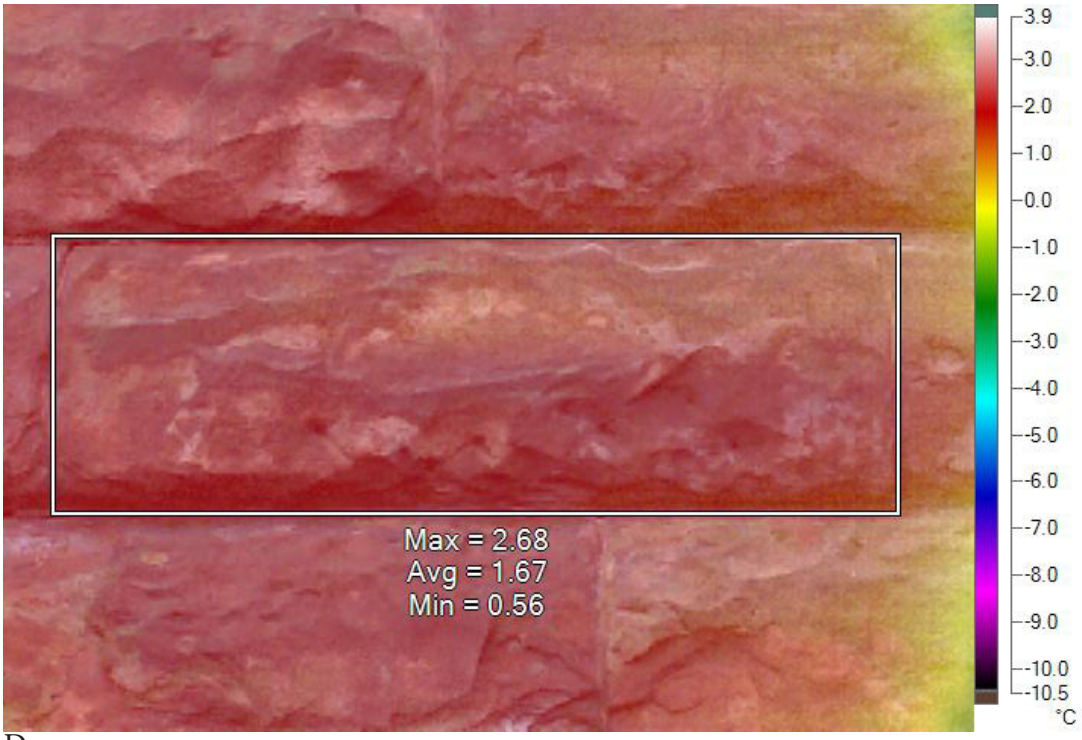


5 minutes

Black biocolonization control
February 7, 2013



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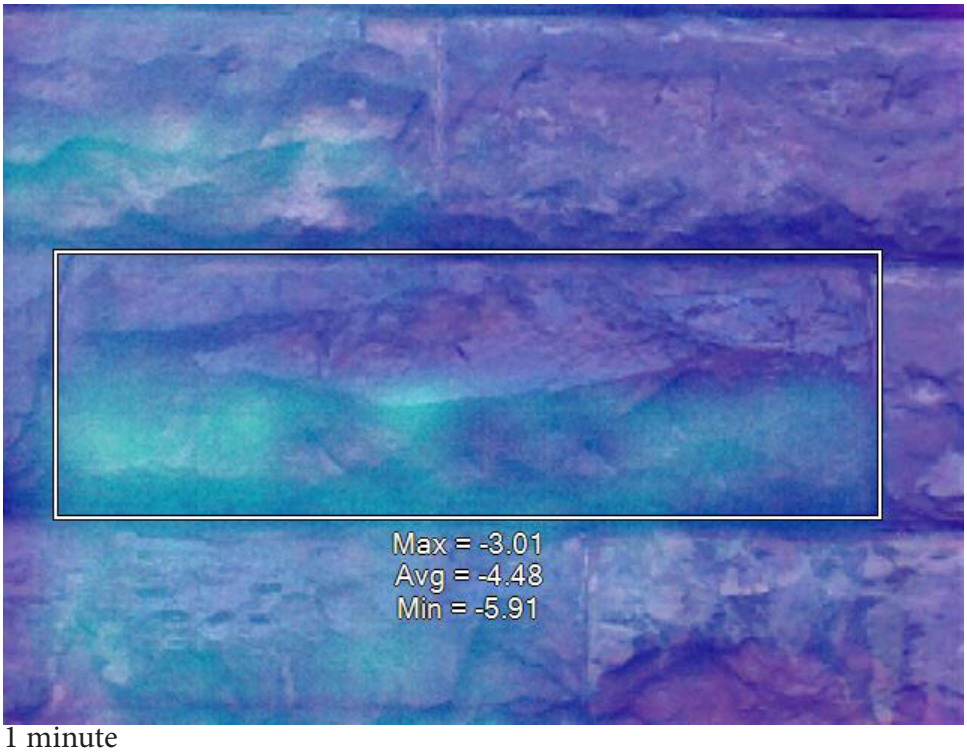
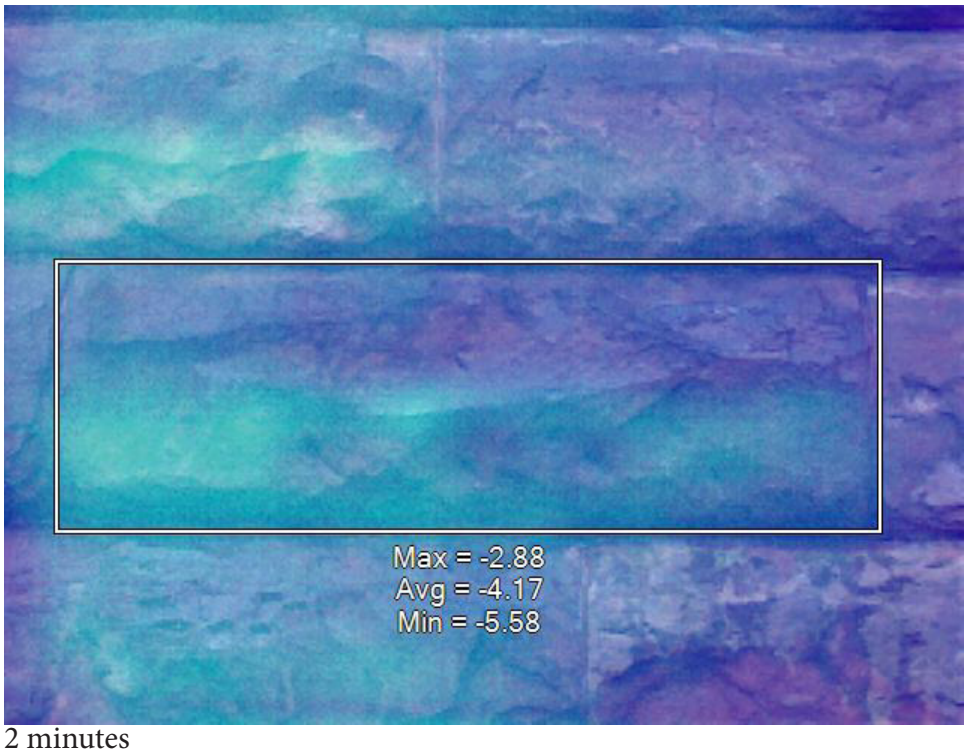
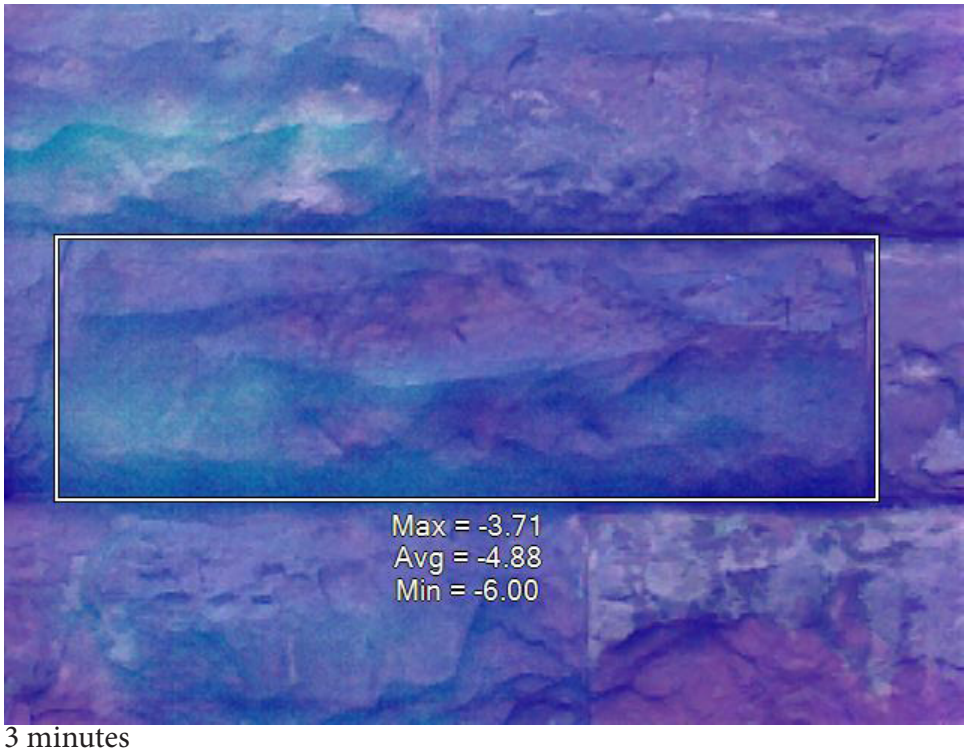


1 minute

Black biocolonization control
February 7, 2013

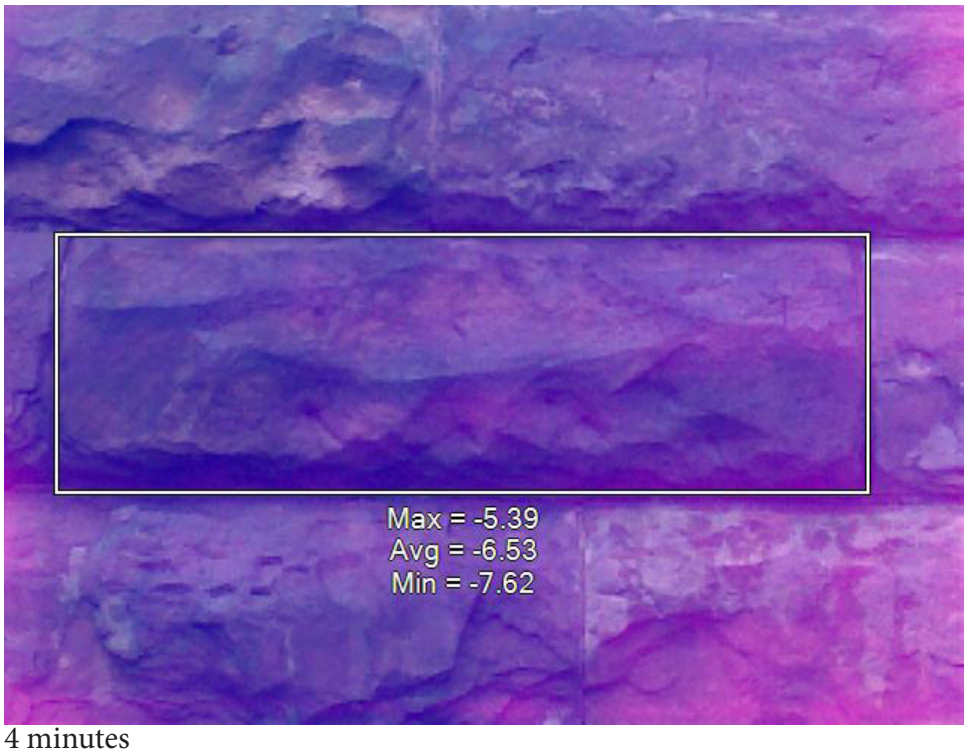


2 minutes

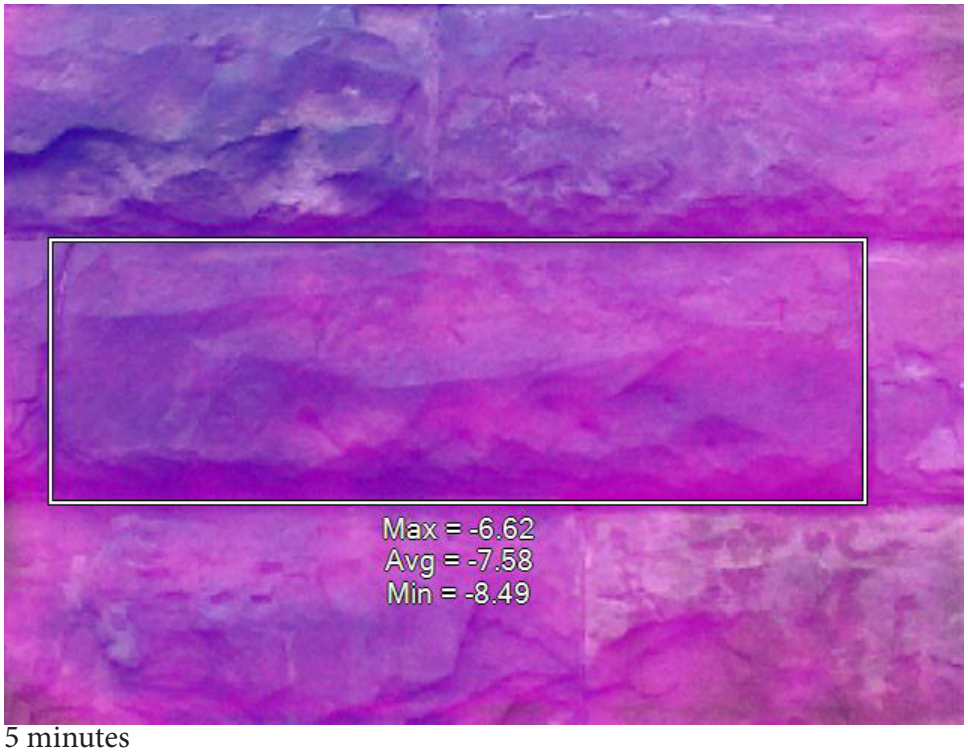


3 minutes

Black biocolonization control
February 7, 2013

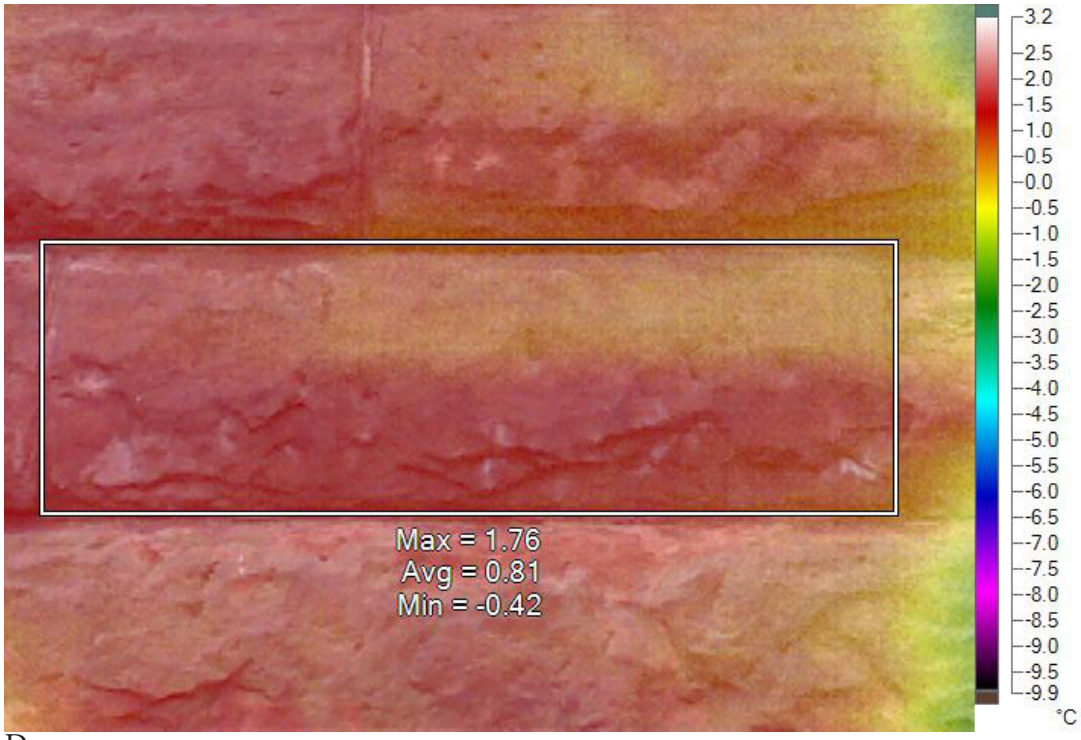


4 minutes



5 minutes

Black biocolonization treated with BioWash
February 7, 2013



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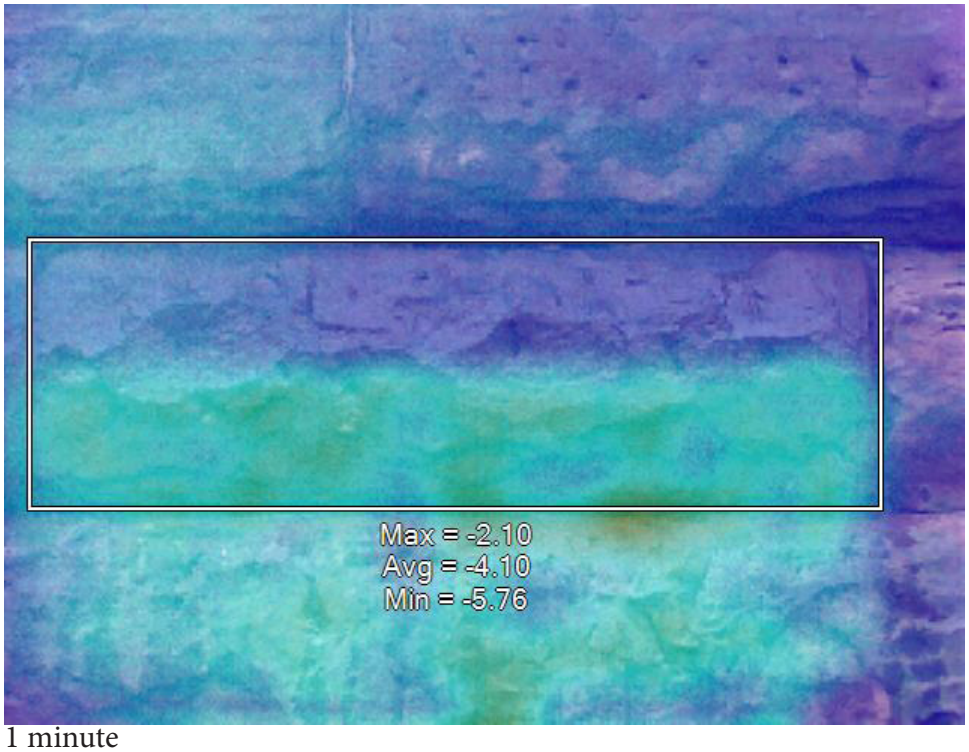
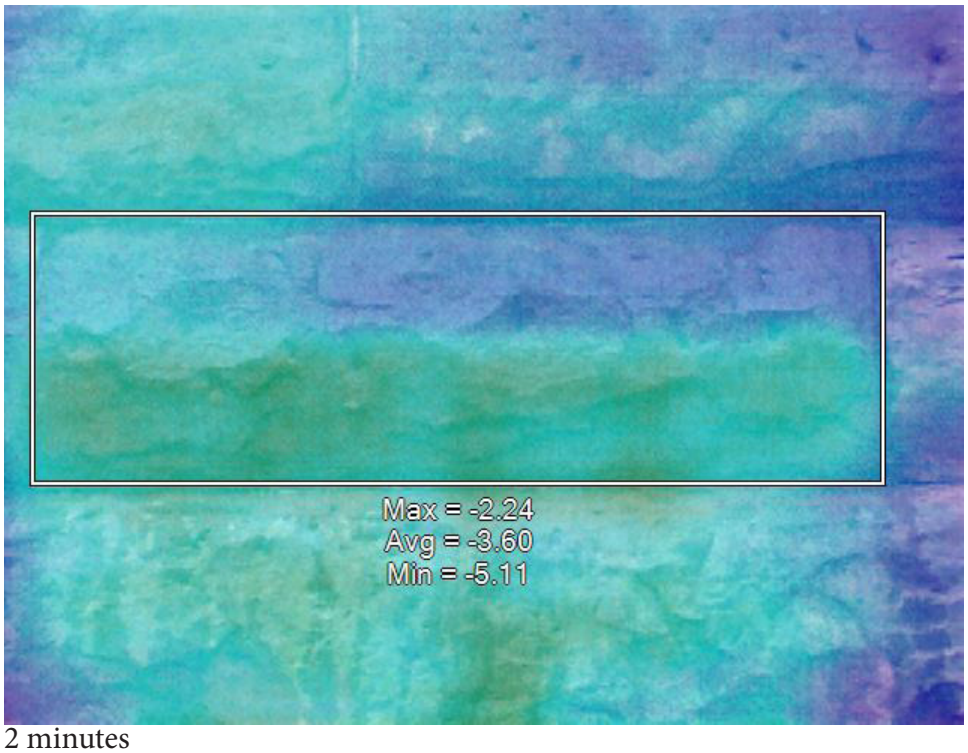
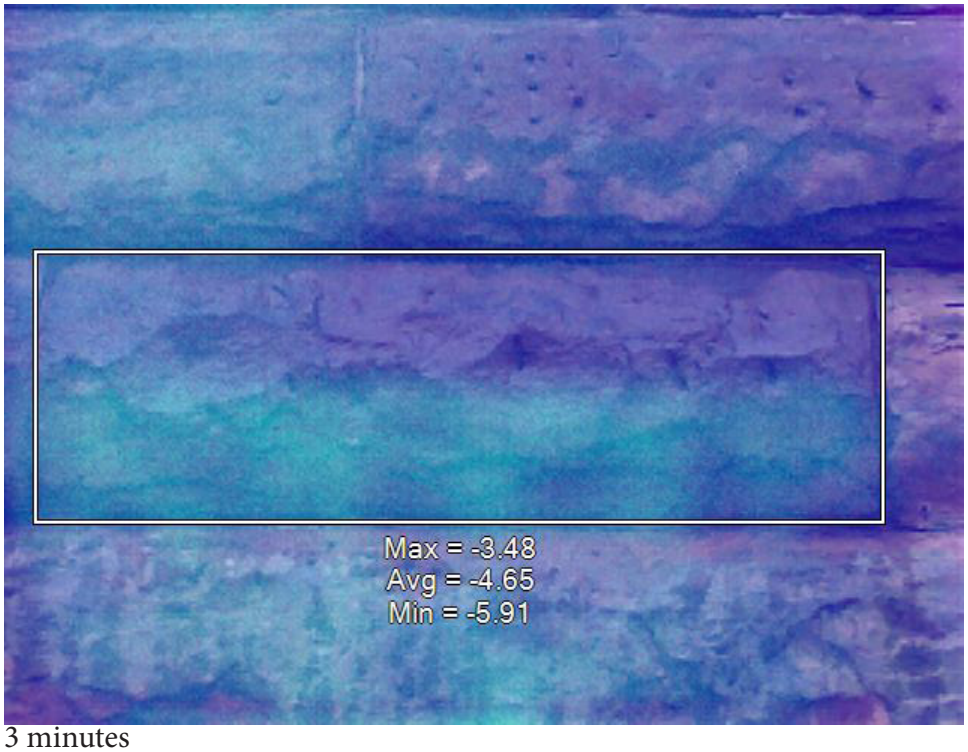


