AN INVESTIGATION INTO THE USE OF UV-C LIGHT IN THE ERADICATION OF CONIDIA ON CALCIUM SULPHATE PLASTER SUBSTRATES

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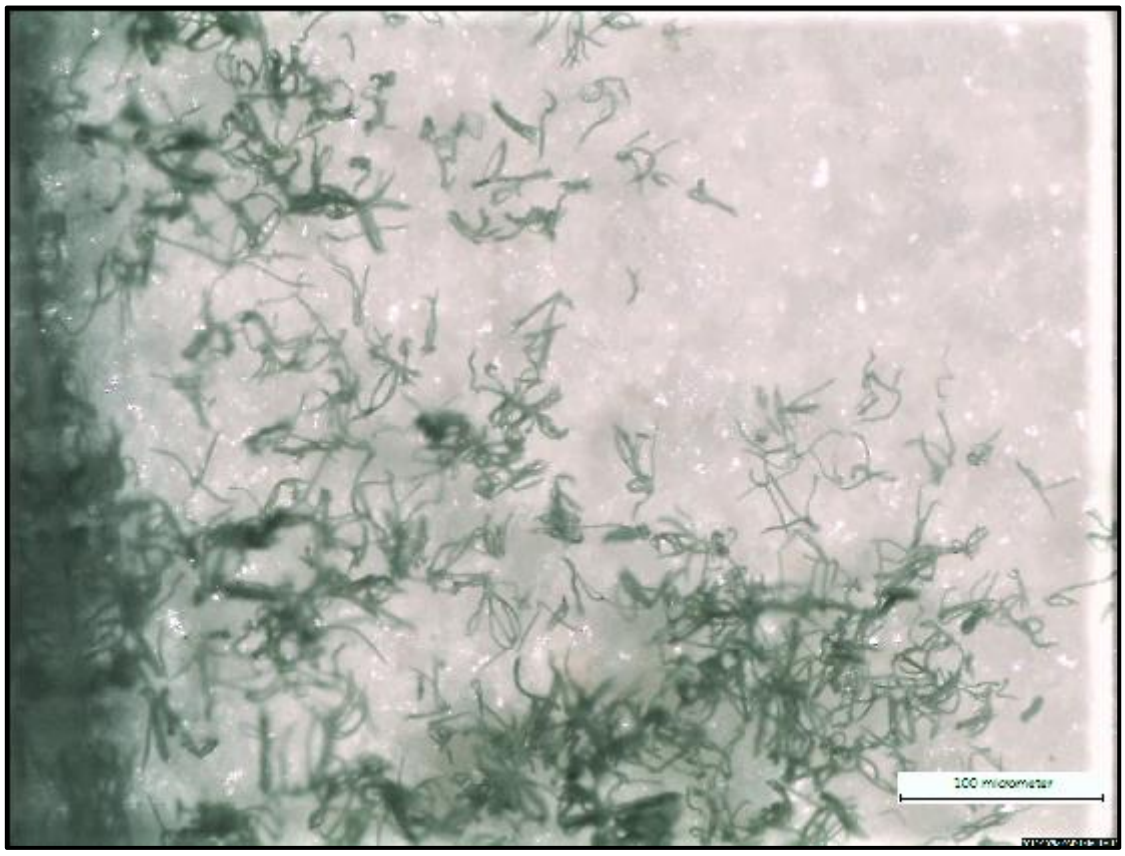

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Acknowledgements

Choosing a conservation thesis topic that involved live mould cultures required a great deal of assistance to execute. I was fortunate to have the support of three fantastic educators who each went the extra distance in their own way to help me through this challenging process. Their patience, humour, and generous expenditure of knowledge, useful feedback, and time inspired me to work beyond my own known boundaries.

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Table of Contents

List of Figures and Tables

All images belong to the author unless otherwise stated.

Figures

Tables

Abstract

The growth of mould on uncoated plaster objects can be a challenging problem for conservators. Conventional cleaning methods, such as the use of chemical fungicides, can damage the porous material. Lowering the humidity and brushing the mould from the surface is the most common treatment used on plaster, but it does not kill the mould. It removes the visible detritus, but the conidia, the reproductive component of mould, remain. The conidia can regenerate, spawning new colonies of mould if the humidity rises again.