1 minute

Black biocolonization treated with BioWash
February 7, 2013

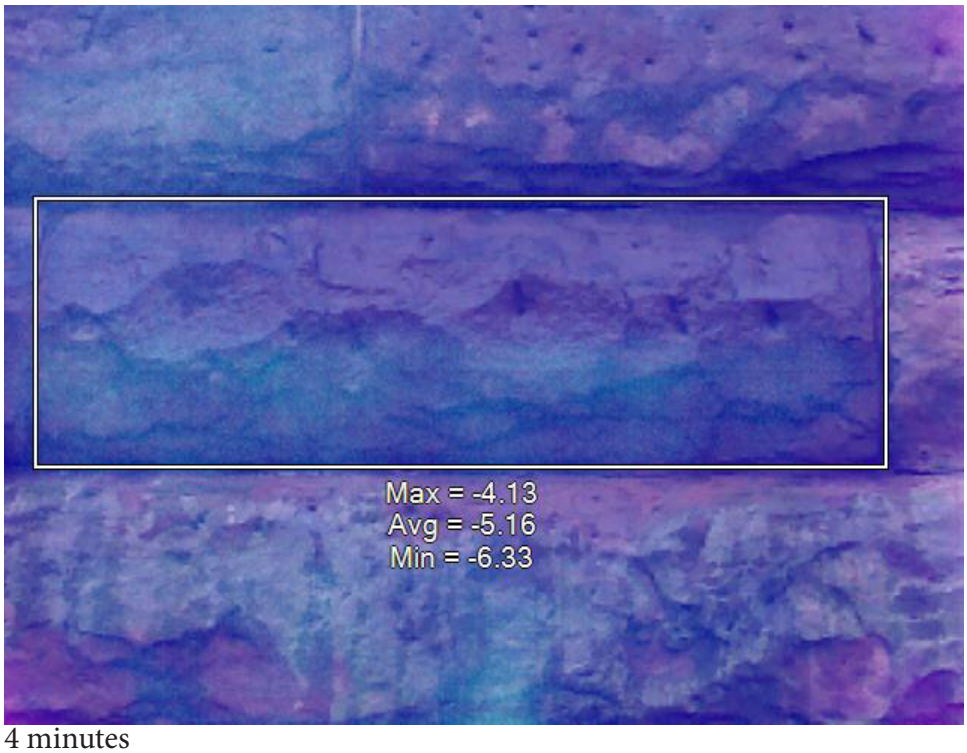


2 minutes

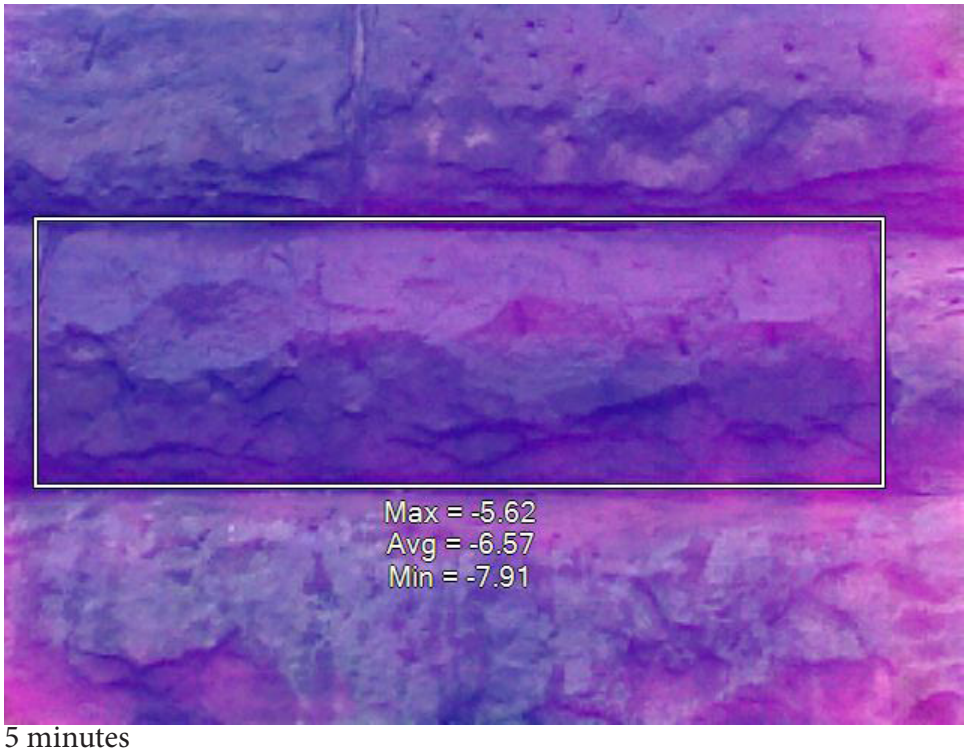


3 minutes

Black biocolonization treated with BioWash
February 7, 2013

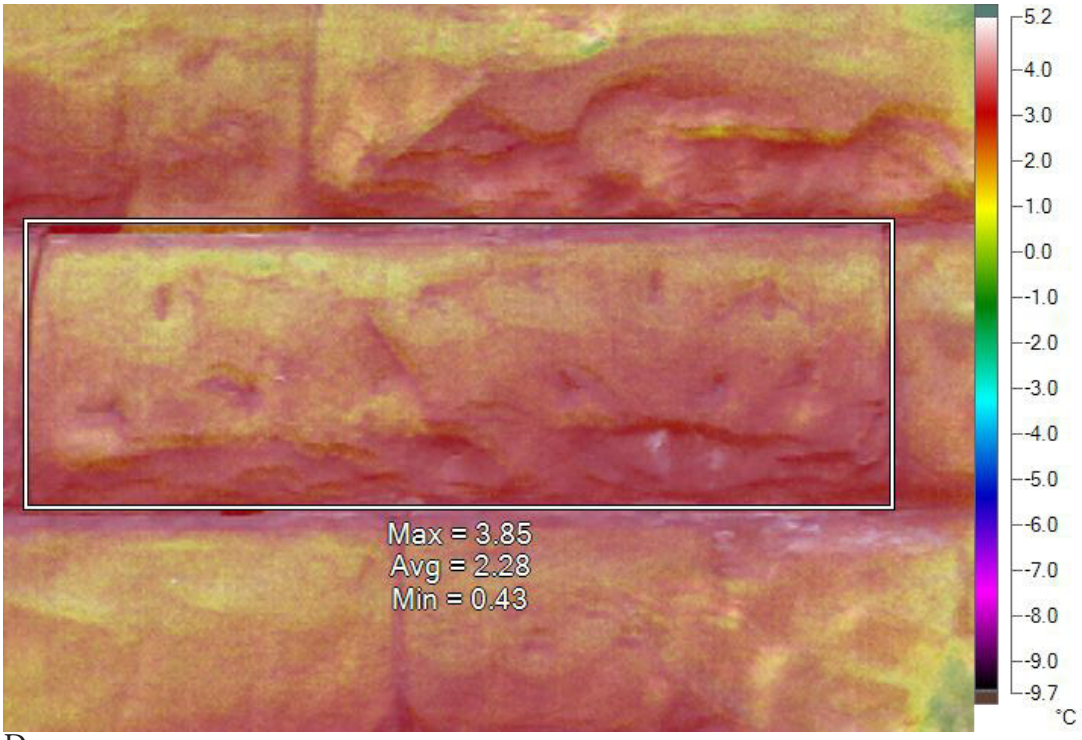


4 minutes



5 minutes

Black biocolonization treated with D/2
March 5, 2013



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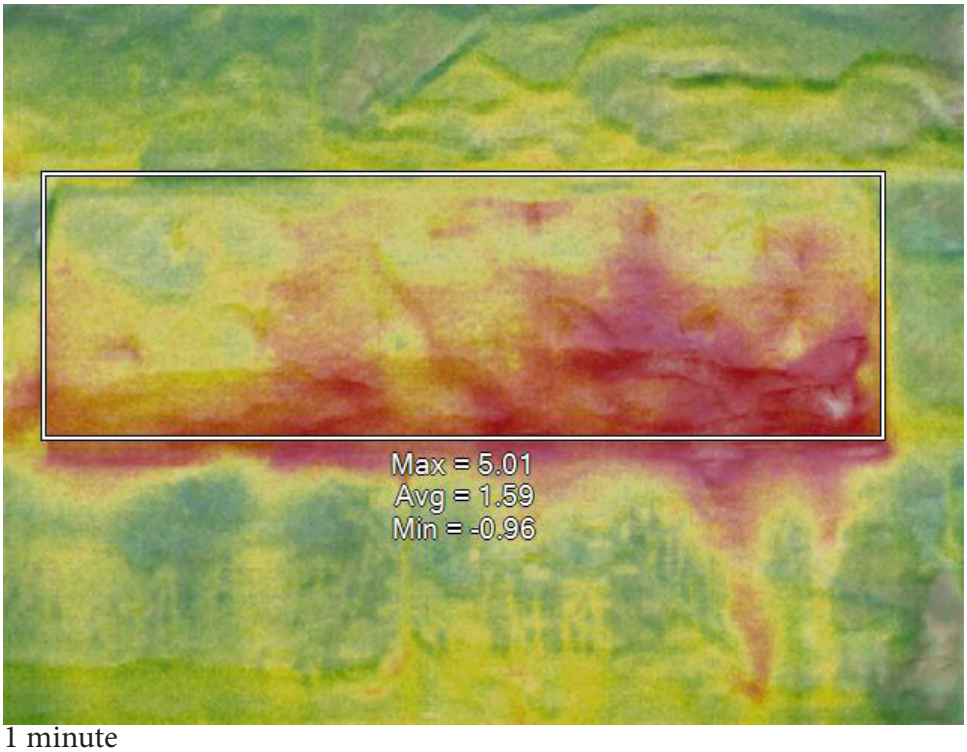
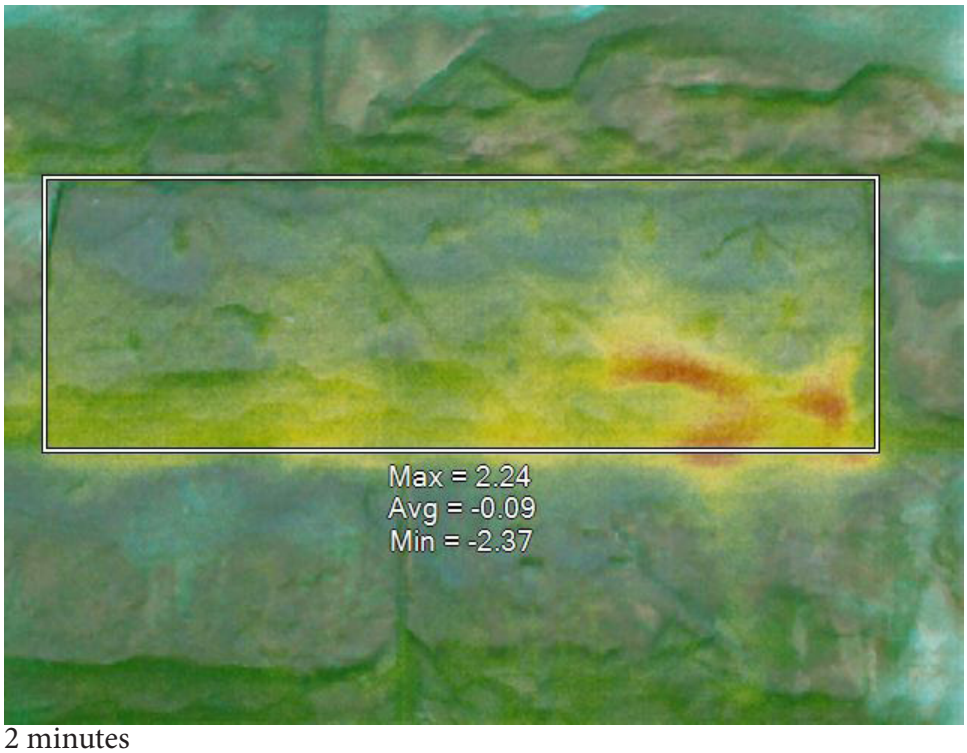
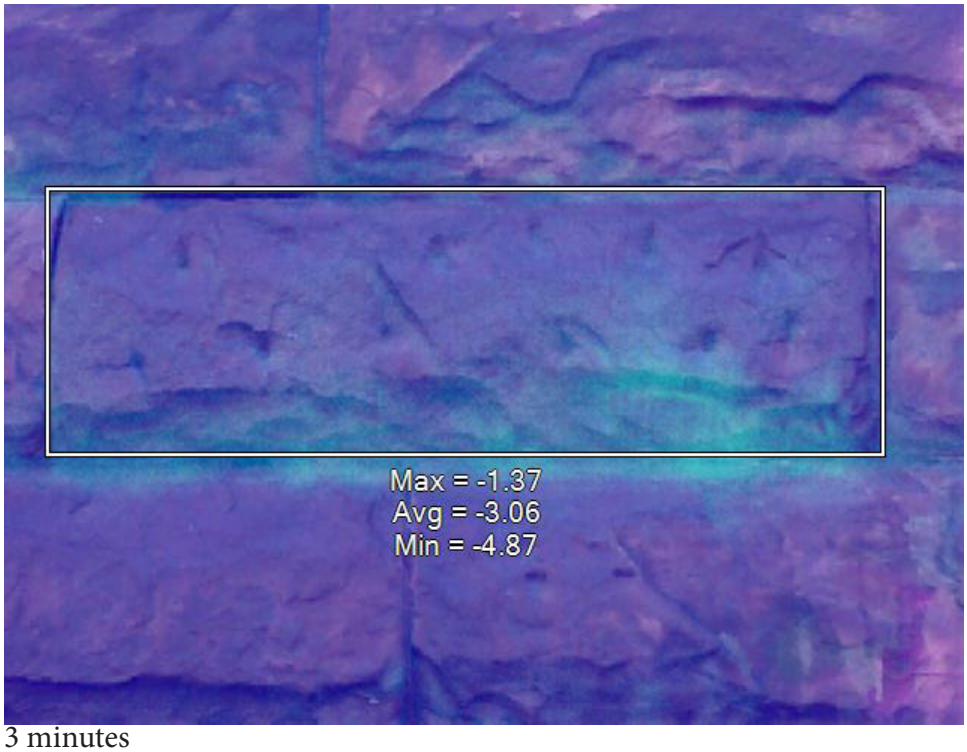


1 minute

Black biocolonization treated with D/2
March 5, 2013

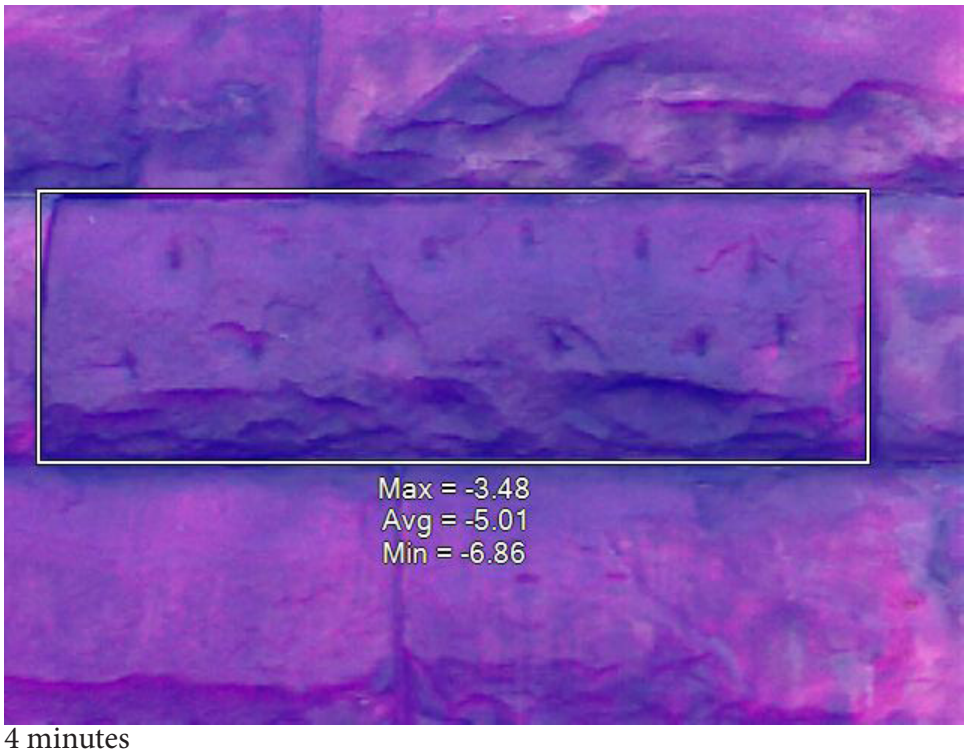


2 minutes

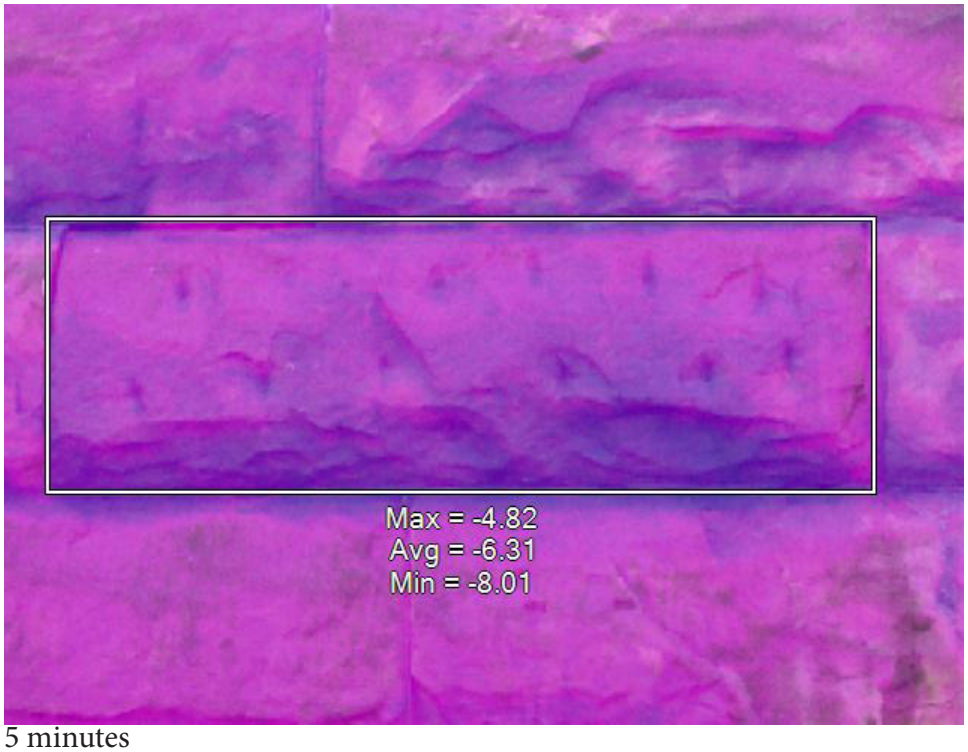


3 minutes

Black biocolonization treated with D/2
March 5, 2013

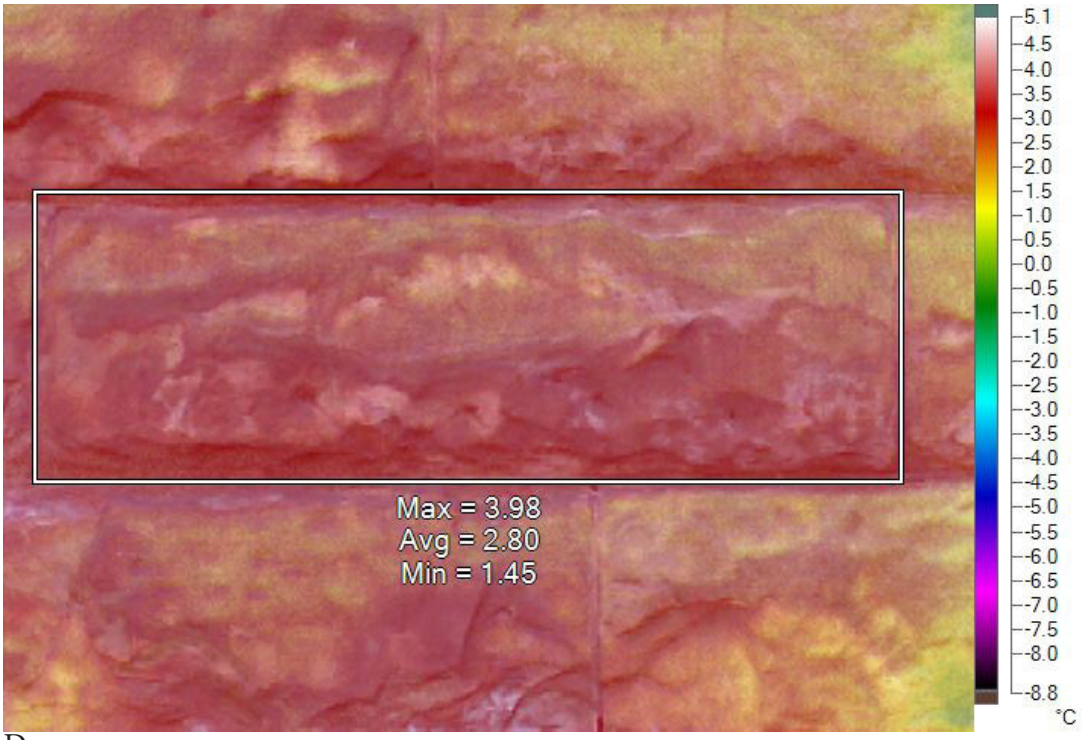


4 minutes

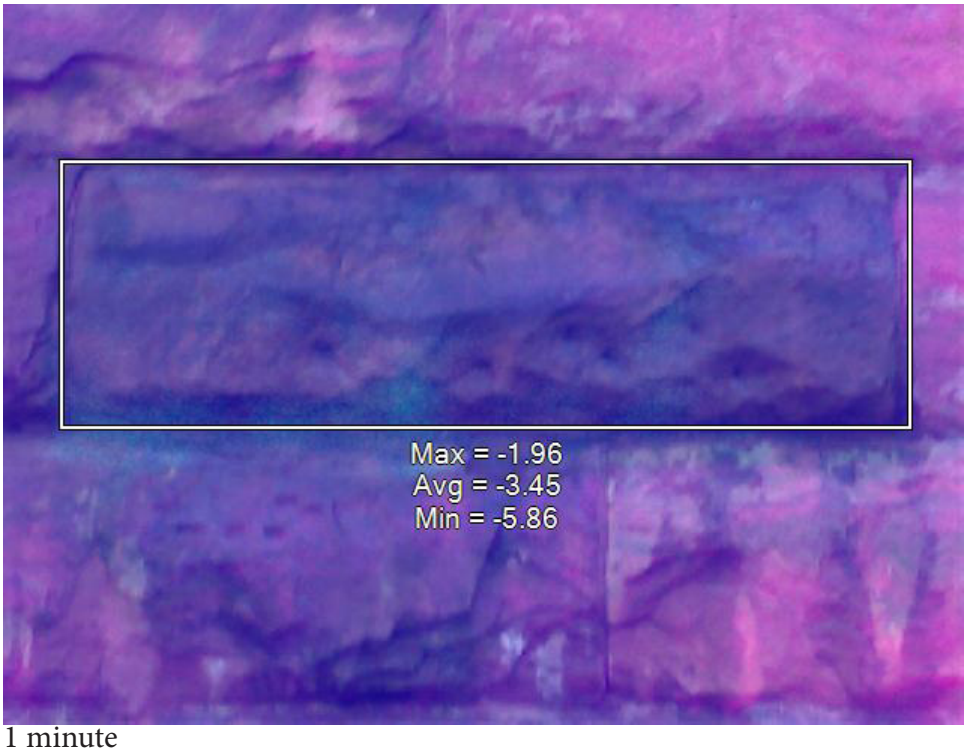


5 minutes

Black biocolonization control
March 5, 2013

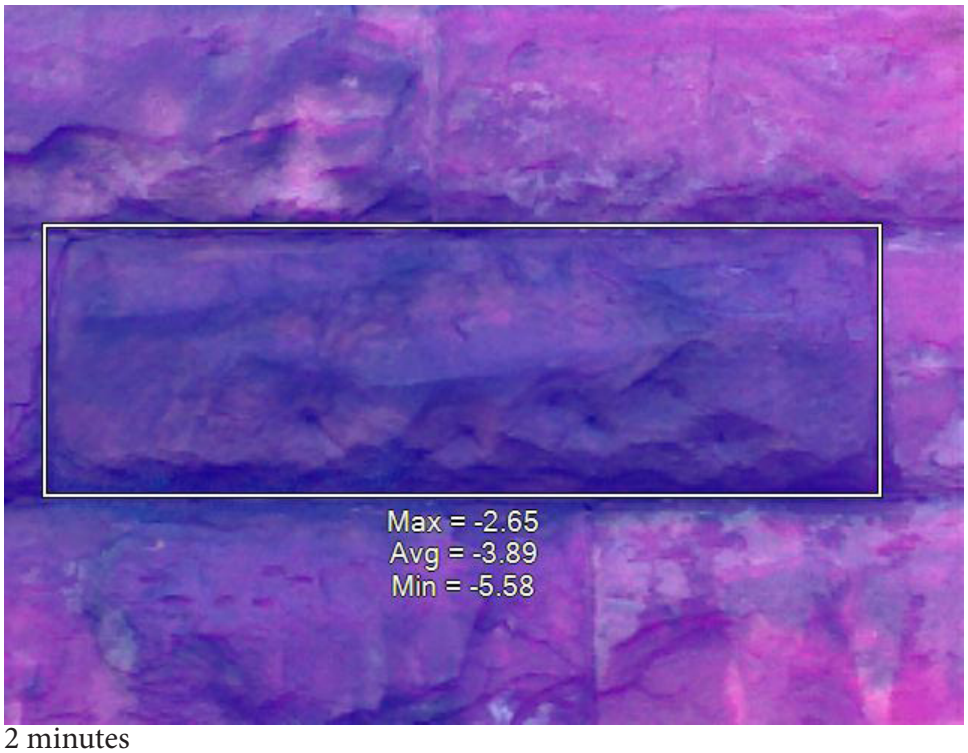


Dry

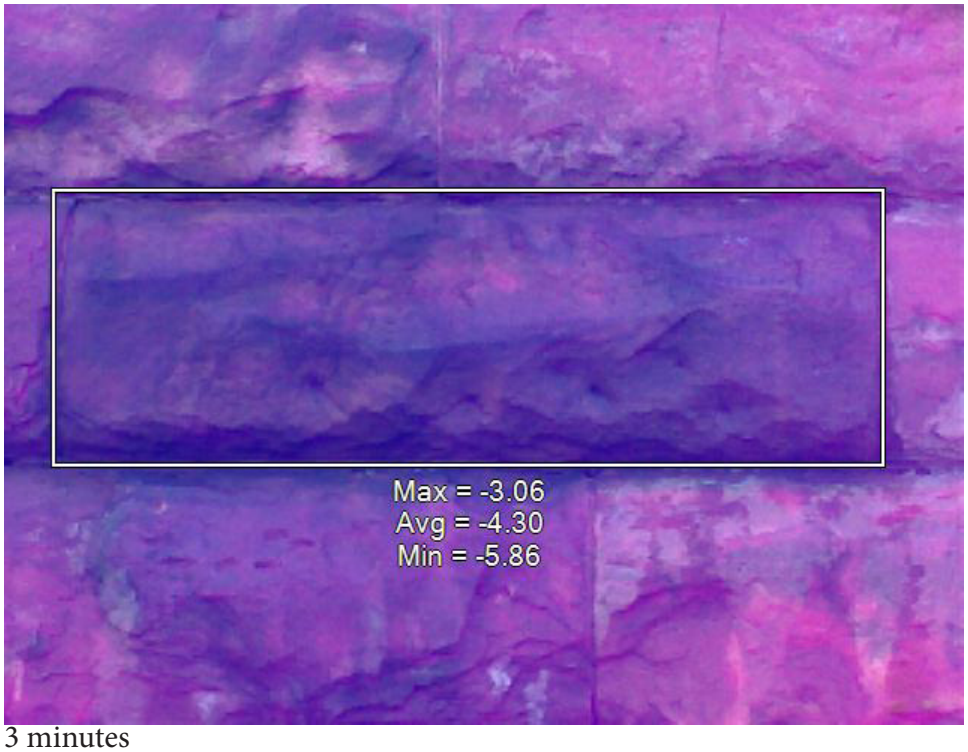


1 minute

Black biocolonization control
March 5, 2013

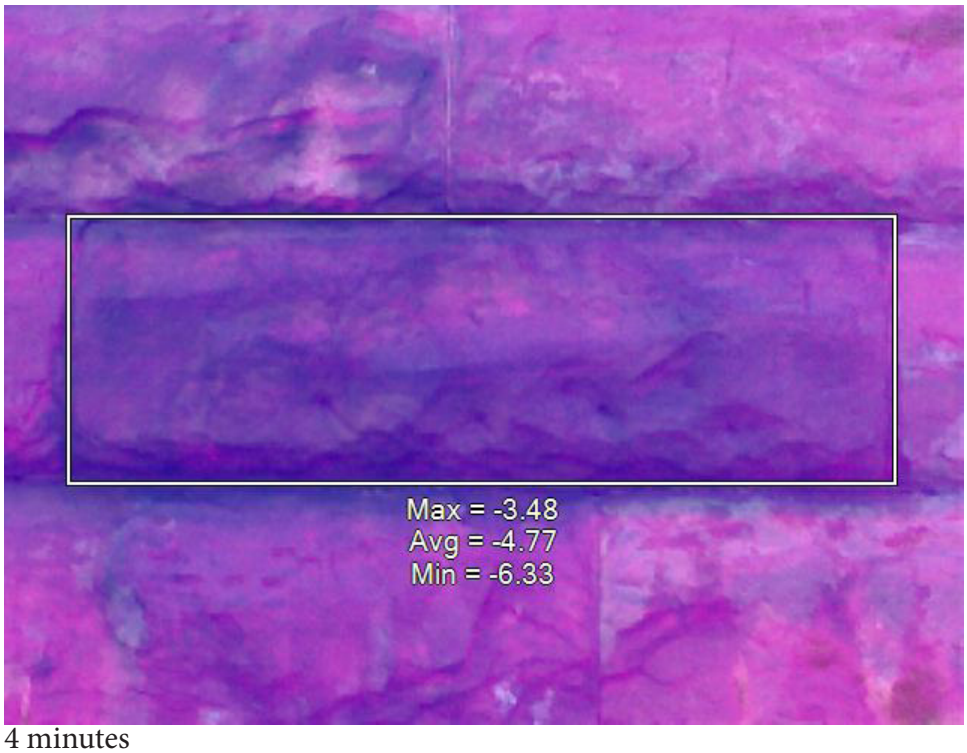


2 minutes

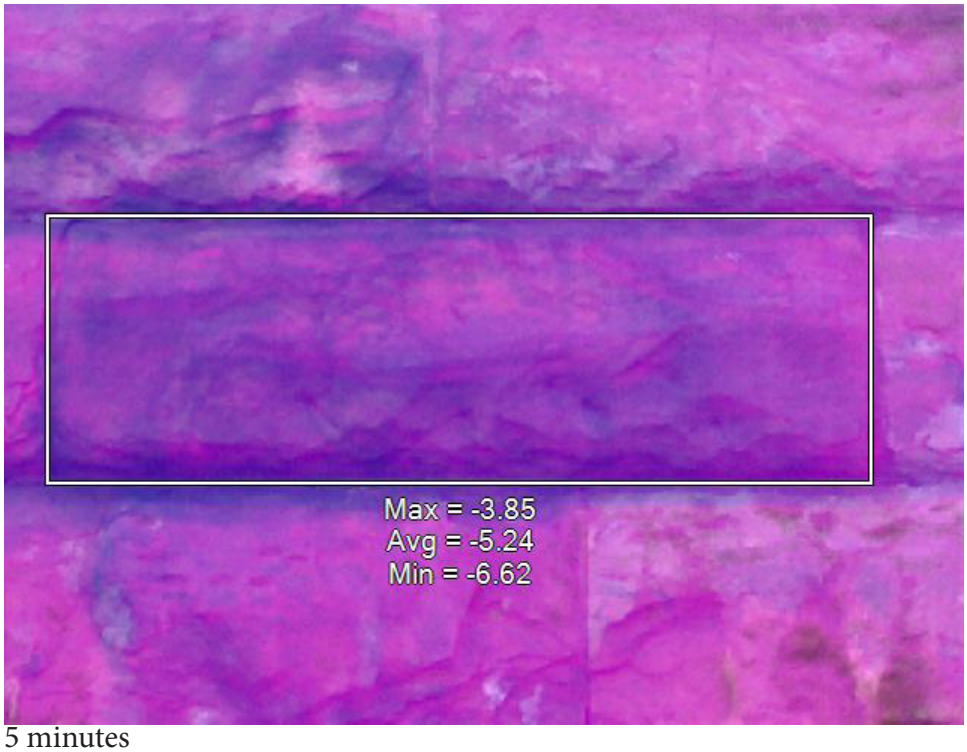


3 minutes

Black biocolonization control
March 5, 2013

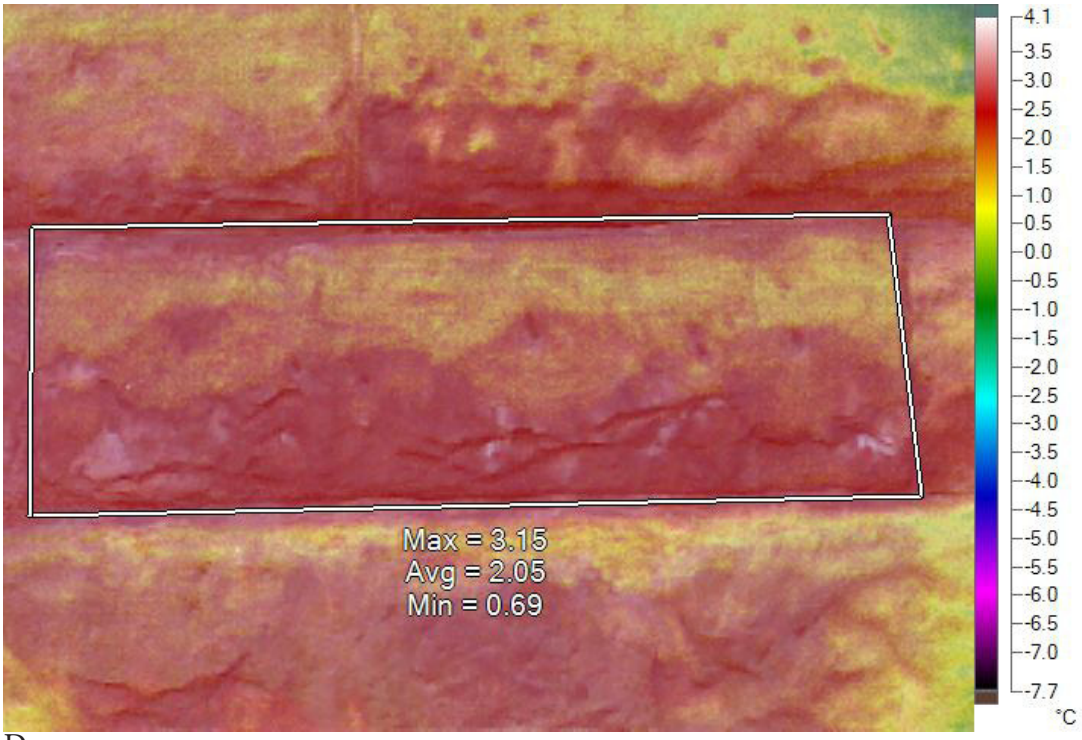


4 minutes

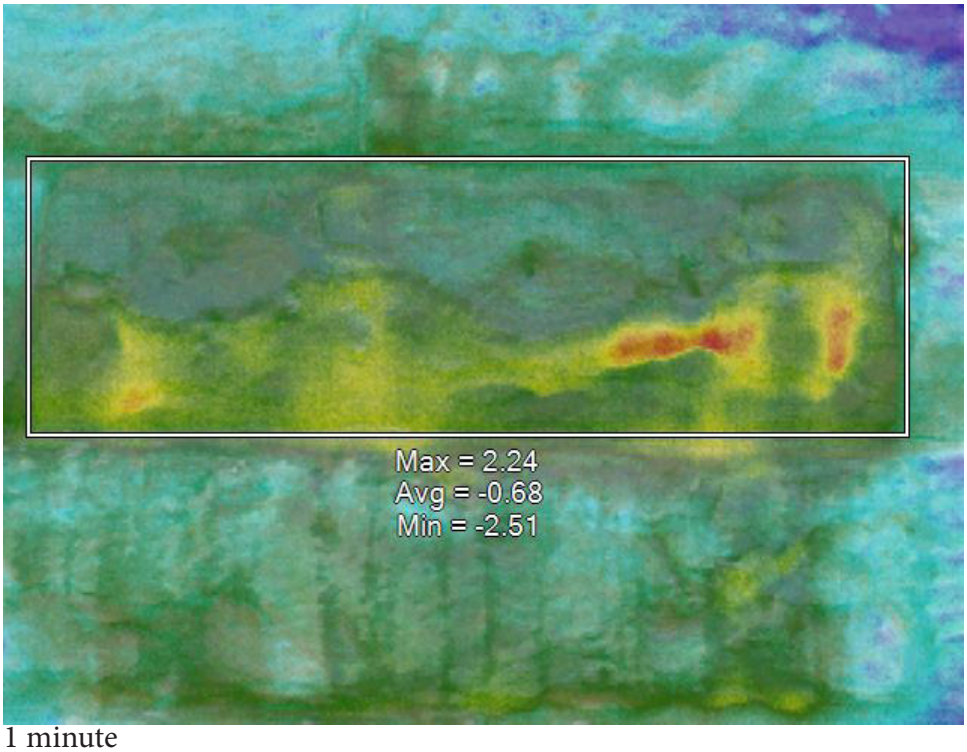


5 minutes

Black biocolonization treated with BioWash
March 5, 2013

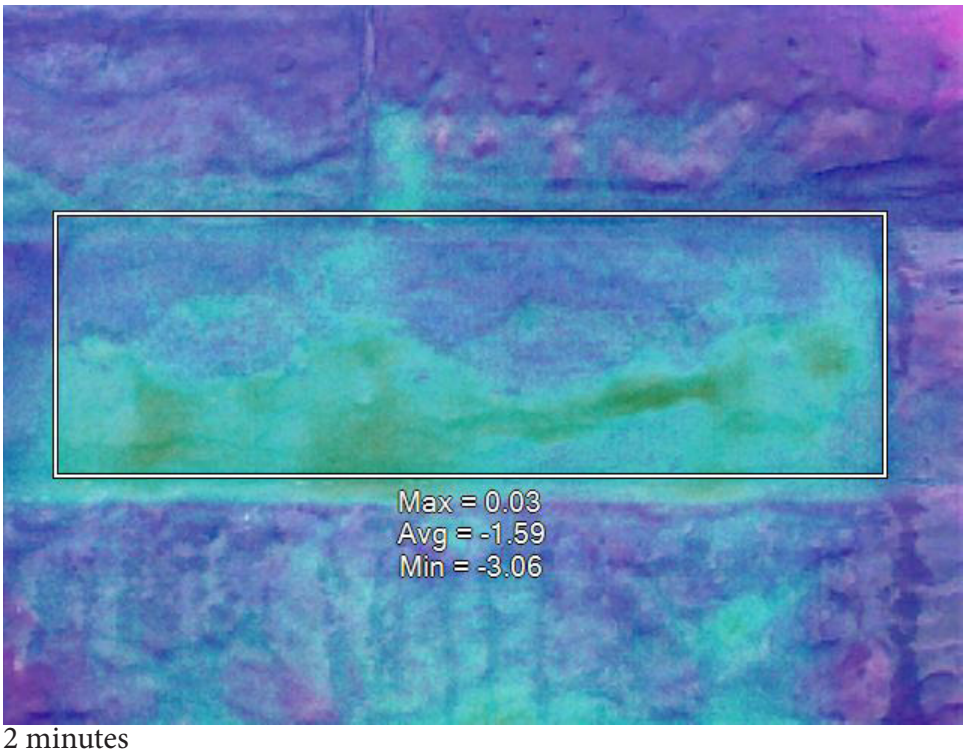


Dry

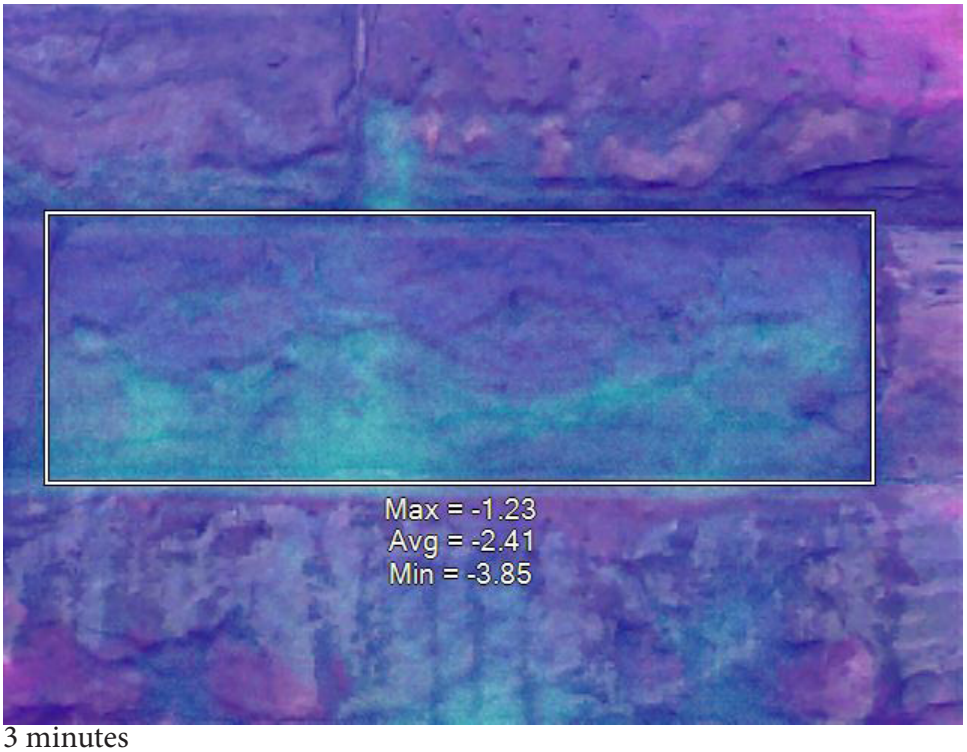


1 minute

Black biocolonization treated with BioWash
March 5, 2013

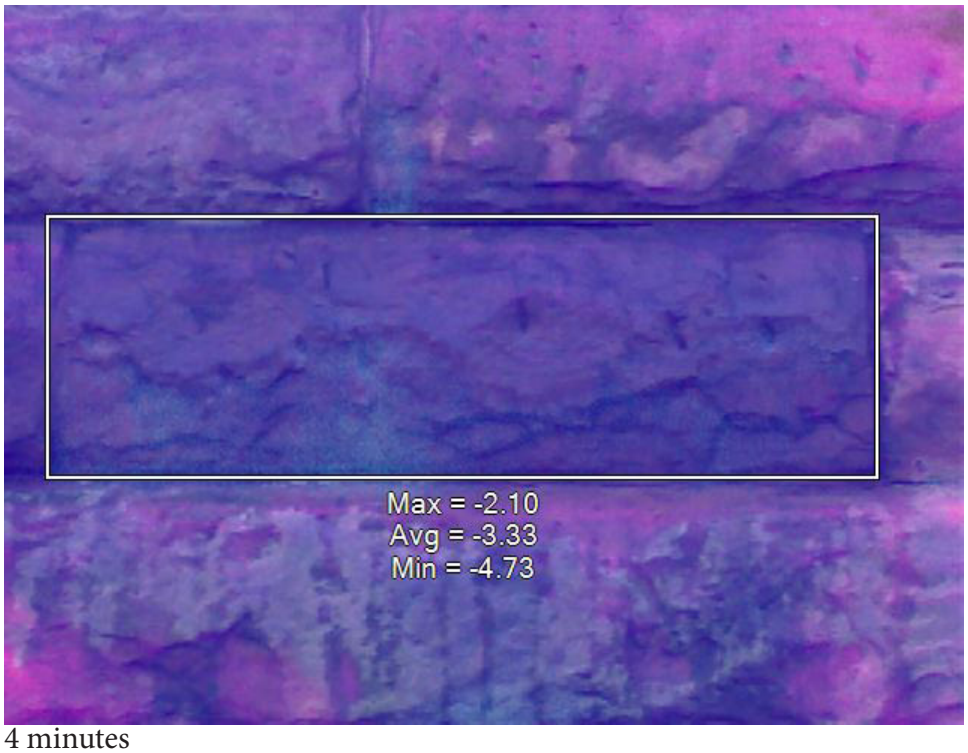


2 minutes

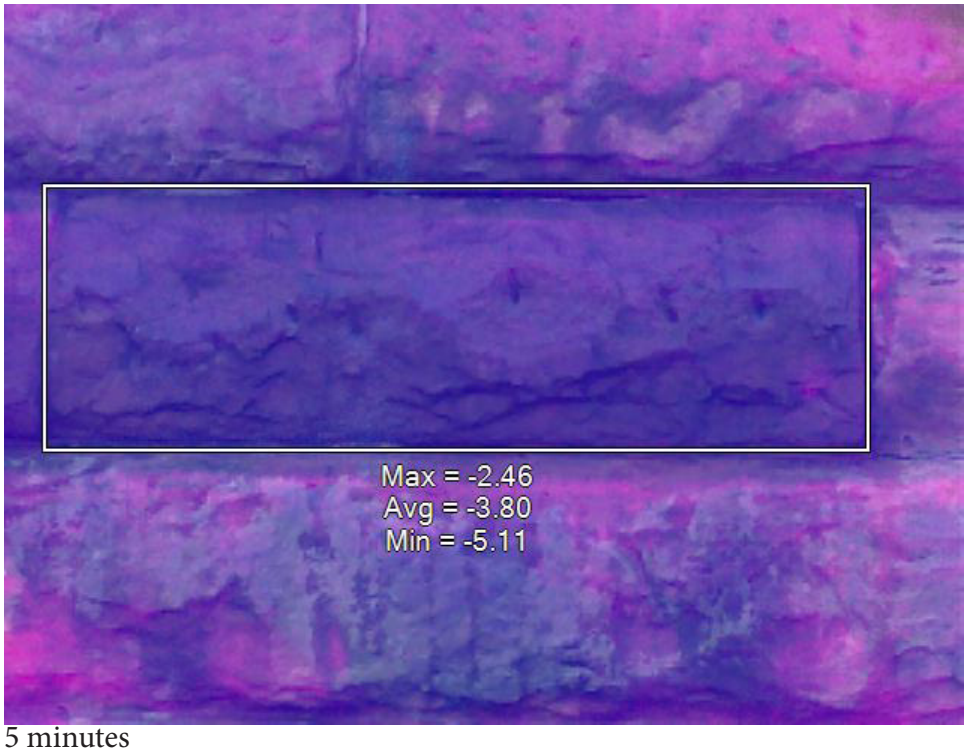


3 minutes

Black biocolonization treated with BioWash
March 5, 2013



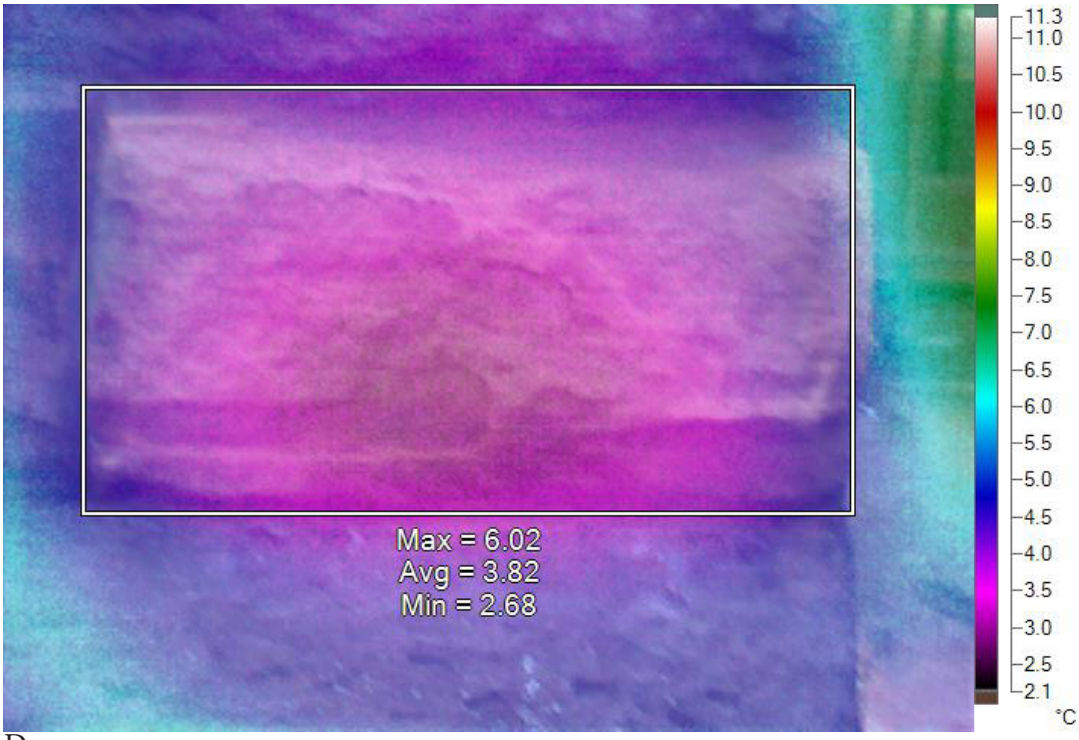
4 minutes



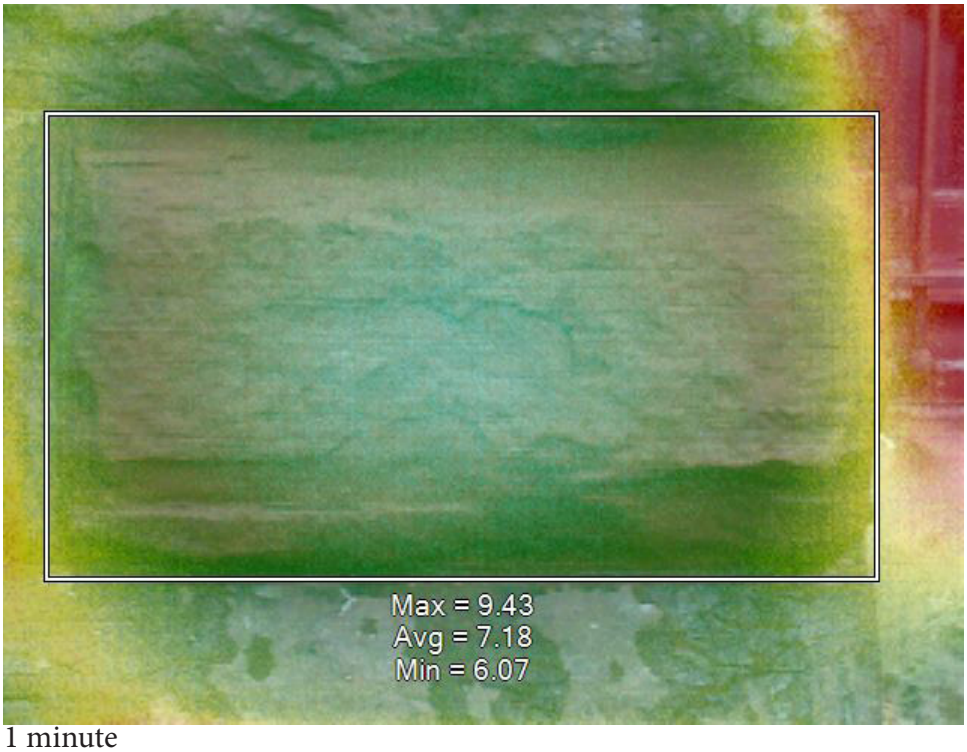
5 minutes