This study examined whether the use of UV-C lamps, used to eradicate germs in hospitals and commercial food settings, is safe and effective to use on uncoated plaster objects. The assimilation of medical and scientific equipment for use in conservation is not unusual, and while conservators are often faced with treating damage caused by mould, working with live cultures for the experiments required the advice of microbiologists. Through an interdisciplinary approach, the laboratory procedures of microbiology were adapted to simulate the conditions and mould growth that a conservator might encounter.

To conduct the experiments, plaster samples were created and inoculated with a common form of mould. One group of samples acted as a control, one group was only dry brushed, and another was dry brushed and exposed to UV-C light at the established kill dosage for the species. Cultures from the samples were taken to determine how much living mould remained on the samples after treatment. To determine if UV-C could change the colour of the plaster, tiles were created and spectrophotometer readings of the colour of the plaster were taken. The samples were then subjected to UV-C light for differing lengths of time, and spectrophotometer readings were taken again.

The results suggest that the UV-C treatment did not alter the colour of the uncoated plaster, and was more effective at reducing the number of conidia on the surface than the dry brushing treatment alone. Certain factors, such as the texture of the plaster, may affect how well the UV-C kills the mould, but in certain applications, UV-C lamps may be used to treat mould growth on uncoated plaster objects.

Introduction

The growth of mould on heritage objects is a relatively common problem across the field of conservation. Any collection undergoing a spike in humidity lasting more than a couple of days can experience a mould outbreak, but resolving the issue can be particularly difficult when conservators are faced with treating highly absorbent materials such as plaster.

In October 2018, one such object, a painted plaster bust, was sent to West Dean College for evaluation and possible treatment. The bust is of Dr. David Livingstone, a 19th century physician, explorer, and missionary (The David Livingstone Trust, 2019). The bust was shattered into more than 50 pieces (*Figure 1*).

Figure 1, Broken plaster bust of Dr. Livingstone sent to West Dean College for examination.

In addition to treatments of reconstruction and filling any areas of loss, the uncoated interior of the bust had significant mould growth, (*Figure 2*). Conventional treatments, such as dry brushing and the use of chemical fungicides[1], were considered, but lack of access to the interior and potential damage to the substrate from chemical exposure were problematic.

[1] Any applied treatment that kills fungi.

Figure 2, Interior view of main cavity of hollow plaster bust with significant mould growth.

Preliminary research revealed little information on treating uncoated plaster with mould growth. Victor Borges, a conservator from the Victoria and Albert Museum with extensive experience of plaster collections, as well as two other conservators, were consulted regarding their preferred treatment methods. Andrea Felice, a master craftsman in the creation of plaster sculpture, was also contacted to determine if artisans dealt with mould outbreaks differently from conservators. The consensus was that lowering humidity and brushing off the dry mould was the usual approach, but they knew it did not kill the mould. They had also used chemical fungicides with varying degrees of success and with the understanding that the chemicals could be dangerous to the plaster, as well as themselves.

However, a lecture at West Dean College included a case study where UV-C lamps were successfully used to treat mould on a collection of stone objects (Stanley et al., 2016). Could the UV-C treatment also be safe and effective for use on plaster? Would it be more effective at preventing regrowth than dry brushing alone? The two major concerns regarding the use of UV-C were whether it could kill the mould or alter the colour of the plaster.

Chapter 1: Mould – An Insidious Problem for Collections

This chapter examines mould as a microorganism, the most common ways that conservators and museum professionals manage mould on artefacts, and the ethical foundation for further treatment of mould growth on uncoated plaster objects.

1.1: The nature of mould

Mould, a type of fungus, is present on most surfaces and in the air around the world (Florian, 2002). It performs important ecological functions in the decay of organic material and has been used to create life-saving medications. While vital to the function of the planet, mould can be problematic for humans and objects of cultural importance when it grows in homes and museums.

The most important properties for mould growth are moisture and basic nutrients, often obtained from the air or dust (Heseltine et al. 2009, p.xiv). According to Mary-Lou Florian, a conservation scientist and author of *Fungal Facts: Solving fungal problems in heritage collections*,

> the majority of fungi that grows on the surfaces of the materials of heritage objects are the conidial fungi. These fungi produce conidia that are airborne and land on surfaces, initiating the surface growth. The life cycle of the conidial fungi starts with a conidium[2] that germinates and produces a vegetative stage on which are produced hundreds of new conidia thereby completing the cycle (Florian 2002, p.31).

The conidia are small spheres that grow on the end of stalks, called hyphae (*Figure 3*). A single conidium can spawn multiple colonies of mould. The conidia are protected by hard outer shells made of chitin, an impermeable natural polymer also found in the exoskeletons of insects and crustaceans (Merzendorfer, 2011). This shell preserves the reproductive capabilities of the mould allowing it to survive after the colony has died or desiccated[3].

In her work with *Penicillium*, Iasmina notes that even with inhospitable conditions, conidia can live for months or years (Iasmina, 2012). This longevity can be particularly damaging to collections because a few days of high humidity can generate an active mould infestation.

[2] Singular of conidia.

[3] The process by which moisture is removed from something.

Figure 3, Microscopic image of mould growing on plaster sample at 7.2x magnification on a Nikon AZ100 microscope. The roundish shapes on the mould are conidia. Each conidium can spawn a new colony of mould.

1.2: Current preventative conservation and treatment methods

Most museums rely on preventative conservation standards to control mould growth in collections. Although there is some debate as to how much fluctuation in a 24-hour period is acceptable, the general consensus among many professional organisations[4] is that temperatures be kept between 15-16° and 25°C with relative humidity (RH) between 45% and 60% (Atkinson, 2014). However, many moulds are xerophilic in nature, meaning they can thrive in conditions below 60% RH (Florian, 2002). The World Health Organization lists several types of *Aspergillus* and *Penicillium* species as 'primary colonizers', meaning they are prominent in most indoor spaces around the world (Heseltine et al., 2009, p.11). Yet both

[4] International Institute for Conservation of Historic and Artistic Works (IIC), International Council of Museums – Committee for Conservation (ICOM-CC), American Institute for Conservation (AIC), Canadian Conservation Institute (CCI), National Museum Directors' Conference (NMDC), the Bizot Group, Smithsonian Institution, American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE).

species are known to be xerophilic and can grow below 60% RH. Therefore, the standards by which most museums model their environmental controls, do not necessarily prevent the growth of some of the most prevalent types of mould, so a museum with exacting environmental controls could experience a mould outbreak. Even museums with sophisticated HVAC[5] systems can experience flooding or equipment failure that could quickly lead to an outbreak of mould. For this reason, environmental controls cannot be relied upon as the sole means to preventing mould growth in collections.

Additionally, plaster objects in collections are particularly susceptible to mould growth because plaster is porous, absorbent, and retains moisture from the air. Historically, organic materials such as rabbit skin glue and beer were mixed into plaster to retard the curing process (Wager, 1970). These substances, as well as nutrients found in dust, provide a food source for the mould (Piñar et al., 2013). The combination of moisture and organic materials creates favourable conditions for mould growth.

In addition to being prone to mould growth, plaster objects can be more difficult to treat for mould than other artefacts. Across the field of conservation, chemical fungicides are used to treat wooden objects, ceramics, paintings, textiles, and paper. A 70% solution of ethanol and water is a common chemical fungicide used by conservators[6]. The solution is inexpensive, easy to make, and relatively safe for conservators to use. However, the effectiveness of the solution is tied to the length of time it is in contact with the substrate (Strang and Dawson, 1991). Sterflinger advises the best results with the ethanol solution requires a contact time of 2-3 minutes (Sterflinger and Piñar, 2013). Because certain types of plaster can break down in alcohol and water, its use could be dangerous to the substrate.