		Average Stone Temperature (°C)					
Time (min)	**DATE**	**Nov.19**	**Nov. 26**	**Dec. 4**	**Jan. 10**	**Feb. 7**	**Mar. 5**
0	Dry	3.82	-2.64	14.34	0.50	-3.14	-5.00
1		7.17	-1.03	14.61	2.22	-3.59	-3.34
2		6.28	-0.72	13.01	1.20	-4.78	-4.32
3		5.65	-0.92	12.15	-0.23	-5.87	-5.16
4		4.82	-1.47	12.02	-0.59	-6.23	-5.55
5		4.37	-1.69	12.10	-1.22	-6.74	-5.88
Environmental Conditions	**Temp (°C)**	11.6	8.8	18.6	8.6	4.0	4.7
	RH (%)	51	31	59	37	30	43
	Wind	+	+	+	++	+	+
	Sunny (s); partly cloudy (pc); cloudy (c)	s	s	pc	s	c	s
Block Condition	**Sunny (O); partly shaded (ps); shaded (s)**	s	s	s	s	s	s
Temperature (°C)	**Water**	20	21	21	21	14	22

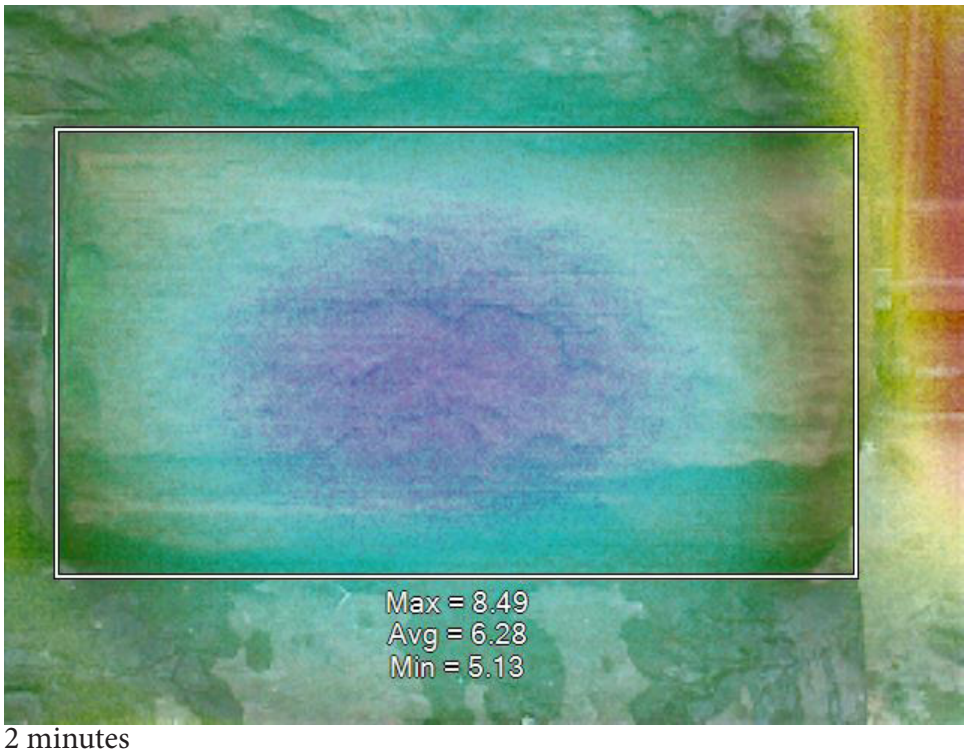
Biocolonization Free
November 19, 2012



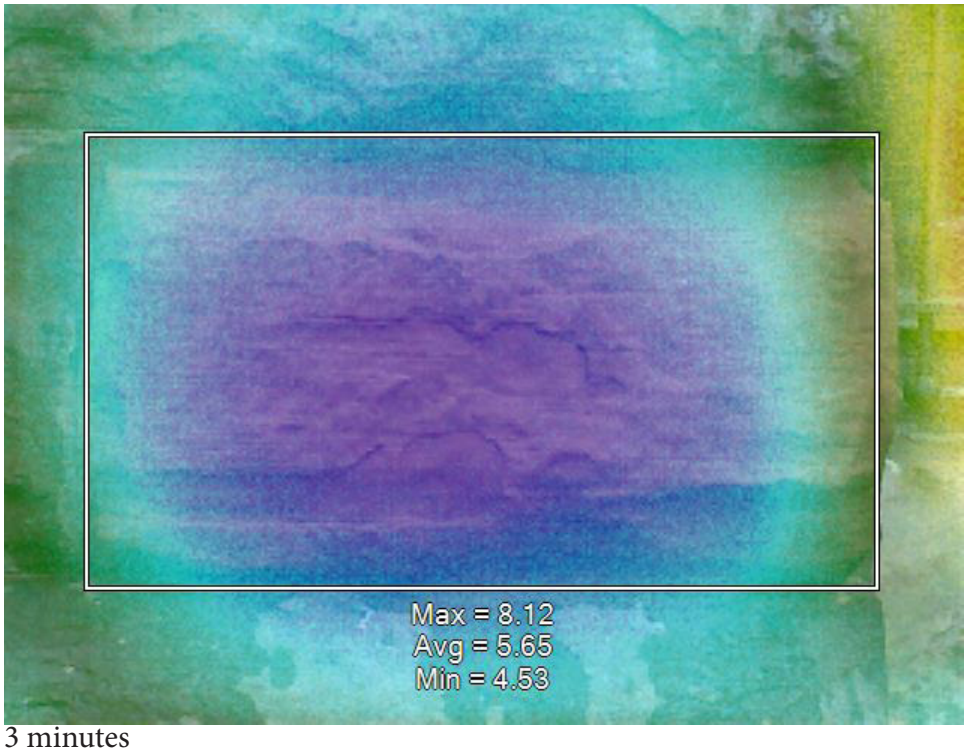
Dry



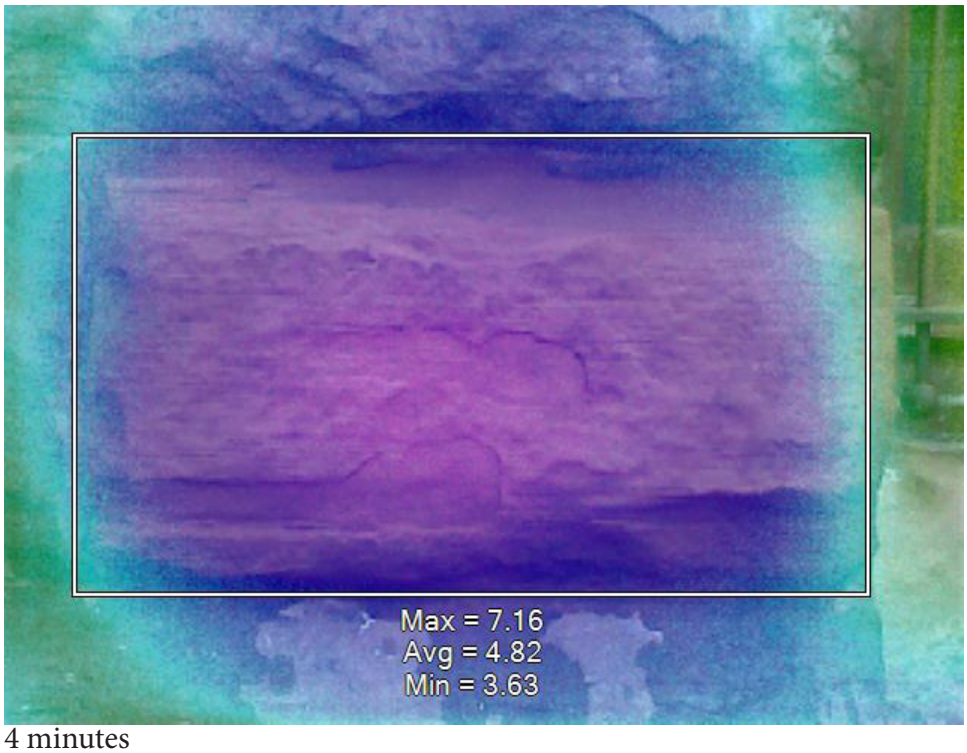
1 minute



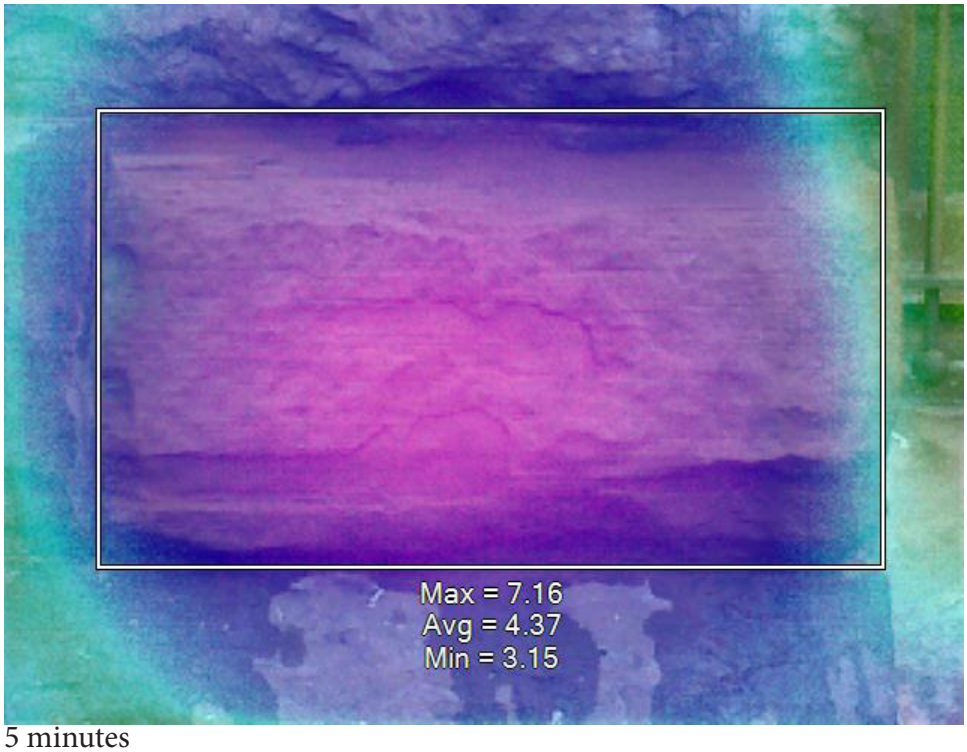
2 minutes



3 minutes

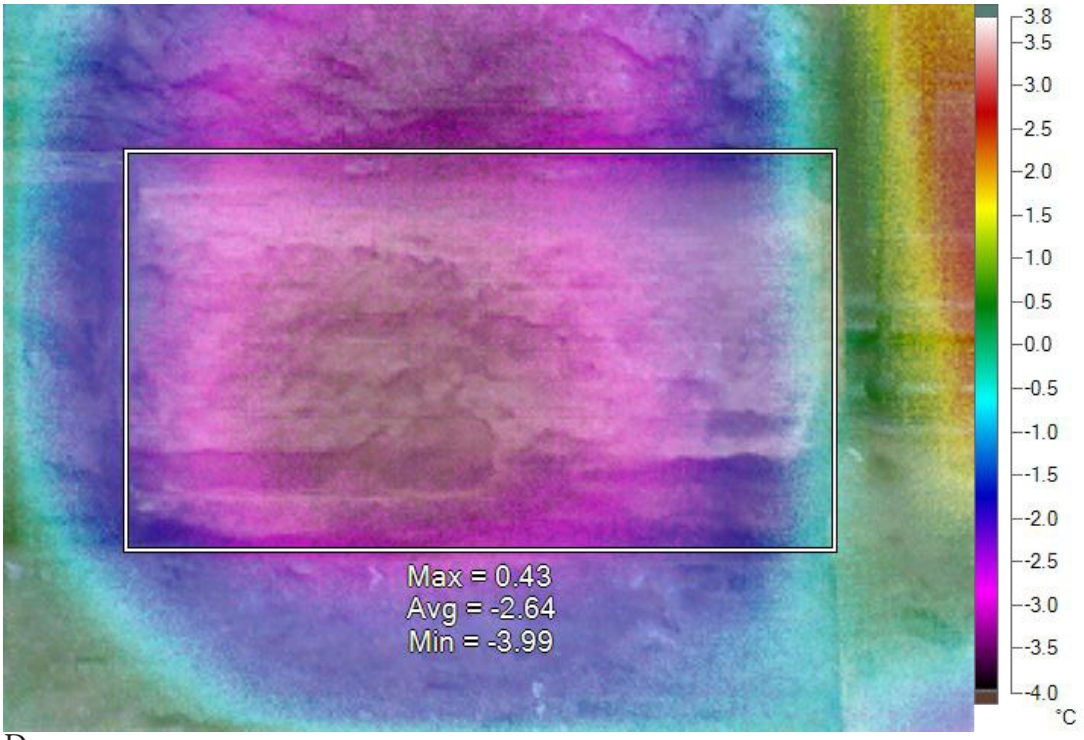


4 minutes



5 minutes

Biocolonization Free
November 26, 2012



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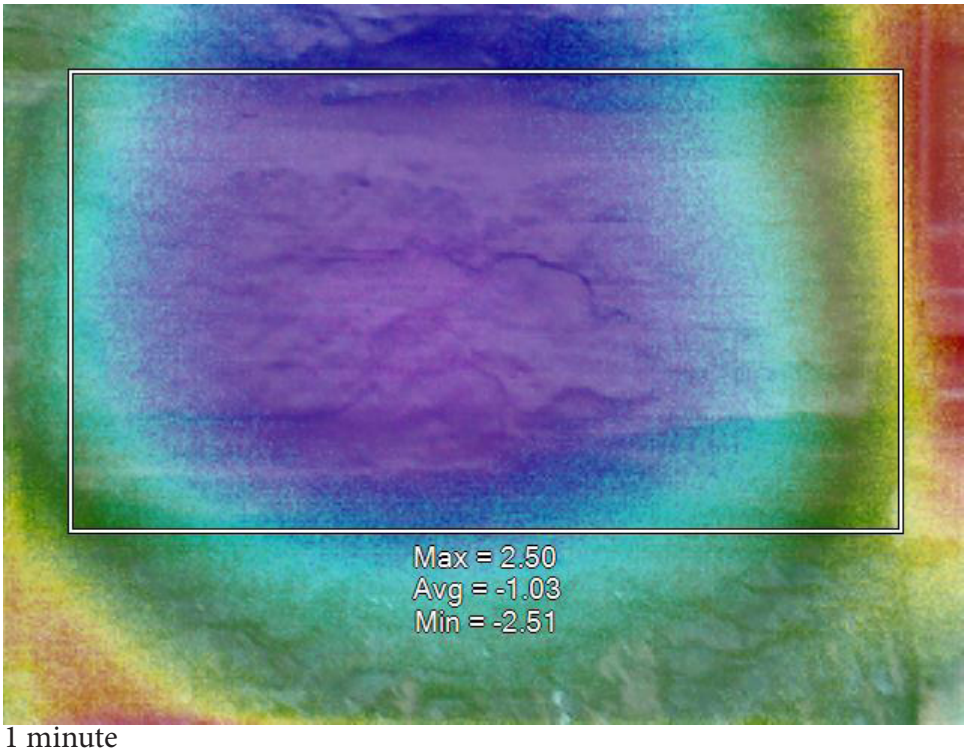

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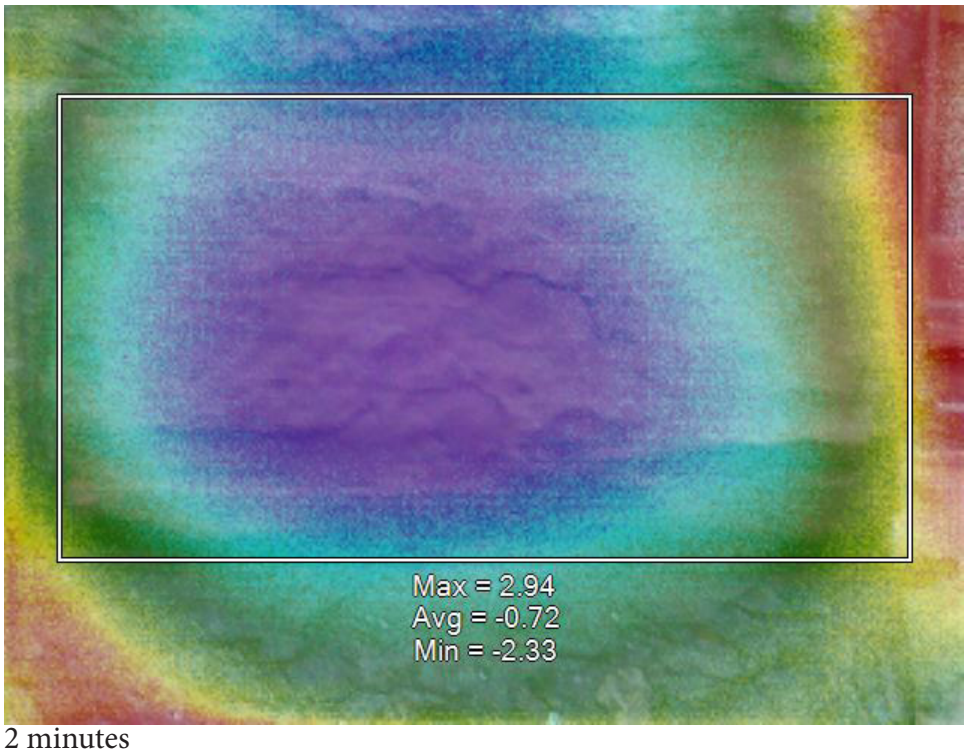

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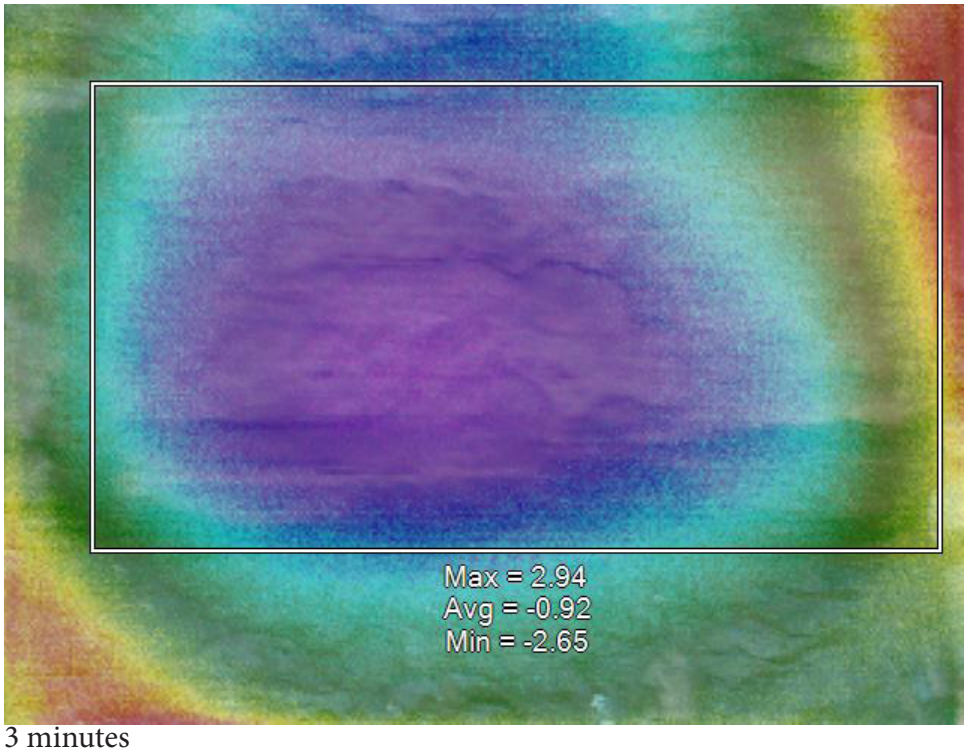