While other fungicides[7] are available, most of them have negative impacts on plaster or pose health risks to the conservator (Borges, 2019). For this reason, many conservators move the object to a space with lower RH which will dry out the mould, then use a soft brush to remove the visible mould, and advise the client to keep the RH below a certain percentage.

[5] Heating, ventilation, and air conditioning system.

[6] The 70% ethanol in water solution was suggested as a common fungicide for mould treatment by (Sterflinger and Piñar, 2013), (Rogawansamy et al., 2015), (Borges 2019), and (Stanley et al., 2016).

[7] Gamma radiation, fumigation with toxic gases, and other chemical fungicides.

Although dry brushing has been shown to supress the regrowth of mould (Stanley et al., 2016), it does not kill the conidia. The ability of the mould to regenerate itself after years of inactivity necessitates a treatment method that attempts to eradicate the conidia rather than simply maintaining its dormancy.

1.3: The ethical foundation for further treatment

One of the first questions a conservator might ask when faced with mould growth on an object is: should it be removed? The aesthetic and original intent of the object are important aspects to consider with treatment, but patinas, old repairs, and fingerprints, also give clues about the history of an object. Section VI of the *Code of Ethics and Guidelines for Practice of the American Institute for Conservation* (AIC) states:

> The conservation professional must strive to select methods and materials that, to the best of current knowledge, do not adversely affect cultural property or its future examination, scientific investigation, treatment, or function (AIC, 2019, p.4).

Advances in technology have afforded conservators the ability to learn more about an object through scientific analysis, leading to an ethical drift[8] in conservation where direct intervention is less frequent (Ashley-Smith, 2018).

It is possible that the mould on the Livingstone bust could give clues about its history, and while the concepts of preventative conservation and indirect intervention[9] are often associated, the AIC *Code of Ethics* also states that a conservator's responsibility in regard to preventive conservation is to 'limit damage or deterioration to cultural property' (AIC, 2019, p.4). For the Livingstone bust, leaving the mould for the sake of future testing must be weighed against the chances of the mould causing further damage.

[8] A progression in philosophy for an entity that has specific guidelines, but over time, shifts occur that create distance from established guidelines and become norms without a definitive demarcation (Ashley-Smith, 2018).

[9] A process by which an object is treated without physically touching or altering it such as, monitoring environmental controls or access (Ashley-Smith, 2018).

In a study examining the growth of fungi on stone, Salvadori and Municchia found that:

> The filamentous structures of fungal hyphae favour their penetration into the substrate, depending on its structure, chemical composition and state of conservation. Fissures, cracks, cavities, pores and grain boundaries represent advantage for penetration and provide a more favourable microhabitat compared to the stone surfaces. Fungi can also perforate intact minerals (Salvadori and Municchia, 2016, p.40).

The study further found that certain species of mould excrete acids and produce melanin[10] that can stain or break down minerals through biodeterioration[11]. These processes weaken the structure and leave pitting, disfiguring the surface and making it more susceptible to further growth (Salvadori and Municchia, 2016).

Weighing the retention of a possibly historical surface against the future safety of an object is an example of an ethical dilemma that is common in conservation. It is why the AIC *Code of Ethics* does not set standard treatments, but encourages conservators to consider all factors and act in good faith to the best of their ability.

Is the mould on the Livingstone bust 'original'? Is it a more recent housekeeping issue? The answer is unknown. What is clear is the potential for further damage. If mould can regenerate months or years after coming into contact with an object, it carries inherent risk of possibly catastrophic proportions. In this case, the risk and potential loss of an artefact outweighs the data that could be gained by retaining the mould for possible future study.

The decision of whether to pursue treatment to avoid the regrowth of mould is further complicated by lack of viable options to kill it. Due to the possible damage that could result from various methods[12] of intervention when treating mould on plaster, dry brushing and manipulating environmental controls has been a common approach.

[10] A naturally produced dark pigment.

[11] The chemical processes that occur when secretions from a microorganism, such as mould or lichens, break down the substrate they inhabit.

[12] Chemical fungicides, gamma radiation, and fumigation with toxic gases such as ethylene oxide (Sterflinger and Piñar, 2013).

While conservators probably understand that dry brushing a plaster sculpture will not kill the mould, they are left with few options that are:

a) safe for use on the plaster
b) safe for use by the conservator
c) affordable
d) reasonably attainable by the average conservator
e) effective in eradicating the mould.

This study examines whether the use of ultraviolet light (UV-C) could be a safe and effective method of treating mould on plaster and could help prevent regrowth of the mould if favourable conditions arise. If UV-C light does kill the conidia without harming the plaster, is relatively safe for the conservator, affordable, and requires little training or extra equipment, it could be a useful tool for conservators faced with this treatment dilemma.

Chapter 2: UV-C Light and the Eradication of Germs

This chapter defines the ultraviolet spectrum and how the UV-C light effectively eradicates mould.

The International Agency for Research on Cancer (IARC) categorises the ultraviolet spectrum into three subtypes: UV-A, long wavelength light that radiates at 315-400 nanometres[13] (nm); UV-B that radiates at 280-315 nm; and UV-C light, the shortest of the wavelengths at 100-280 nm (IARC, 2012). UV-C lamps operating at approximately 253.7 nm (*Figure 4*) are the most effective at killing mould because they destroy the nucleic acids within the conidia that are essential in reproduction (Kowalski, 2009).

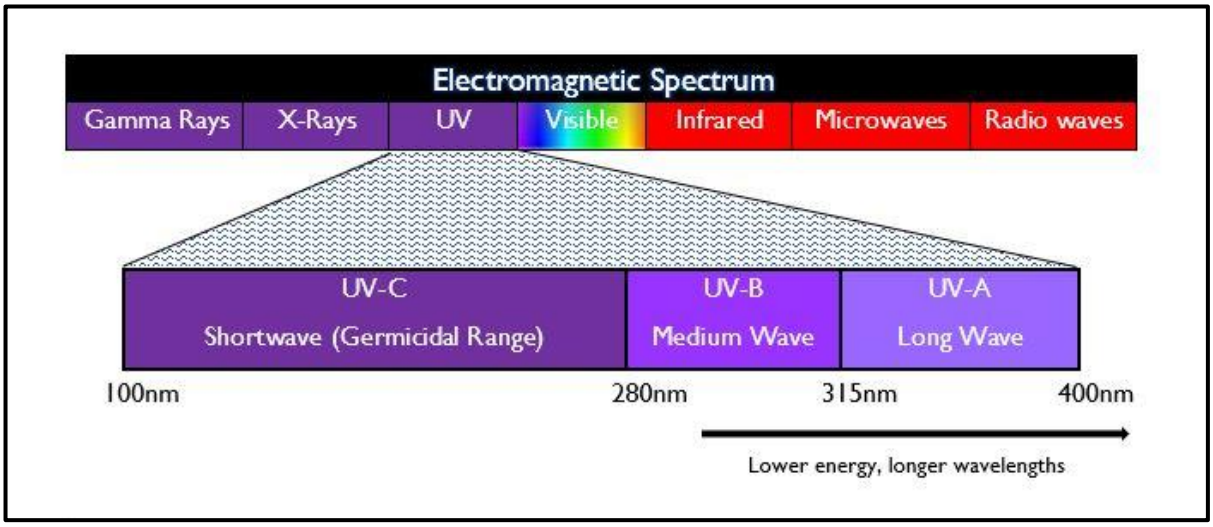

Figure 4, Diagram of electromagnetic spectrum with ultraviolet range.

The technology that utilises UV-C light in the eradication of germs is UVGI, or Ultraviolet Germicidal Irradiation. UVGI has long been used for the eradication of microorganisms, including mould, in medical facilities and places where food is served (Brickner et al., 2003). The West Dean College cafeteria employs a UVGI lamp in the serving area (*Figure 5*). The technology has seldom been considered for use in conservation due to the negative effects to light-sensitive materials, such as paper or painted surfaces. However, uncoated plaster presumably has no colour to fade and might not be susceptible to the same type of damage as other artefacts.

[13] 'One thousand-millionth of a metre' (OED Online, 2019).