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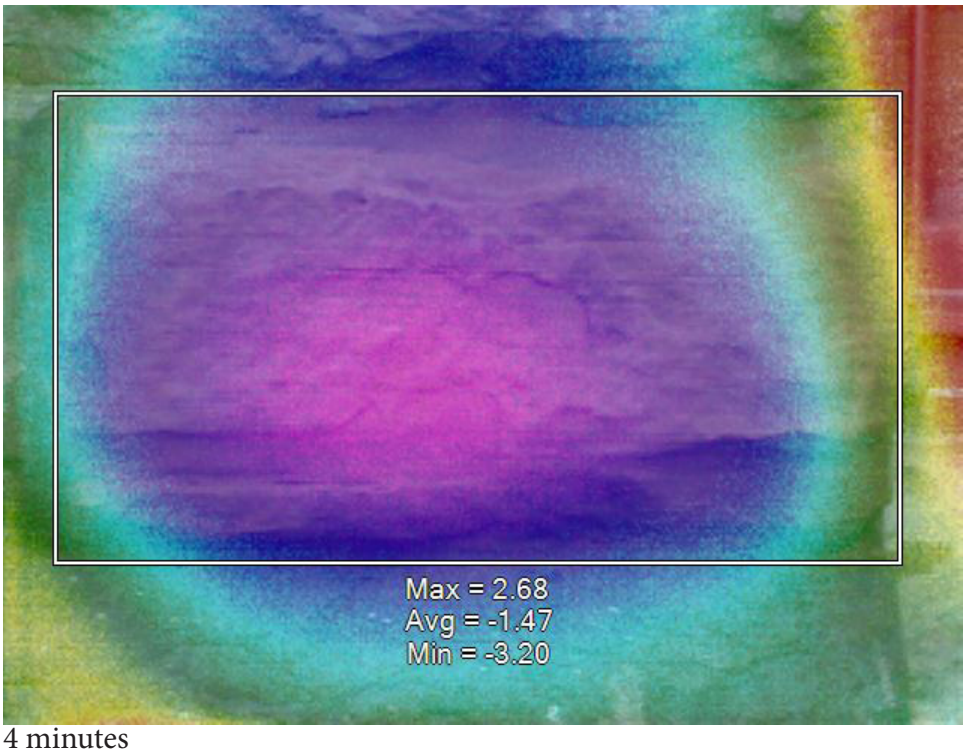

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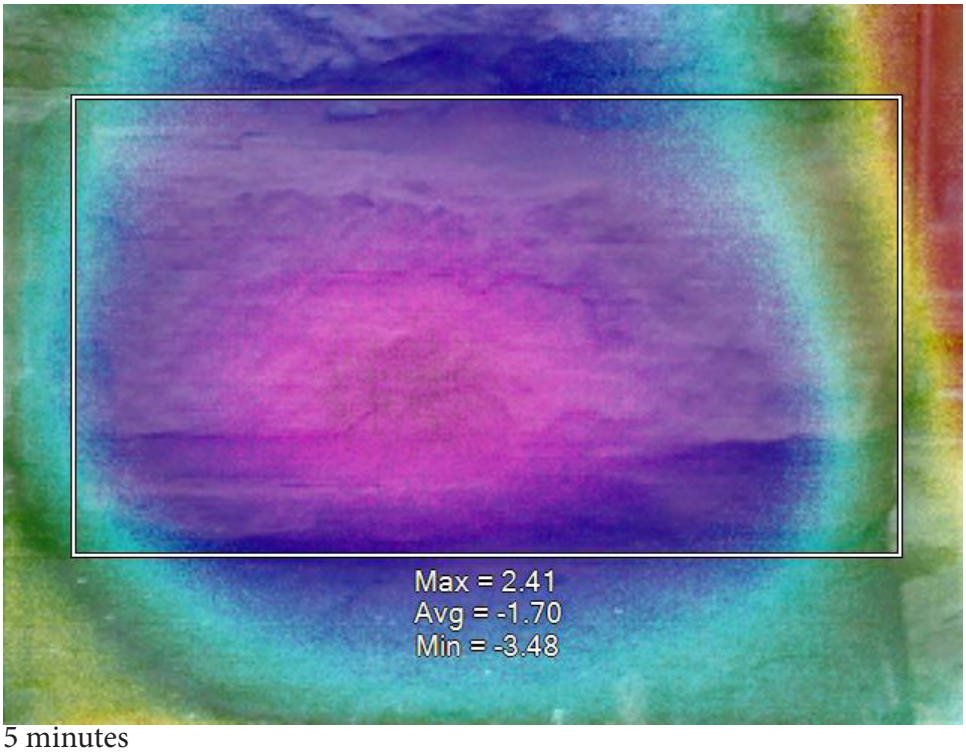

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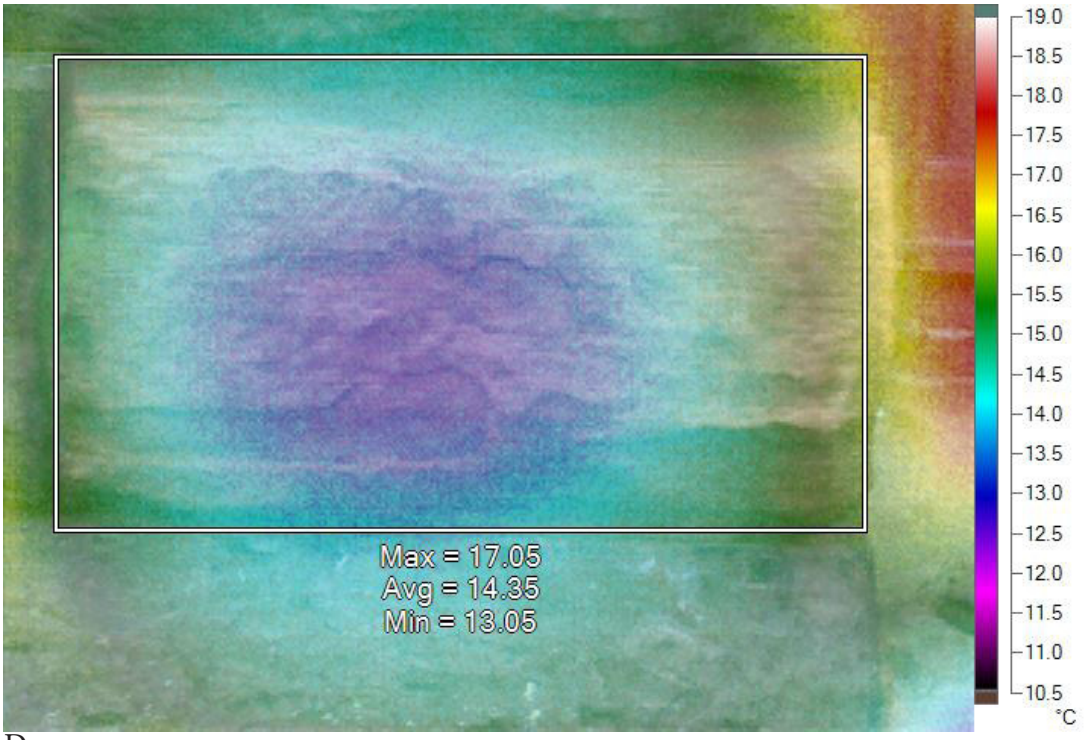
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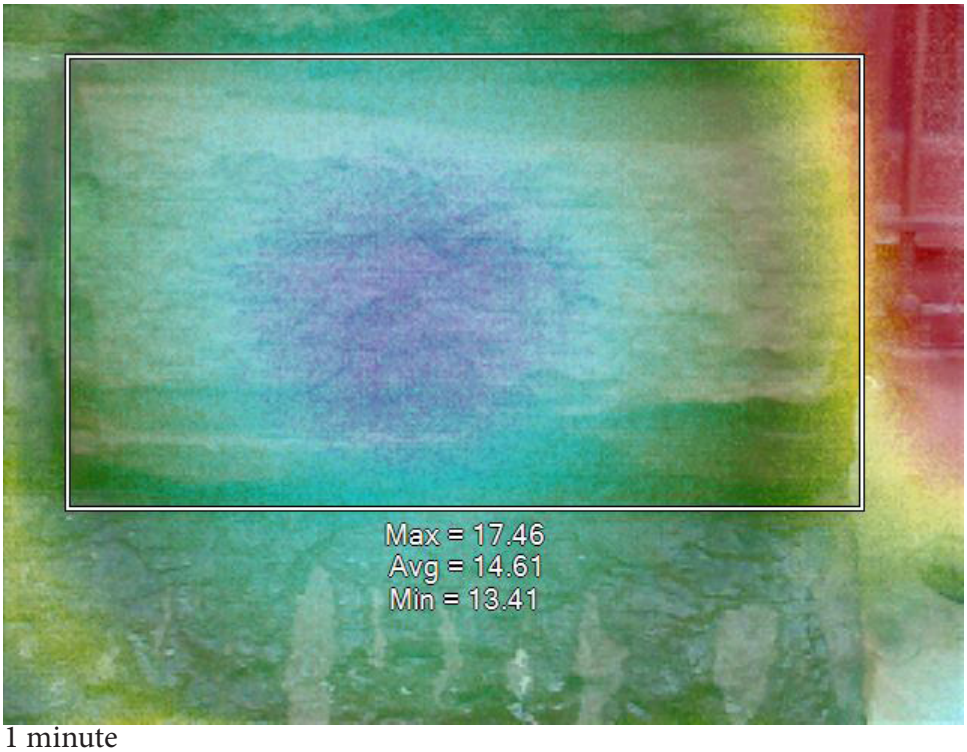
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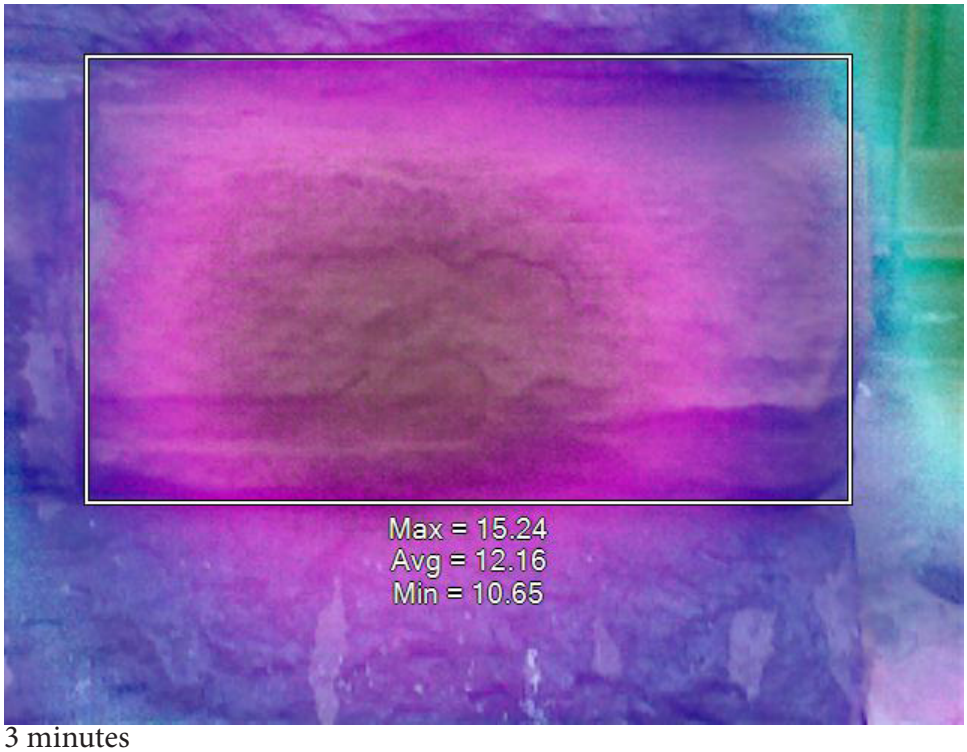

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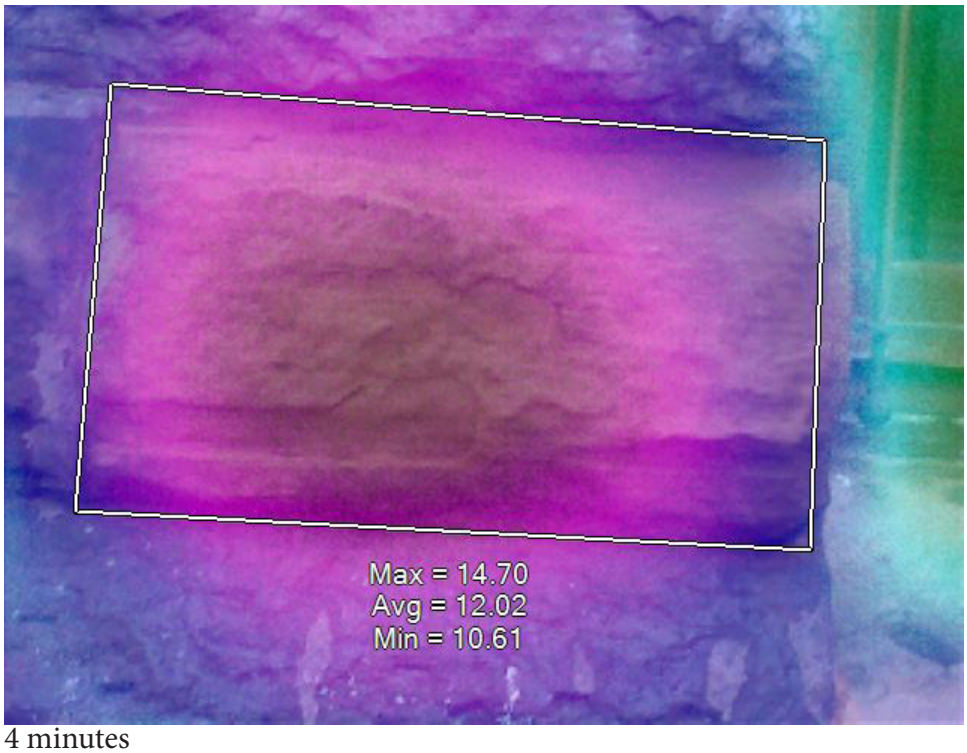

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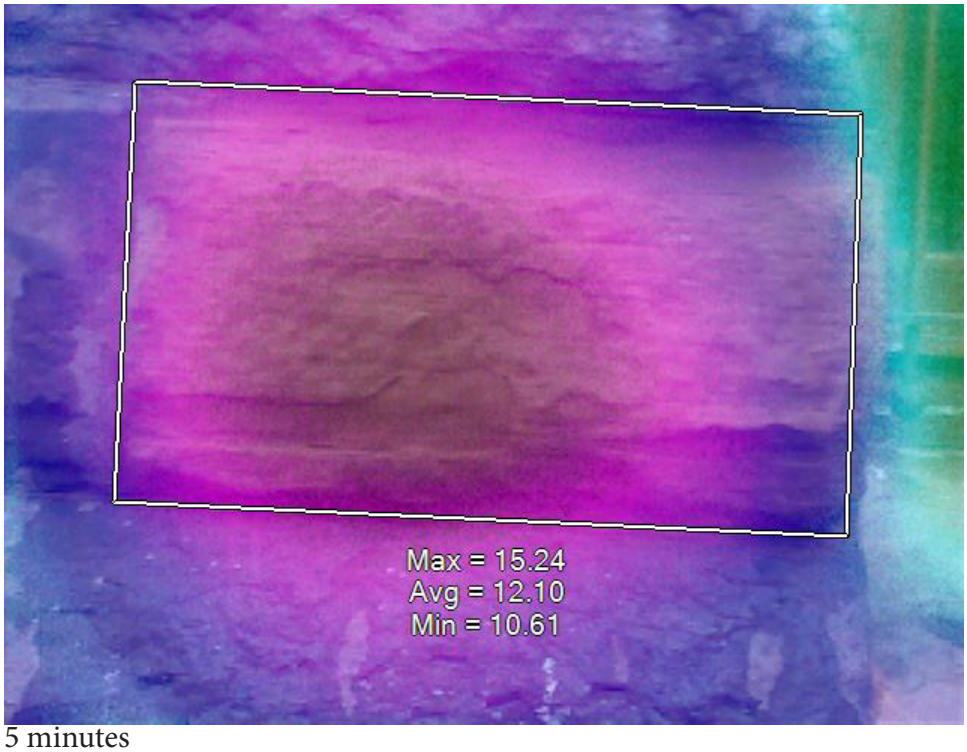

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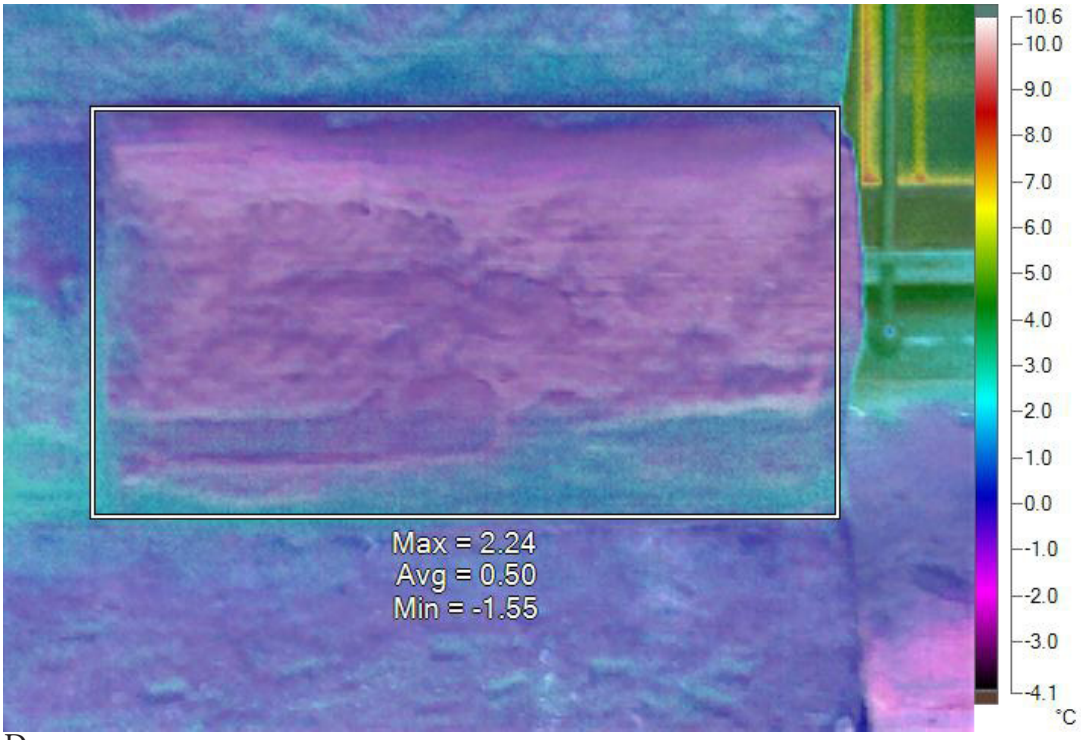
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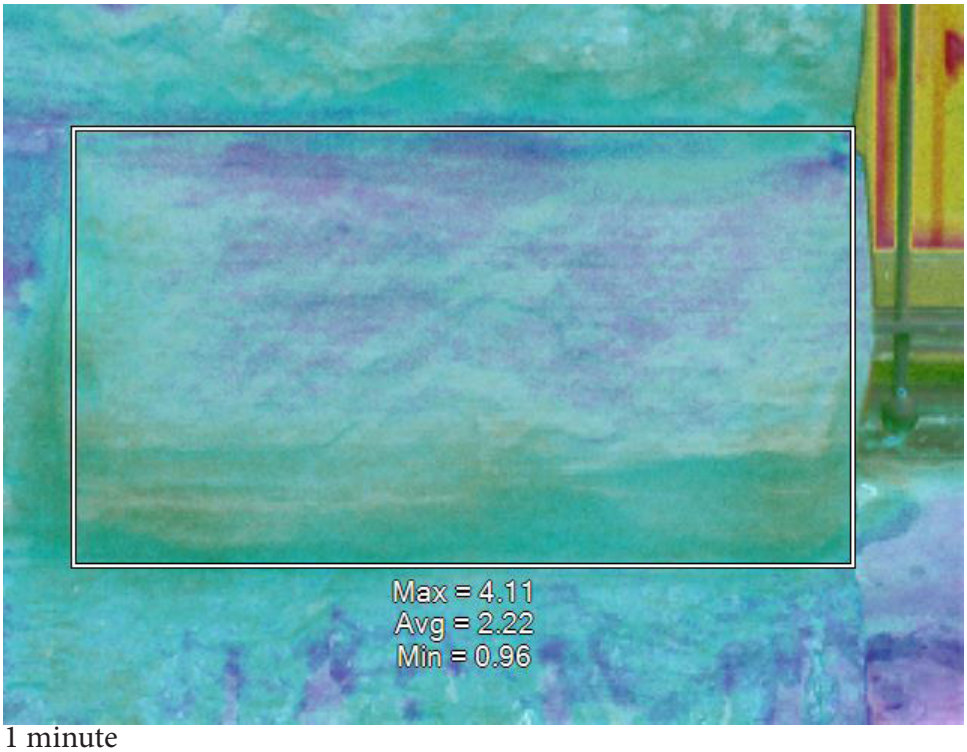
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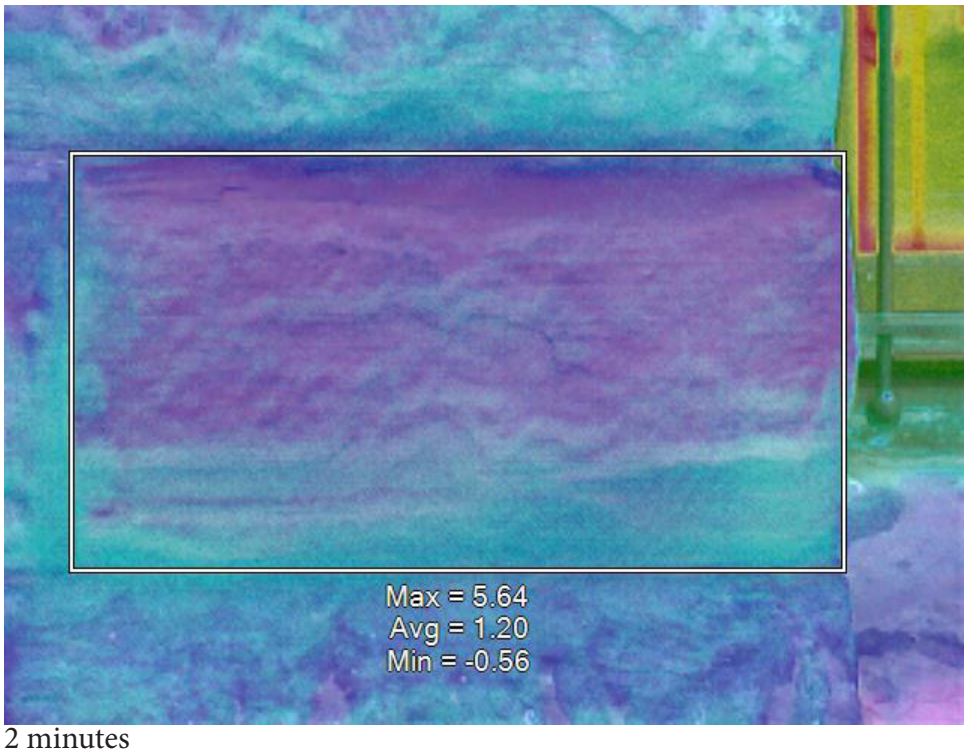

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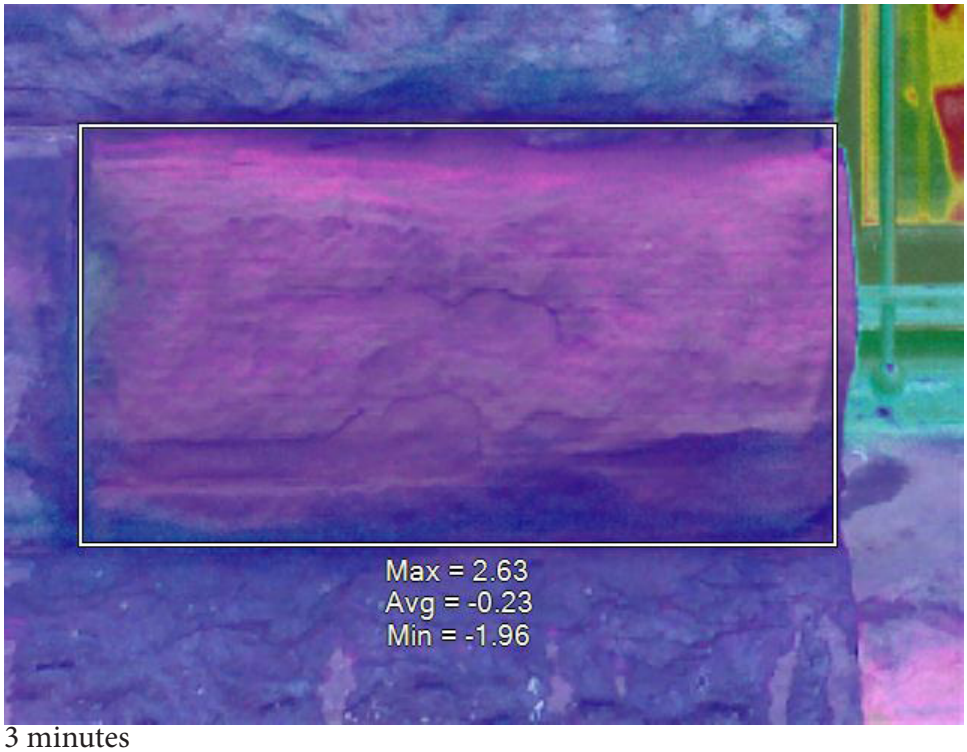

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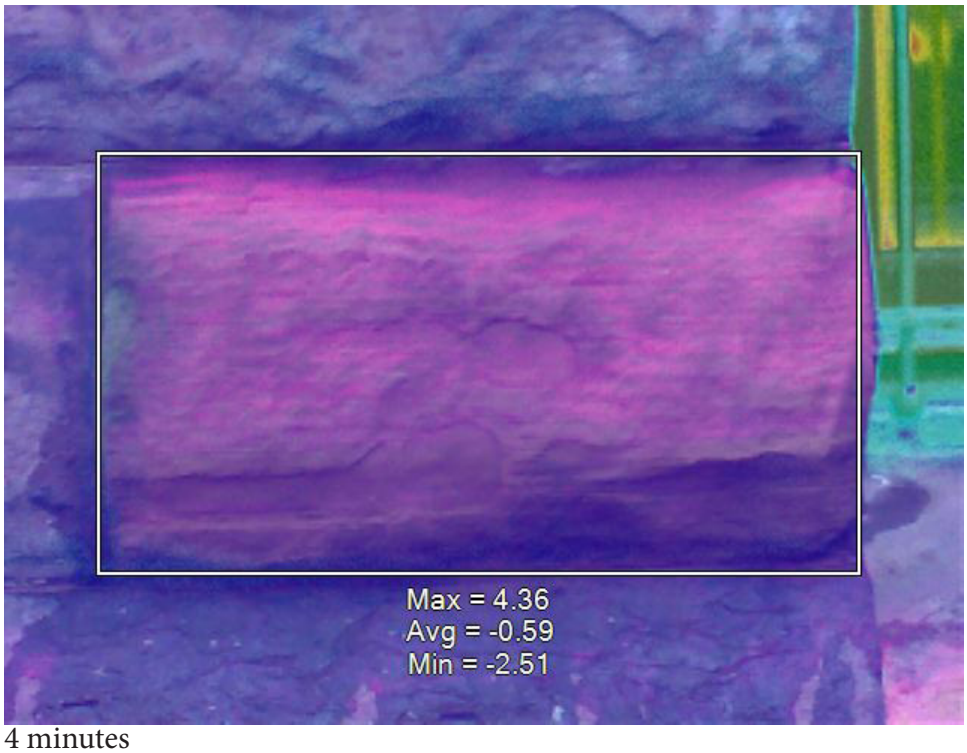

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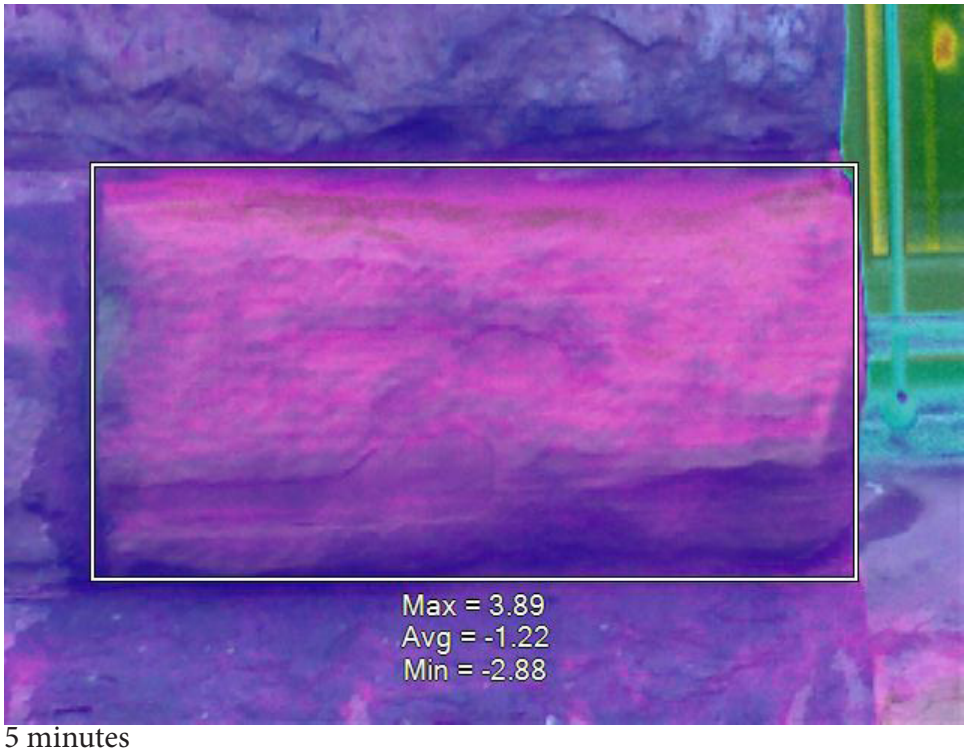

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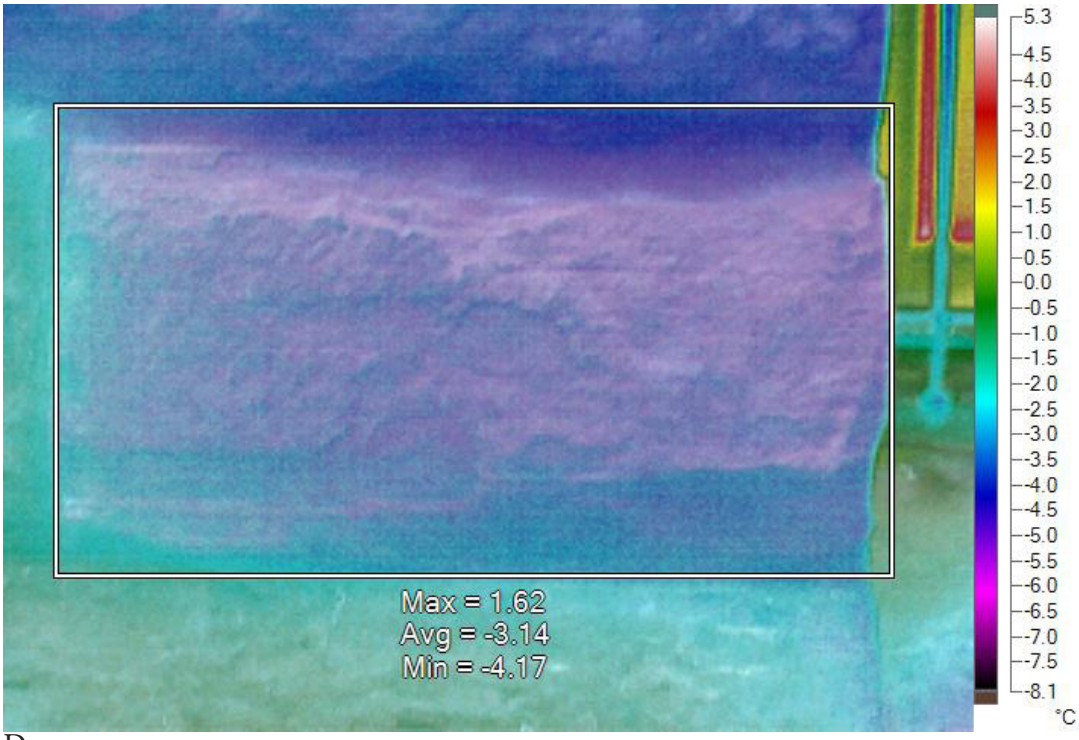
Biocolonization Free
February 7, 2013



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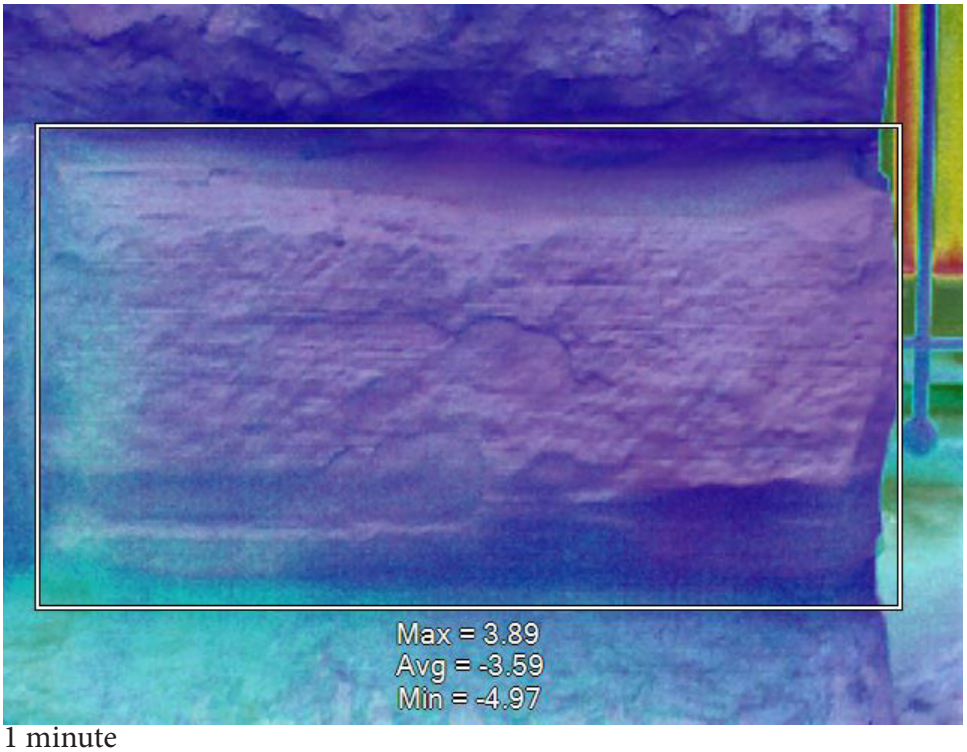

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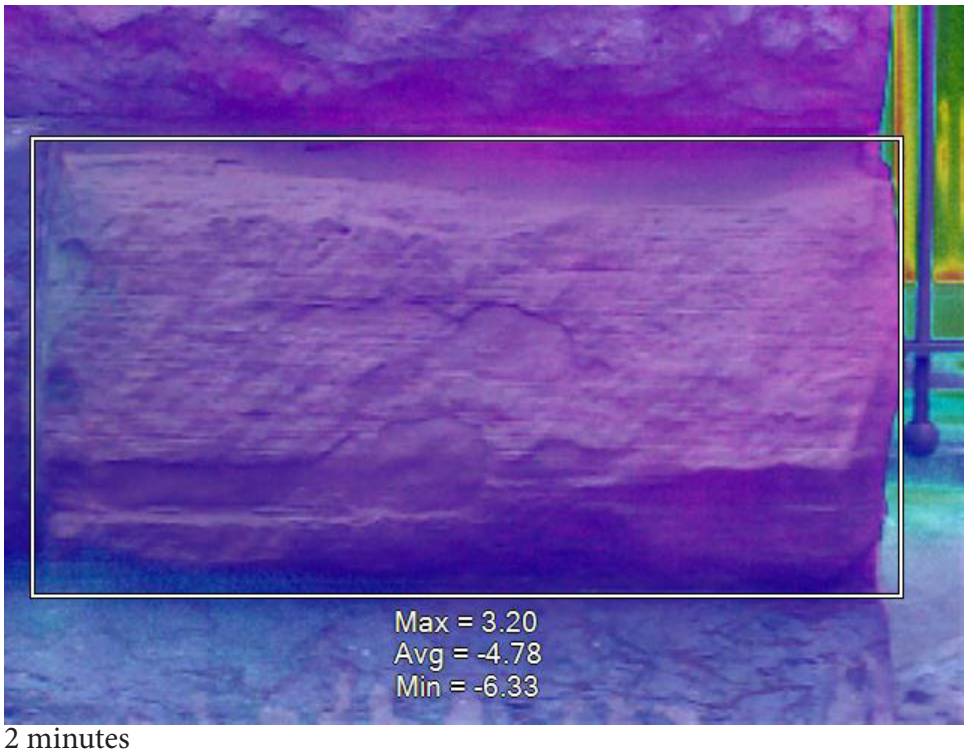

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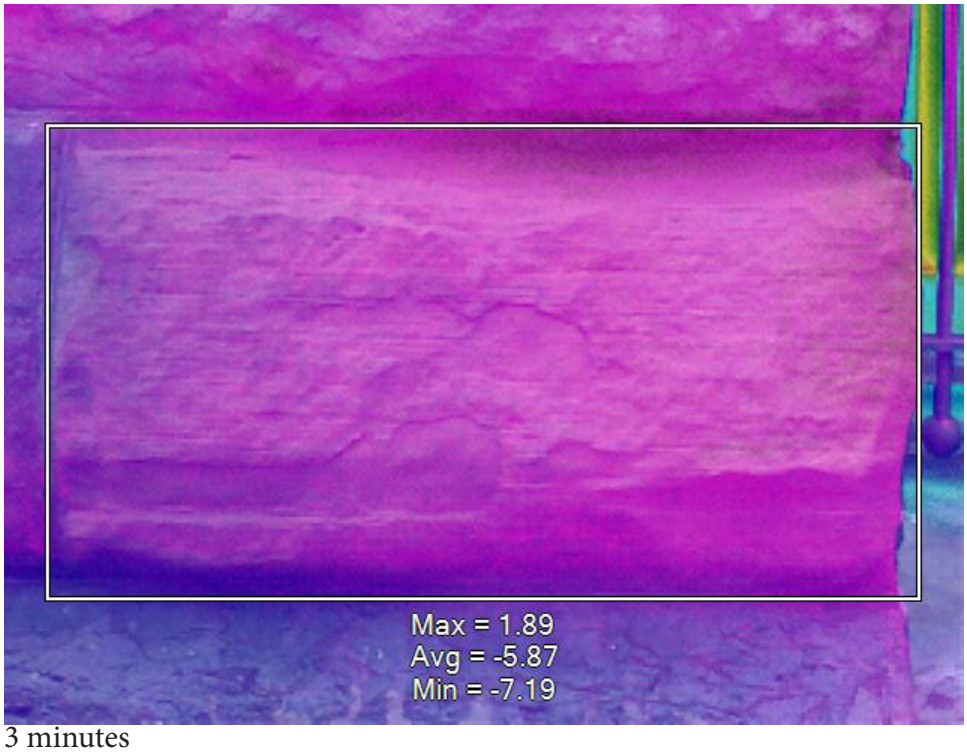

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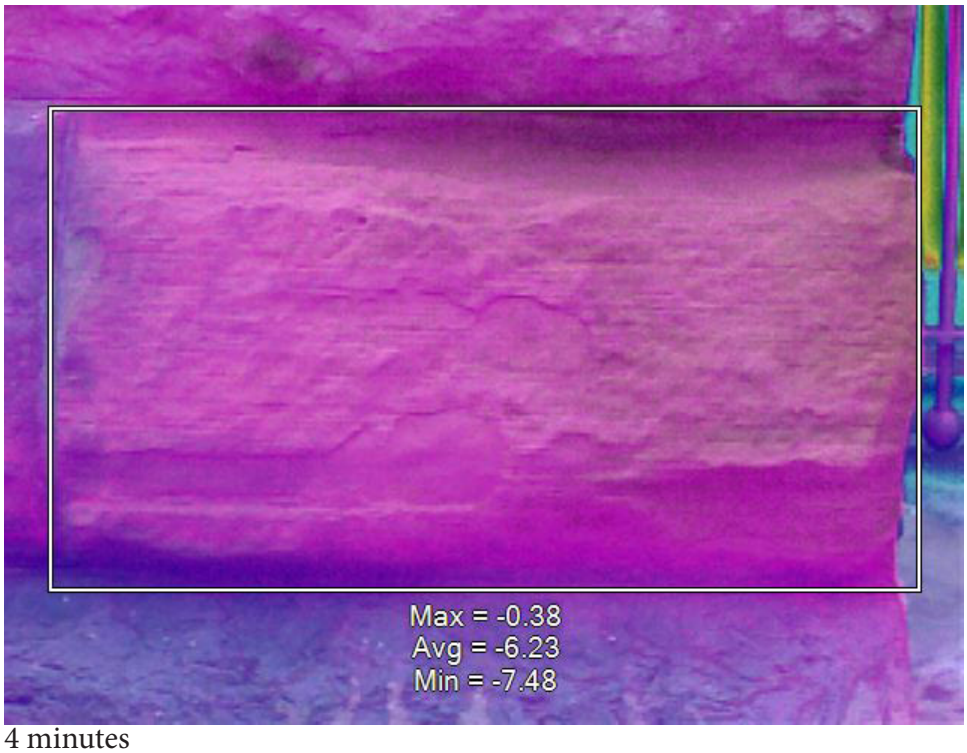

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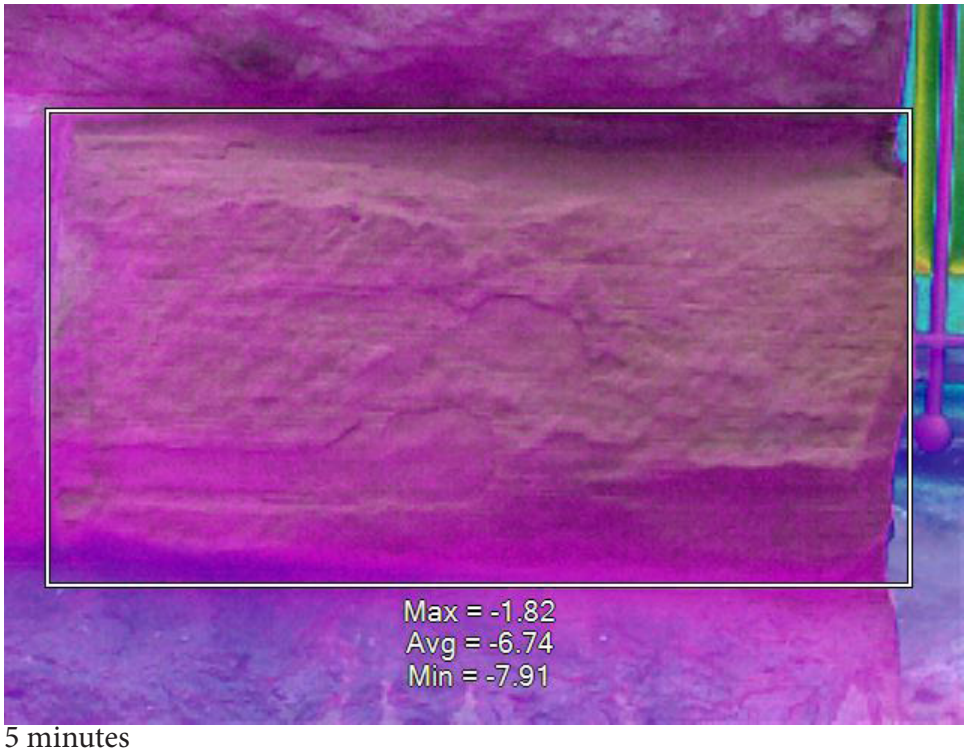

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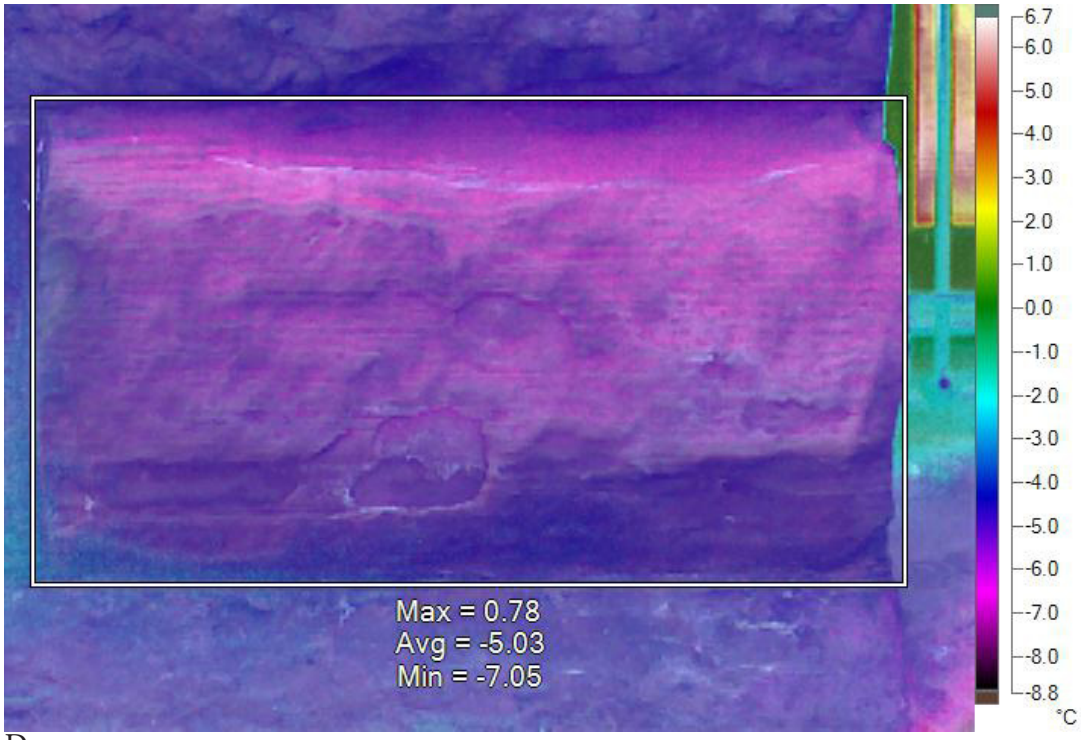

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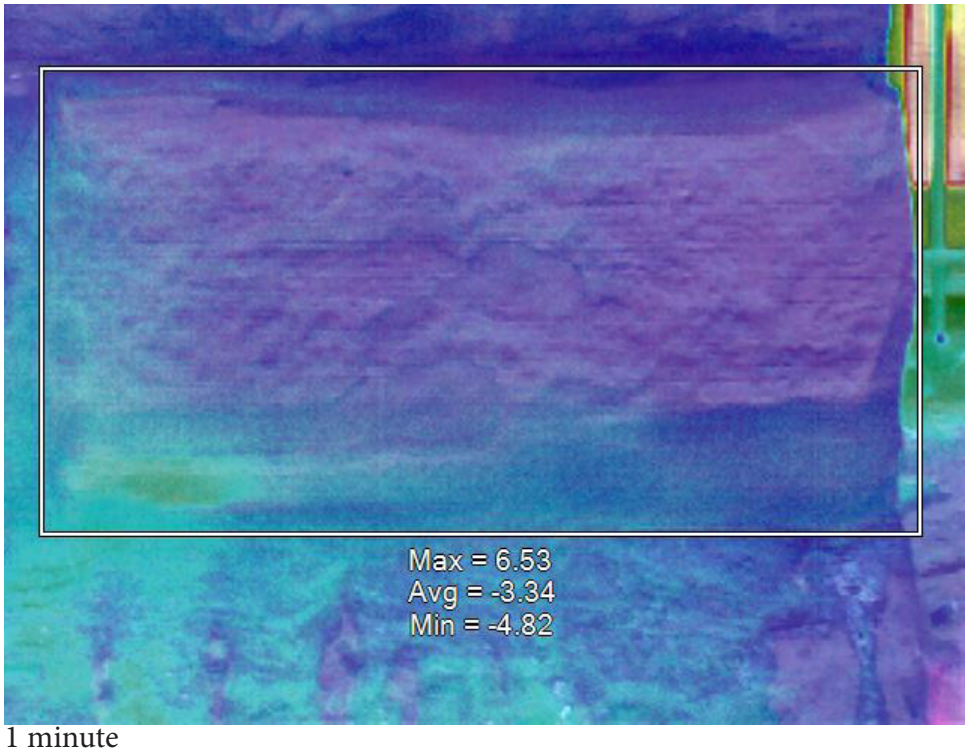

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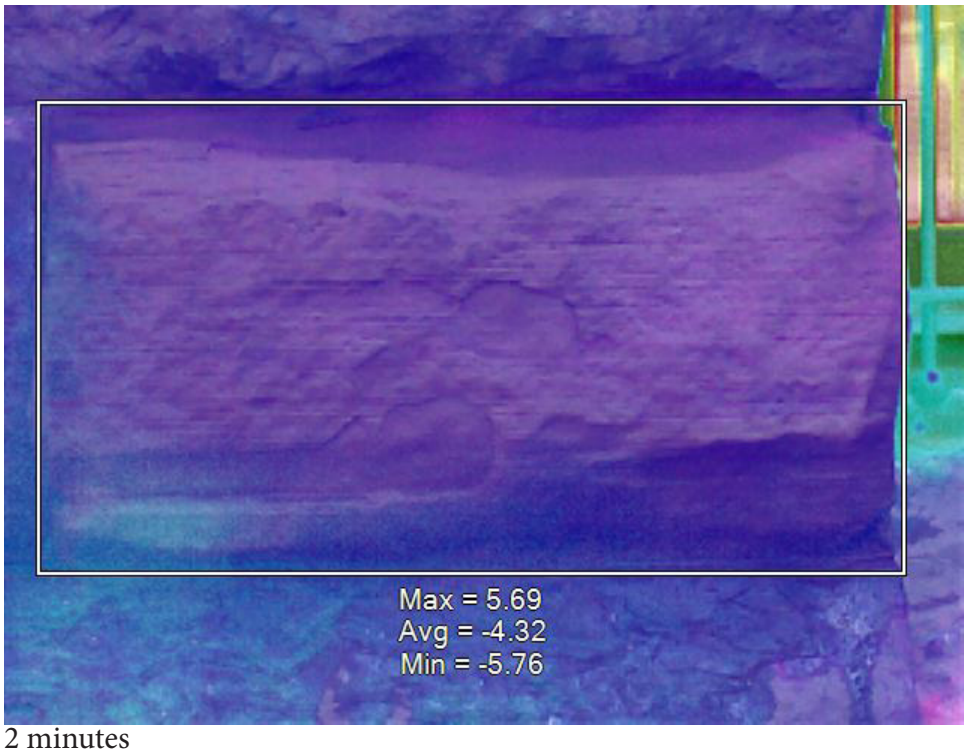

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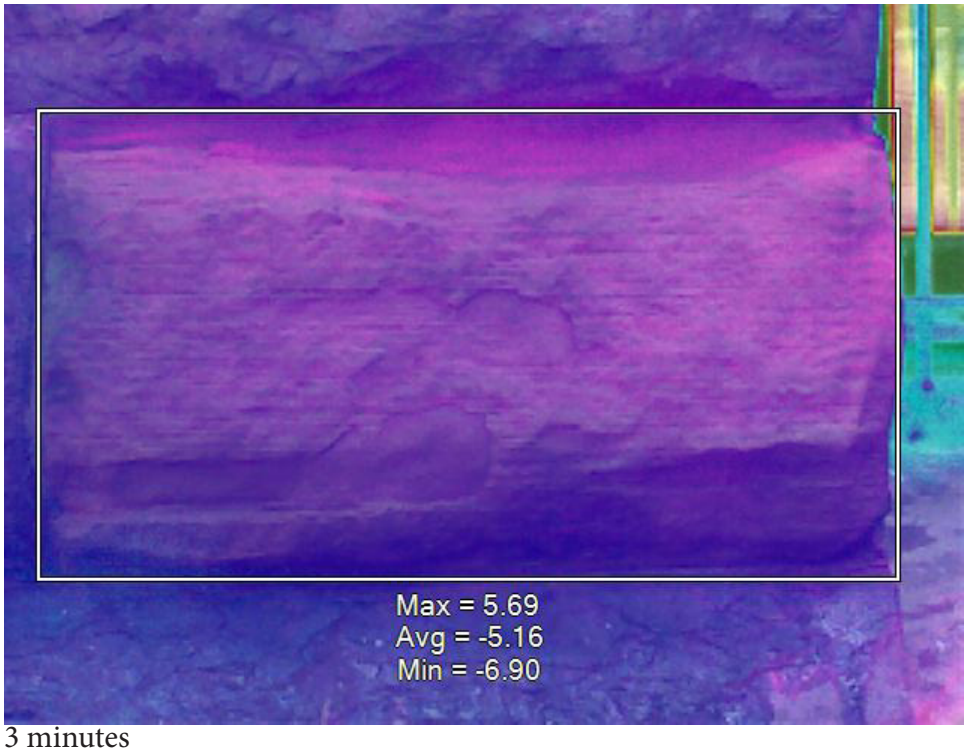

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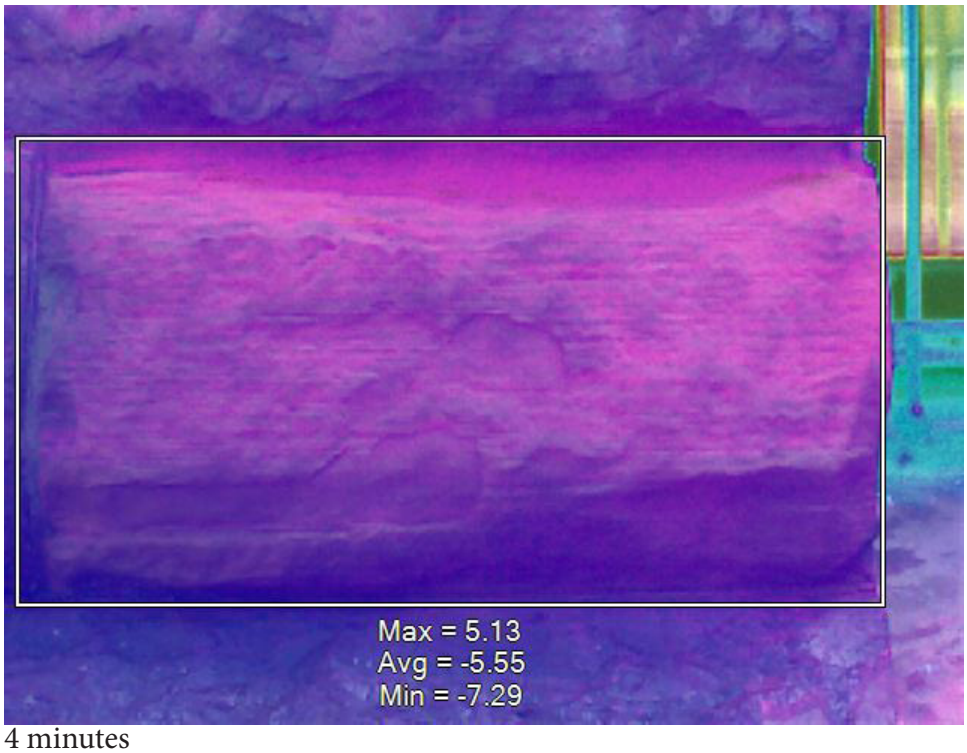

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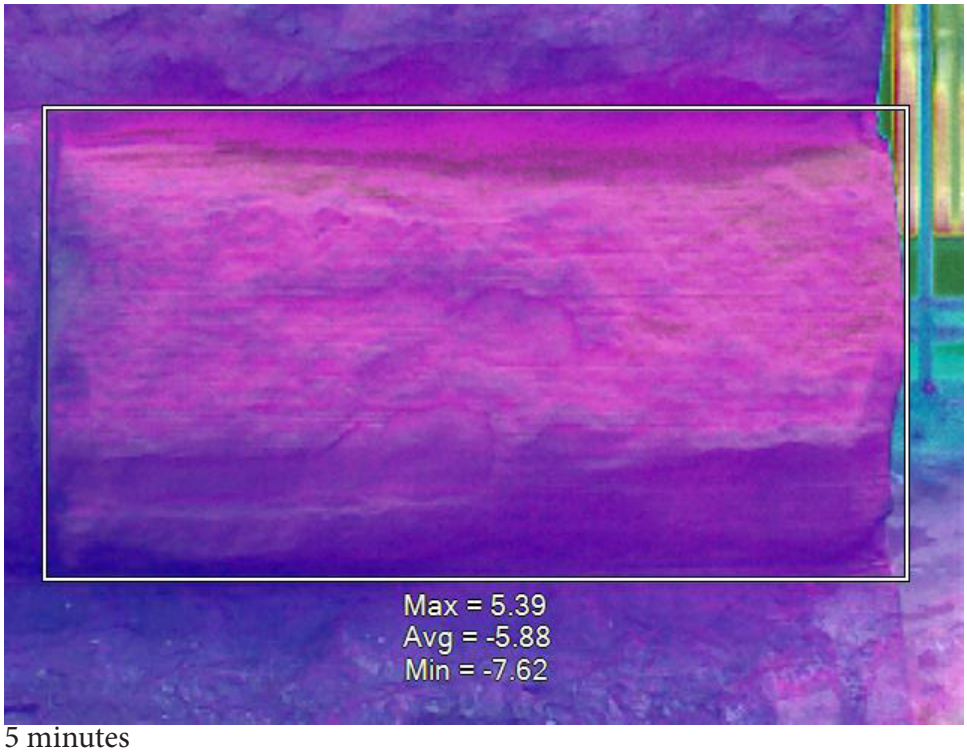

5 minutes

Appendix C. Product Information

Material Suppliers

LimeWorks
PO Box 151
Milford Square, PA 18935
215-536-6706
www.limeworks.us
(3) sample size bottles of D/2 Biological Solutions

PROSOCO Inc.
3741 Greenway Circle
Lawrence, KS 66046
1-800-255-4255
www.prosoco.com
(1) sample size bottle of Enviro Klean BioWash

D/2 Biological Solution

Discover the D/2 difference!

D/2 Biological Solution is a biodegradable, easy to use liquid that removes stains from mold, algae, mildew, lichens and air pollutants. It is effective on marble, granite, limestone, brownstone, travertine, masonry, terra cotta, concrete, stucco, wood, and other architectural surfaces including monuments, sculpture and headstones. A contact time of only 10 to 15 minutes followed by scrubbing with a soft nylon or natural bristle brush will loosen most biological and air pollutant staining.

D/2 Biological Solution is effective for removing harmful biological and air pollutant staining from many building materials including masonry, marble, granite, limestone, brownstone, travertine, terra cotta, concrete, stucco, wood, canvas and vinyl & aluminum siding.

Features and Benefits

- **Fast acting**: 10 to 15 minutes contact time for great results.
- **Biodegradable**
- **Contains no acids, salts, or chlorine**
- **pH neutral**
- **Will not etch metals or glass**
- **Safer to use around plantings**
- **Is not a hazardous material and requires no special handling or protection**
- **Use full strength, no in-field mixing required**
- **Shelf life of 5 years**

Application Procedures

Always do a spot test sample before proceeding with project. D/2 works best when air and surface temperatures are 45°F or above. Use D/2 undiluted for best results. In the event of excessive plant exposure, rinse all plants and water in all planted ground areas.

Immediate Result Method

1. Apply D/2 Biological Solution with a brush, roller, hand pump sprayer (garden style pump sprayer) or low pressure power sprayer.
2. Allow undiluted D/2 to remain on the surface 10-15 minutes.
3. Apply additional D/2 as necessary to maintain a wet surface.
4. Scrub with soft nylon or natural bristle brush. **DO NOT USE METAL BRUSH.**
5. Lightly mist with water and continue scrubbing.
6. Rinse thoroughly with clean, potable water.

No Scrub/No Rinse Method

1. Apply D/2 Biological Solution with a brush or pump sprayer to a dry surface. Do not pre-wet the surface.
2. Allow to dry. Repeat if there are heavy biological deposits.

D/2 works with the elements and results occur within one week to one month depending on severity of growth and weather conditions. The surface will become cleaner over time as the subsurface biological growth dies and releases.

Safety Information

D/2 Biological Solution is non-mutagenic, and contains no carcinogenic compounds as defined by NTP, IARC, or OSHA. It is considered essentially non-toxic by swallowing, as it has an oral LD50 of 2.0 g/kg of body weight. No special ventilation is required during use.

D/2 Biological Solutions, Inc. | PO Box 3746, Westport, MA 02790 | (917) 693-7441 | d2bio.com

Packaging and Coverage

D/2 Biological Solution is available in 1 gallon and 5 gallon containers, and 55 gallon drums. The area that can be treated with one gallon of D/2 will vary considerably as a function of the nature and extent of biological deposits, as well as the physical characteristics of the surface. Typical coverage to remove medium deposits will vary from 250 to 350 square feet per gallon.

Technical Data

Physical Form . . . . . Transparent, low viscosity liquid

Color . . . . . Almost colorless

pH 9.5

Specific Gravity 1.01g/cc

Solubility in Water . . . . . Complete

Vapor Pressure . . . . . 25 mm Hg @ 20°C

10/2012

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GUIDELINE FOR WRITING SPECIFICATIONS WHEN USING

D/2 Biological Solution

Select Relevant Section

Division 04900-Masonry Restoration and Cleaning

Part 1 – GENERAL

1.1 RELATED DOCUMENTS

A. The Contract Documents shall govern work of this section. Provide materials, labor, equipment, and services necessary to furnish, deliver, and install all work of this section as shown on the drawings, as specified herein, and/or as required by job conditions.

1.2 SUMMARY OF WORK

A. This section includes, but is not limited to, the following:

   a. Removal of satins from biological growth by chemicals from all historic surfaces including smooth and ornamental wood, metal, masonry, concrete, and brick. Mock- ups will determine the best appropriate method.

B. Visual Requirements:

   a. Maintain aesthetic or historic qualities of Project by protecting Work designated to remain.

1.3 REFERENCES

A. Manufacturer's specifications and instructions.

1.4 SUBMITTAL

A. Submit each item in this Article according to the Conditions of the Contract and Division 1 Specification Sections.

D/2 Biological Solutions, Inc. | PO Box 3746, Westport, MA 02790 | (917) 693-7441 | d2bio.com

B. Product Data: Submit manufacturer's specifications and installation instructions for products used including finishing materials and methods.

C. Submit manufacturer's technical data sheet for product indicated including recommendations for their application and use.

D. Submit a work plan describing capture, storage, and disposal as required and/or governed by any and all local, state, and/or federal laws, codes, and regulations.

E. Samples: Provide sample installation of product. Locations per the owner or owner's representative's directions.

1.5 QUALITY ASSURANCE

A. Mock-ups: Prepare sample of D/2 Biological Solution on the appropriate substrate indicated. See 1.6 Test Panels.

B. Provide at least one person who shall be present at all times during the execution of the work of this section, who shall be thoroughly familiar with the specified requirements, and the materials and methods needed for their execution, and who shall direct all work performed under this section.

C. Provide adequate numbers of workers skilled in the necessary crafts and properly informed of the specialized methods and materials to be used in this work.

1.6 TEST PANELS

A. The Contractor shall arrange for providing test panels. Minimum size of test panels shall be a 5 ft. by 5 ft. area. Manufacturer's application instructions shall be followed. Allow a minimum of 7 days drying time before inspection or longer if possible. Some forms of biological staining will continue to diminish for as long as three to four weeks or longer. Test panel shall serve as the performance standard and remain available for comparison during the cleaning process.

B. Contractor shall prepare a written report detailing results of testing including description of methods employed, materials, concentration of cleaner, dwell times and other elements of test procedures as defined above.

C. Each test panel must be carefully labeled, charted, and photographed. Approved test panels will become a part of the Work, and serve as the quality standard for similar type work on this project.

D. Notify the owner's representative seven (7) days in advance of the dates and time when the test panels will be installed.

1.7 PROJECT/SITE CONDITIONS

A. Contractor shall be responsible for protecting all existing adjacent materials such as doors, windows, flashings, roofing, and other existing materials that are not intended to be treated.

B. Contractor shall be responsible for the repair of all damaged adjacent materials due to the execution of the work at no additional expense to the owner. Repairs shall be made by qualified mechanics skilled in the type of repairs required, to the satisfaction of the owner's representative.

C. Protect adjacent areas and surfaces not being treated with barriers suitable for the product being used. Appropriate care should be taken at air intakes, air conditioning vents and similar openings that may come in contact with the product.

D. Take appropriate precautions to avoid harm to building occupants, pedestrians and nearby property.

E. Safety: For any number of reasons it is essential to maintain a high degree of worker and occupant safety while working with biological solution.

PART 2 – PRODUCTS

2.1 MATERIALS

A. D/2 Biological Solution

   a. Non-toxic, biodegradable, biological solution with a neutral pH shall be used. Acids, caustics, and chlorine bleach based products must not be used. Acceptable products available through LimeWorks.us: (215) 536-6706, Bonstone Materials Corporation: (262) 363-9877, and additional approved distributors of D/2 Biological Solution (see http://d2bio.com).

B. Miscellaneous Equipment

   a. Natural bristle brushes

   b. Soft clean rags

   c. Clean, potable water

   d. Rubber gloves

   e. Eye and skin protection

f. Low-pressure applicator, such as pump sprayer or battery powered sprayer. g. Pressure washers using 600 psi or less.

PART 3 – EXECUTION

3.1 GENERAL APPLICATION OF INITIAL CHEMICAL TREATMENT

A. Follow manufacturers' instructions (See Data Sheet). B. Work sections that can easily be applied in one shift.

C. Clearly mark or identify time of application and dwell time.

D. Wash down in the same sequence of sections in which product was applied.

E. Thoroughly rinse application areas and surrounding adjacent surfaces.

3.2 CLEAN UP

A. During the work, remove from the site discarded materials, rubbish, cans and rags at the end of each workday.

B. Upon completion of work, remove all protective coverings and coatings, and clean window glass and other spattered surfaces

C. Rinse treated areas to clean and remove all biological growth and chemicals.

PART 4 – CONTRACTOR QUALITY CONTROL

4.1 QUALITY CONTROL

A. The implementation of a Contractor Quality Control Program does not relieve the Contractor from the responsibility to provide work in accordance with the Contract Documents, applicable codes, regulations, and governing authorities. The Contractor Quality Control Program shall include, but not be limited to, the elements herein. These elements are provided only as a minimum starting point for the Contractor to use to generate the complete Contractor's Quality Control Program.

END OF SECTION

10/2012

D/2 Biological Solutions, Inc. | PO Box 3746, Westport, MA 02790 | (917) 693-7441 | d2bio.com

Section 1: PRODUCT & COMPANY IDENTIFICATION

Product Name: D/2 Biological Solution

Manufactured By:
D/2 Biological Solution
PO Box 3746
Westport, MA 02790
(917) 693-7441
http://d2bio.com

Emergency Phone: Chem-Tel 24-Hour Emergency Service: (800) 255-3924

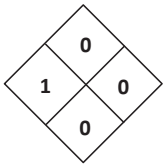
Use of Product: D/2 Biological Solution is a biodegradable, easy to use liquid that removes stains from mold, algae, mildew, lichens and air pollutants. It is effective on marble, granite, limestone, brownstone, travertine, masonry, terra cotta, concrete, stucco, wood, and other architectural surfaces including monuments, sculpture and headstones.

Section 2: HAZARDS IDENTIFICATION

D/2 Biological Solution is a colorless liquid with a very faint detergent-like odor.
It is non-flammable, non-combustible, non-explosive, and non-reactive.

Hazard Rating (NFPA/HMIS)	
Health = 1*	Reactivity = 0
Fire = 0	Special = 0

* Mild eye irritant, non-mutagenic and non-carcinogenic



Rating Scale		
0 = Minimal	1 = Slight	2 = Moderate
3 = Serious		4 = Severe

Eye Contact: Eye Irritant.
Skin Contact: Prolonged skin contact with D/2 Biological Solution may irritate the skin. Repeated daily application to the skin without rinsing, or continuous contact of D/2 Biological Solution on the skin may lead to irritation.
Ingestion: Essentially non-toxic. May cause stomach or intestinal upset if swallowed.
Inhalation: No adverse effects expected under typical use conditions. Adequate ventilation should be present when using D/2 Biological Solution over a prolonged period of time. Open windows or ventilate via fan or other air-moving equipment if necessary. Mucous membranes may become irritated by concentrate mist.
Carcinogens: No ingredients are listed by OSHA, IARC, or NTP as known or suspected carcinogens.
Medical Conditions: No medical conditions are known to be aggravated by exposure to D/2 Biological Solution.

Section 3: COMPOSITION/INFORMATION ON INGREDIENTS

Ingredients	CAS Number	OSHA PEL ACGIH TLV
Surfactants	Proprietary	None established
Wetting Agents	Proprietary	None established
Buffers	Proprietary	None established

Section 4: FIRST AID MEASURES

If in Eyes: Immediately rinse the eye with large quantities of cool water; if present, contact lenses should be removed after 5 minutes of rinsing; continue rinsing 10-15 minutes more. Both upper and lower lids should be lifted to facilitate thorough rinsing.

If on Skin: Minimal effects, if any, from diluted product; rinse skin with water, rinse shoes and launder clothing before reuse. Reversible reddening may occur in some dermal-sensitive users; thoroughly rinse area.

If Inhaled: Use in well-ventilated area, or use adequate protection from inhaling mist during spray applications. Prolonged exposure of workers to concentrate-mist during spray application may cause mild irritation of nasal passages or throat. If this happens, relocate workers to fresh air.

If Ingested: Give several glasses of milk or water to dilute; do not induce vomiting. If stomach upset occurs, consult physician.

Section 5: FIRE FIGHTING MEASURES

Extinguishing Media: Not flammable/non-explosive. No special procedures required.

Special Fire Fighting Procedures: None required.

Section 6: ACCIDENTAL RELEASE MEASURES

Personal Precautions: Avoid contact with eyes. Do not rub eyes with hands during cleanup. No special precautions for dermal contact are needed. Wash hands thoroughly after cleaning up spill or leak.

Procedure to follow in case of spill or leak: Evacuate area. Identify source of leak or spill and contain with sand, earth, or containment bin. Then proceed to clean up spill or leak.

Method for cleaning up: Recover all usable material. Residual may be removed by wipe or wet mope. Rinse area with plenty of water and mop to sanitary sewer.

Section 7: HANDLING AND STORAGE

No special handling is required. Keep in a closed plastic container. Store at ambient temperature. Avoid contact with eyes. Wash hands thoroughly after handling. **This product is non-hazardous for storage and transport according to the U.S. Department of Transportation Regulations.**

This material does not meet the definition of a hazardous material according to 49 CFR, ICAO, IMDG and the UN Orange Book.

Section 8: EXPOSURE CONTROLS/PERSONAL PROTECTION

Precautionary measures: No special requirements under normal use conditions.

Exposure Limits: The D/2 Biological Solution formulation presents no health hazards to the user, other than mild eye irritancy.

Eye protection: Caution, including reasonable eye protection, should always be used to avoid eye contact where splashing may occur, such as during spray applications.

Respiratory Protection: No special precautions required.

Ventilation: No special ventilation is required during normal use.

Skin protection: No special precautions required; rinse completely from skin after contact.

Section 8: EXPOSURE CONTROLS/PERSONAL PROTECTION (cont'd)

General hygiene conditions: There are no known hazards associated with this material when used as recommended. The following general hygiene considerations are recognized as common good industrial hygiene practices:

- Avoid breathing vapor or mist.
- Avoid contact with eyes.
- Wash thoroughly after handling and before eating, drinking, or smoking.

Section 9: PHYSICAL AND CHEMICAL PROPERTIES

Appearance:	Clear Liquid	**Freezing Point:**	-9 °C (16 °F)
Odor:	Very faint detergent-like odor	**Boiling Point:**	98 °C (209 °F)
pH:	9.5	**Specific Gravity:**	1.011
Evaporation Rate:	0.4 (butyl acetate = 1)	**Vapor Pressure:**	20.7 mm Hg
Water Solubility:	100%	**Vapor Density:**	1.3 (air = 1)

Section 10: STABILITY AND REACTIVITY

Stability: Stable.

Materials to Avoid: Contains ammoniated compounds – do not mix with bleach, tub & tile cleaner, mold/mildew removers, or chlorinated compounds.

Hazardous Decomposition Products: None expected

Section 11: TOXICOLOGICAL INFORMATION

Toxicity Data: Available from relevant laboratory testing of ingredients or similar mixtures.

Acute Toxicity: Oral LD50: >2.0 g/kg body weight Dermal LD50: Not estimated

Eye Irritation: With or without rinsing with water, the irritation scores in rabbits at 24 hours did not exceed 17 (mild irritant) on a scale of 110 (extremely irritating); all scores were normal at seven days.

Dermal Irritation: In a standard test on rabbits, mild irritation was found at 72 hours; well-defined reddening was observed at 7 and 14 days after exposure.

Dermal Sensitization: No allergic reactions occurred in guinea pigs treated with D/2 Biological Solution.

Carcinogenicity: D/2 Biological Solution contains no carcinogenic compounds as defined by the National Toxicology Program (NTP), the international Agency for Research on Carcinogens (IARC), or the Occupational Health and Safety Administration (OSHA).

Section 12: ECOLOGICAL INFORMATION

Biodegradability: All components are inherently biodegradable.

Ecotoxicity: Not Tested.

Section 13: DISPOSAL CONSIDERATIONS

Unused Product: Dilute with water 1:10 (1 part D/2 to 10 parts water) and dispose by sanitary sewer.

Used Product: Used product may be hazardous depending on the cleaning application and resulting contaminants.

Empty Containers: Triple-rinse with water and offer for recycling if available. Otherwise, dispose as non-hazardous waste.

Dispose of used or unused product, and empty containers in accordance with the local, State, Provincial, and Federal regulations for your location. Never dispose of used degreasing rinsates into lakes, streams, and open bodies of water or storm drains.

Section 14: TRANSPORT INFORMATION

This product is non-hazardous for storage and transport according to the U.S. Department of Transportation Regulations. D/2 Biological Solution requires no special labeling or placarding to meet U.S. Department of Transportation requirements.

IATA Proper Shipping Name: Detergent solution
Hazard Class: Nonhazardous
UN Number: Not Required

Section 15: REGULATORY INFORMATION

Reportable components: None. The U.S. Environmental Protection Agency (EPA) has determined that propylene glycol ethers are not included within the listed category "glycol ethers" under either EPCRA §313 Toxic Release Inventory or Clean Air Act §112 Hazardous Air Pollutants (both lists include only ethylene glycol ethers). Nor are propylene glycol ethers included in the various EPA Resource Conservation and Recovery Act, and Clean Water Act lists, nor the California Proposition 65 lists.

All components are listed on: EINECS and TSCA Inventory
No components listed under: Clean Air Act Section 112
RCRA Status: Not a hazardous waste.
CERCLA Status: No components listed
TSCA TRI Reporting: Not required / Not listed
CA PROP. 65 Status: No components listed

Section 16: OTHER INFORMATION

Technical information contact:

D/2 Biological Solution
PO Box 3746
Westport, MA 02790
(917) 693-7441
http://d2bio.com

DISCLAIMER: All information appearing herein is based upon data obtained by the manufacturer and recognized technical sources. Judgments as to the suitability of information herein for purchaser's purposes are necessarily purchaser's responsibility. Therefore, although reasonable care has been taken in the preparation of this information, D/2 Biological Solutions, inc. or its distributors extends no warranties, makes no representations and assumes no responsibility as to the suitability of such information for application to purchaser's intended purposes or for consequences of its use.

EnviroKLEAN®

BioWash®

biological soiling remover for monuments & gravestones

OVERVIEW

Enviro Klean® BioWash removes mold and mildew staining and atmospheric staining that disfigures and degrades many types of construction materials. BioWash® is a highly efficient alternative to aggressive cleaners traditionally used on interior and exterior masonry, stone and tile surfaces.

BioWash® can also be applied safely to non masonry substrates such as wood, painted surfaces, metal, plastic and glass. Simply dilute with clean water as directed, and apply BioWash® to the surface. A short contact time, gentle scrubbing and a water rinse are normally enough to remove light-to-moderate soiling and staining typically encountered on building surfaces and monuments.

SPECIFICATIONS

For all PROSOCO product specifications visit www.prosoco.com and click on "SpecBuilder" or "Solution Finder."

ADVANTAGES

- Safe for landscape plantings and grass.
- Safe for interior use in occupied buildings.
- Effective on all types of stone, concrete and brick masonry.
- Non-fuming, low-odor formulation.
- Needs no substrate neutralization.
- Minimal precautions required for handling and storage.
- Easy to apply with brush, roller or coarse spray.
- Biodegradable.
- Concentrated for economy.
- Safe and effective on wood, painted surfaces, metal, glass and plastic.

Limitations

- For removal of heavy biological or atmospheric soiling, consult your PROSOCO representative, or call Customer Care - technical support, toll-free at (800) 255-4255.

REGULATORY COMPLIANCE

VOC Compliance

Enviro Klean® BioWash® is compliant with all national, state and district regulations

TYPICAL TECHNICAL DATA

FORM	Clear, low-odor liquid. Slight amber color
SPECIFIC GRAVITY	1.00
pH	5.5–6.5
WT/GAL	8.34 lbs
ACTIVE CONTENT	Not applicable
TOTAL SOLIDS	Not applicable
VOC CONTENT	Not applicable
FLASH POINT	Not applicable
FREEZE POINT	32°C (0°C)
SHELF LIFE	3 years in tightly sealed, unopened container
SOLUBILITY IN WATER	Complete

BioWash®

PREPARATION

Protect people, vehicles, property and all surfaces not set for cleaning from product, splash, rinse, residue, fumes and wind drift. Protect and/or divert traffic if needed.

Drain water from architectural structures (such as fountains) before application. Carefully brush or scrape loose surface debris, and heavy growths of moss, ivy, or other contaminants from the dry surface.

Fragile or Deteriorated Surfaces

Fragile or deteriorated stone may require reduced rinsing pressure, or even stone consolidation to avoid further damage.

Severely deteriorated limestone and marble may be strengthened enough for thorough cleaning by treatment with Conservare® HCT. HCT also prolongs the service life of acid-soluble stone by dramatically increasing its resistance to acid rain. Consult your PROSOCO representative, or call Customer Care - technical support, toll-free at (800) 255-4255 for more information on use of HCT in conjunction with BioWash®.

Surface and Air Temperatures

Cleaning effectiveness is reduced when surface and air temperatures fall below 50°F (10°C). Do not apply at temperatures below 40°F (4°C). If freezing conditions exist before application, let masonry thaw.

Equipment

Apply using a soft-bristled brush, roller or coarse spray. Rinse with enough water and pressure to flush spent cleaner and dissolved soiling from the masonry surface and surface pores without damage. Inadequate rinsing leaves residues which may stain the cleaned surface.

Masonry-washing equipment generating 400–1000 psi with a water flow rate of 6–8 gallons per minute is the best water/pressure combination for rinsing porous masonry. Use a 15–45° fan spray tip. Heater water (150–180°F; 65–82°C) may improve cleaning efficiency.

Use adjustable equipment for reducing water flow rates and rinsing pressure as needed for sensitive surfaces. Rinsing pressures greater than 1000 psi and fan spray tips smaller than 15° may permanently damage sensitive masonry. Water flow rates less than 6 gpm may reduce cleaning productivity and contribute to uneven cleaning results.

Storage and Handling

Store in a cool, dry place. Always seal container after dispensing. Do not alter or mix with other chemicals. Published shelf life assumes upright storage of factory-sealed containers in a dry place. Maintain temperature of 45–100°F (7–38°C). Keep from freezing. Do not double stack pallets. Dispose of in accordance with local, state and federal regulations.

APPLICATION

Before use, read "Preparation" and "Safety Information."

ALWAYS TEST for suitability and results before overall cleaning. Test using the following application procedures. Let test area dry thoroughly before inspection.

NOTE: Many types of biological soiling change color when exposed to BioWash®. Most surface discoloration will disappear soon after thorough water rinsing and weathering.

Dilution

Adjust dilution rate based on testing. Always pour cold water into empty bucket first, then carefully add product.

Type of Soiling	*Concentrate : Water*
• Light biological staining	1:10
• Moderate biological staining	1:5
• Heavy biological staining	use in concentrate

ALWAYS TEST

ALWAYS TEST a small area of each surface to confirm suitability and desired results before starting overall application. Test with the same equipment, recommended surface preparation and application procedures planned for general application.

Coverage Rates

One gallon of diluted BioWash® treats 80–240 square feet based on surface texture, weather conditions at the time of application, and the severity of soiling.

Application Instructions

1. Working from the bottom to the top, apply generously to dry surface until surface is thoroughly wet.
2. Leave on the surface for 2–3 minutes. If needed, apply more to keep the surface wet.
3. Mist treated surfaces with water and gently scrub with a non-metallic, short-fibered scrub brush to loosen biological soiling.
4. Working from the bottom to the top, rinse thoroughly with clean water. Reduce rinsing pressure as needed for fragile or deteriorated stone. See "Fragile or Deteriorated Surfaces" in "Preparation" section.
5. If used on food-contact surfaces (such as, but not limited to picnic benches or bench-table combos, food-stand counters, eating- or food-preparation surfaces, etc.) a potable water rinse must follow cleaning.

It may take several days for the full cleaning effect to be realized. When practical, allow two or more weeks for biological soiling to disappear. Repeat as necessary to remove remaining biological soiling.

Cleanup

Clean tools and equipment with fresh water.

EnviroKLEAN®

BioWash®

PRODUCT DATA SHEET
PROSOCO
SINCE 1939

SAFETY INFORMATION

Enviro Klean® BioWash® is a water-reduced cleaning product. Use appropriate safety equipment and job site controls during handling and application. Read the full label and MSDS for precautionary instructions before use.

First Aid

Ingestion: Seek medical attention.

Eye Contact: Rinse thoroughly for 15 minutes. Get medical assistance if irritation persists.

Skin Contact: Remove contaminated clothing and rinse thoroughly. Get medical attention if irritation persists. Launder contaminated clothing before reuse.

Inhalation: Remove to fresh air. Get medical attention as necessary.

24-Hour Emergency Information:
INFOTRAC at 800-535-5053

WARRANTY

The information and recommendations made are based on our own research and the research of others, and are believed to be accurate. However, no guarantee of their accuracy is made because we cannot cover every possible application of our products, nor anticipate every variation encountered in masonry surfaces, job conditions and methods used. The purchasers shall make their own tests to determine the suitability of such products for a particular purpose.

PROSOCO, Inc. warrants this product to be free from defects. **Where permitted by law, PROSOCO makes no other warranties with respect to this product, express or implied, including without limitation the implied warranties of merchantability or fitness for particular purpose**. The purchaser shall be responsible to make his own tests to determine the suitability of this product for his particular purpose. PROSOCO's liability shall be limited in all events to supplying sufficient product to re-treat the specific areas to which defective product has been applied. Acceptance and use of this product absolves PROSOCO from any other liability, from whatever source, including liability for incidental, consequential or resultant damages whether due to breach of warranty, negligence or strict liability. This warranty may not be modified or extended by representatives of PROSOCO, its distributors or dealers.

CUSTOMER CARE

Factory personnel are available for product, environment and job-safety assistance with no obligation. Call 800-255-4255 and ask for Customer Care.

Factory-trained representatives are established in principal cities throughout the continental United States. Call Customer Care at 800-255-4255, or visit our web site at www.prosoco.com, for the name of the Enviro Klean® representative in your area.

BEST PRACTICES

Drain water from architectural structures (such as fountains) before application. Carefully brush or scrape loose surface debris, and heavy growths of moss, ivy, or other contaminants from the dry surface.

Fragile or deteriorated stone may require reduced rinsing pressure, or even stone consolidation to avoid further damage.

Masonry-washing equipment generating 400–1000 psi with a water flow rate of 6–8 gallons per minute is the best water/pressure combination for rinsing porous masonry. Use a 15–45° fan spray tip. Heater water (150–180°F; 65–82°C) may improve cleaning efficiency.

Many types of biological soiling change color when exposed to BioWash®. Most surface discoloration will disappear soon after thorough water rinsing and weathering.

It may take several days for the full cleaning effect to be realized. When practical, allow two or more weeks for biological soiling to disappear. Repeat as necessary to remove remaining biological soiling.

Never go it alone. For problems or questions, contact your local PROSOCO distributor or field representative. Or call PROSOCO technical Customer Care toll-free at 800-255-4255.

Enviro Klean® BioWash® Cleaning Specification

Specifier Note: The information provided below is intended to guide the Architect in developing specifications for products manufactured by PROSOCO, Inc. and should not be viewed as a complete source of information about the product(s). The Architect should always refer to the Product Data Sheet and MSDS for additional recommendations and for safety information.

Specifier Note: Paragraph below is for PART 1 GENERAL, Quality Assurance.

Test Area

Test a minimum 4 ft. by 4 ft. area on each type of masonry. Use manufacturer's application instructions. Let the test panel dry 3 to 7 days before inspection. Keep test panels available for comparison throughout the cleaning project.

Specifier Note: Paragraphs below are for PART 2 PRODUCTS, Manufacturers and Products.

Manufacturer: PROSOCO, Inc., 3741 Greenway Circle, Lawrence, KS 66046. Phone: (800) 255-4255; Fax: (785) 830-9797. E-mail: CustomerCare@prosoco.com

Product Description

Enviro Klean® BioWash® removes mold and mildew staining and atmospheric staining that disfigures and degrades many types of construction materials. BioWash® is a highly efficient alternative to aggressive cleaners traditionally used on interior and exterior masonry, stone and tile surfaces. BioWash® can also be applied safely to non masonry substrates such as wood, painted surfaces, metal, plastic and glass. Simply dilute with clean water as directed, and apply BioWash® to the surface. A short contact time, gentle scrubbing and a water rinse are normally enough to remove light-to-moderate soiling and staining typically encountered on building surfaces and monuments.

Technical Data

FORM: Clear, low-odor liquid, slight amber color

SPECIFIC GRAVITY: 1.00

pH: 5.5 to 6.5

WT/GAL: 8.34 pounds

ACTIVE CONTENT: not applicable

TOTAL SOLIDS: not applicable

VOC CONTENT: not applicable

FLASH POINT: not applicable

FREEZE POINT: 32 degrees F (0 degrees C)

SHELF LIFE: 3 years in tightly sealed, unopened container

SOLUBILITY IN WATER: Complete

Limitations

- For removal of heavy biological or atmospheric soiling, consult your PROSOCO representative, or call Customer Care - technical support, toll-free at (800) 255-4255.

Specifier Note: Paragraphs below are for PART 3 EXECUTION, Installation.

Application

Before applying, read "Preparation" and "Safety Information" sections in the Manufacturer's Product Data Sheet for BioWash®. For light biological soiling, mix 1 part BioWash® with up to 10 parts clean water. For moderate biological soiling, mix 1 part BioWash® with up to 5 parts clean water. For heavy biological soiling, use in concentrate.

1. Working from bottom to top, apply generously to dry surface until surface is thoroughly wet.

2. Leave on the surface for 2-3 minutes. If needed, apply more to keep the surface wet.

3. Mist treated surfaces with water and gently scrub with a non-metallic, short-fibered scrub brush to loosen biological soiling.

4. Working from bottom to top, rinse thoroughly with clean water. Pressure rinsing is highly effective at removing all product and biological soiling from surfaces. Reduce rinsing pressure as needed for fragile or deteriorated stone. Severely deteriorated stone may require consolidation prior to cleaning. The best combination of rinsing pressure and water volume is provided by masonry washing equipment generating 400-1000 psi with a water flow rate of 6-8 gallons per minute delivered through a 15-45 degree fan spray tip. Equipment should be adjustable to reduce water flow rate and rinsing pressure as required for controlled cleaning of more sensitive surfaces. See also "Equipment" section of the Product Data Sheet.

5. If used on food-contact surfaces (such as, but not limited to picnic benches or bench-table combos, food-stand counters, eating-or food-preparation surfaces, etc.) a potable water rinse must follow cleaning.

Note: It may take several days for the full cleaning effect to be realized. When practical, allow two or more weeks for biological soiling to disappear. Repeat as necessary to remove remaining biological soiling.

MATERIAL SAFETY DATA SHEET

PROSOCO, Inc.

I PRODUCT IDENTIFICATION

MANUFACTURER'S NAME AND ADDRESS: PROSOCO, Inc.
3741 Greenway Circle
Lawrence, KS 66046

EMERGENCY TELEPHONE NUMBERS:
8:00 AM – 5:00 PM CST Monday-Friday: 785/865-4200
NON-BUSINESS HOURS (INFOTRAC): 800/535-5053

PRODUCT TRADE NAME: **Enviro Klean® BioWash**

II INGREDIENT INFORMATION

INGREDIENT NAME: ACTIVE:	(COMMON NAME)	CAS NO.	ACGIH TLV/TWA	OSHA PEL/TWA
Di-(C8-10)-alkyl dimethyl ammonium chlorides	(Quaternary compounds)	68424-95-3	None established	None established
Alkyl dimethyl benzyl ammonium chloride (C12-16)	(Quaternary compounds)	68424-85-1	None established	None established
Nonyl Phenol Ethoxylate	(Nonionic surfactant)	9016-45-9	None established	None established

III PHYSICAL DATA

	BOILING POINT (°F)	VAPOR PRESSURE (mm Hg)	VAPOR DENSITY (Air = 1)	EVAPORATION RATE
Di-(C8-10)-alkyl dimethyl ammonium chlorides	Not Determined	Not Determined	Heavier than air	Slower than ethyl ether
Alkyl dimethyl benzyl ammonium chloride (C12-16)	Not Determined	Not Determined	Heavier than air	Slower than ethyl ether
Nonyl Phenol Ethoxylate	>201°F	Not Determined	Heavier than air	Slower than ethyl ether

	SPECIFIC GRAVITY	pH	SOLUBILITY IN WATER	APPEARANCE AND ODOR
Enviro Klean® BioWash	1.00	5.5-6.5	100%	Clear liquid, low odor

IV FIRE AND EXPLOSION HAZARD DATA

EMERGENCY OVERVIEW

Enviro Klean® BioWash is a clear, low odor liquid. This product may cause moderate eye irritation. May cause mild skin irritation after prolonged contact. Material is stable and will not burn. Nontoxic by inhalation. Inhalation of concentrate mists may cause upper respiratory irritation.

FLASH POINT (METHOD): Material is stable and will not burn.

FLAMMABLE LIMITS: Material is stable and will not burn.

EXTINGUISHING MEDIA: Not flammable/nonexplosive.

SPECIAL FIRE FIGHTING PROCEDURES: No special procedures required.

UNUSUAL FIRE AND EXPLOSION HAZARDS: None required.

V HEALTH HAZARD DATA

HUMAN HEALTH EFFECTS: BioWash is a moderate eye irritant; mucous membranes may become irritated by concentrate mist.

Prolonged skin contact with BioWash may irritate the skin. Repeated application to the skin without rinsing or continuous contact of BioWash on the skin may lead to irritation. Allergic reactions are not anticipated.

PRIMARY ROUTES OF EXPOSURE: Skin, eyes, inhalation, ingestion.

CARCINOGEN INFORMATION: Not listed (OSHA, IARC, NTP).

MEDICAL CONDITIONS AGGRAVATED BY OVEREXPOSURE: Allergic reactions are not anticipated.

EFFECTS OF OVEREXPOSURE: None expected based upon the available toxicity data.

EYE CONTACT: This product may be irritating to the eyes. Caution, including reasonable eye protection, should always be used to avoid eye contact where splashing may occur, such as during spray applications.

SKIN CONTACT: May cause skin irritation. Gloves recommended for prolonged exposure. Rinse completely from skin after contact. Repeated or prolonged contact may cause moderate to severe irritation.

INHALATION: Mists may be irritating to the respiratory tract and mucous membranes.

INGESTION: Ingestion may cause irritation of the mouth, throat and gastrointestinal tract. Ingestion of this product may result in central nervous system effects including headache, sleepiness, dizziness, slurred speech and blurred vision.

EMERGENCY AND FIRST AID PROCEDURES:

EYE CONTACT: Immediately rinse the eye with large quantities of cool water; continue 15 minutes or until the material has been removed. Both upper and lower lids should be lifted to facilitate thorough rinsing. Seek medical attention at once.

SKIN CONTACT: Concentrate may cause irritation. Minimal effects, if any, from diluted product. Rinse skin with water, rinse shoes and launder clothing before reuse. Wear protective gloves if long-term exposure is likely. If irritation persists, get medical attention.

INHALATION: Prolonged exposure of workers to concentrate-mist during spray application may cause reversible irritation of nasal passages or throat. Relocate workers to fresh air. If symptoms persist, get medical attention.

INGESTION: Give several glasses of milk or water to dilute; do not induce vomiting. Depending on volume ingested relative to size of individual can cause nausea and diarrhea. Get immediate medical attention.

VI REACTIVITY DATA

STABILITY: Stable.

CONDITIONS TO AVOID: None.

INCOMPATIBILITY (MATERIALS TO AVOID): Chlorine. Product should not come into contact with chlorinated products, or other strong oxidizers.

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS: At thermal decomposition temperatures, carbon monoxide, carbon dioxide, and oxides of nitrogen.

VII SPILL OR LEAK PROCEDURES

SPILL, LEAK, WASTE DISPOSAL PROCEDURES: Recover usable material by convenient method. Residual may be removed by wipe or wet mop.

WASTE DISPOSAL METHODS: Fully soluble in water and with dilution is biodegradable. If disposed by sanitary sewer or drain, diluted solutions should not harm sewage-treatment microorganisms. Dispose of in accordance with all applicable local, state, and federal laws. Do not reuse container. Drain container before disposal in household trash.

VIII SPECIAL PROTECTION INFORMATION

RESPIRATORY PROTECTION: No special requirements under normal use conditions. Wear a NIOSH approved dust/mist respirator, when mists are present.

VENTILATION: No special ventilation is required during use.

PROTECTIVE CLOTHING: If you experience dermal sensitivity, wear protective clothing such as long-sleeved work shirt and pants, work boots and neoprene gloves to avoid prolonged skin contact. Do not allow clothing to become saturated with product. If work practices cannot be adjusted to avoid excess clothing saturation, splash resistant or Tyvek® clothing and boots may be required.

PROTECTIVE GLOVES: Use Neoprene or PVC gloves as necessary to avoid prolonged contact.

EYE PROTECTION: Safety glasses with side shields are recommended during use. If work practices or application technique cause a risk of splashing or excessive wind drift, then splash- resistant goggles may be required.

OTHER PROTECTIVE EQUIPMENT: Access to eyewash is recommended. Provide fresh water for rinsing skin.

IX SPECIAL PRECAUTIONS

WORK PRACTICES: Proper work practices and planning should be utilized to avoid contact with workers, passersby, and non-masonry surfaces. Do not atomize during application. Beware of wind drift. See the Product Data sheet and label for specific precautions to be taken during use. Smoking, eating and drinking should be discouraged during the use of this, or any chemical product. Wash hands thoroughly after handling.

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE: No special precautions required. This product is non-hazardous for storage and transport by all modes of transport. Store in a cool and dry place.

OTHER PRECAUTIONS: None.

X REGULATORY INFORMATION

SHIPPING: Non-hazardous for transport by all modes.

SARA 313 REPORTABLE:

CHEMICAL NAME	CAS	UPPERBOUND CONCENTRATION % BY WEIGHT
N/A		

CALIFORNIA PROPOSITION 65: Contains no chemicals listed under Proposition 65.

XI OTHER

MSDS Status:

Date of Revision: April 18, 2002

For Product Manufactured After: N/A – new product

Changes: Section III – pH corrected..

Item #: 41055

Approved By: Regulatory Department

DISCLAIMER:

The information contained on the Material Safety Data Sheet has been compiled from data considered accurate. This data is believed to be reliable, but it must be pointed out that values for certain properties are known to vary from source to source. **PROSOCO, Inc. expressly disclaims any warranty express or implied as well as any liability for any injury or loss arising from the use of this information or the materials described.** This data is not to be construed as absolutely complete since additional data may be desirable when particular conditions or circumstances exist. It is the responsibility of the user to determine the best precautions necessary for the safe handling and use of this product for his unique application. This data relates only to the specific material designated and is not to be used in combination with any other material. Many federal and state regulations pertain directly or indirectly to the product's end use and disposal of containers and unused material. It is the purchaser's responsibility to familiarize himself with all applicable regulations.

DATE OF PREPARATION: April 18, 2002

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