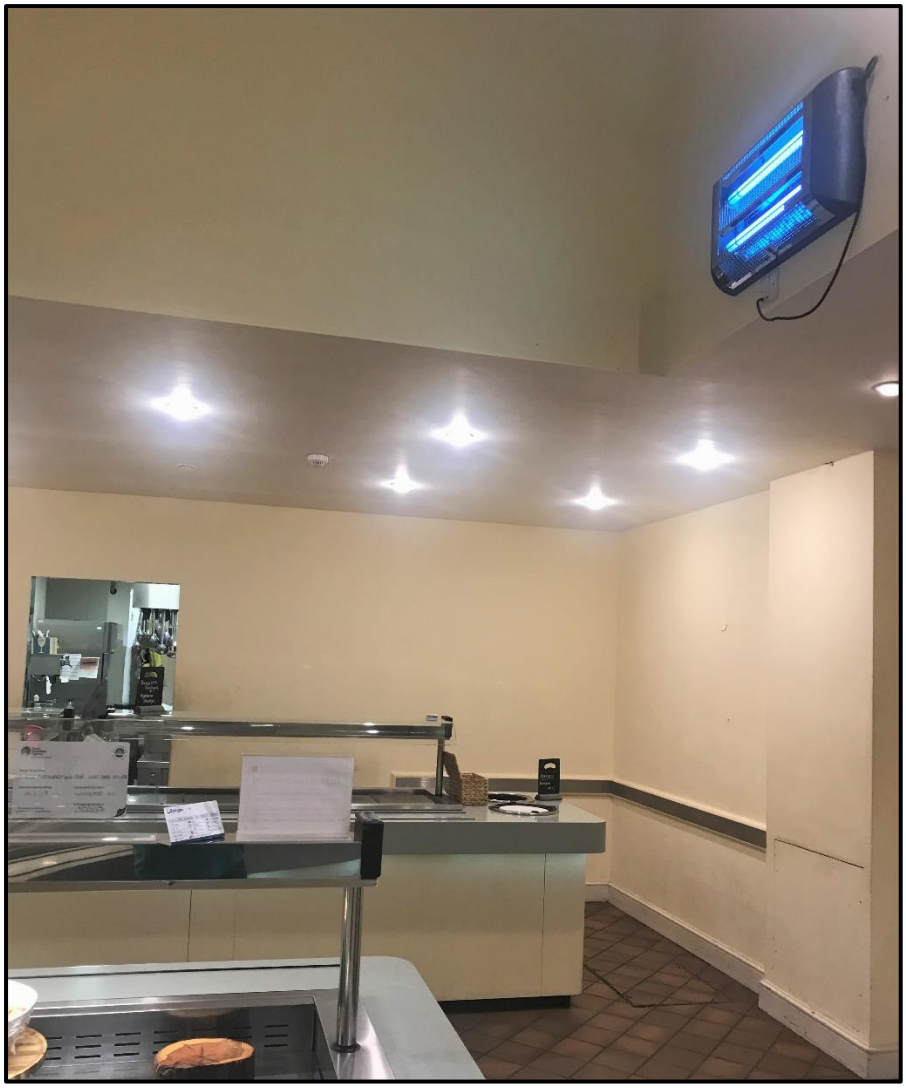

Figure 5, UVGI lamp in serving area of West Dean College Cafeteria.

In a study that successfully used UV-C light to treat algae[14] growth on the plaster[15] walls of the St. Stephanus Church in Pilsum, Germany, the conservators found that UV-C had no effect on the colour of the plaster or wall paint (Van Der Molen et al., 1980), although no scientific quantification of this result was given. While algae is a different organism than mould, its susceptibility to UV-C light operates in a similar way (Zerek, 2014) and could be effective in treating mould on plaster.

[14] Algae and fungi are both microorganisms, but algae and fungi differ in that algae are autotrophs, creating their own food source through photosynthesis. Fungi are considered heterotrophs, feeding off the nutrients in a substrate or local environment (Panawala, 2017).

[15] The type of plaster or whether it contained pigment was not discussed in the study. The affected areas were described as 'modern plaster and whitewash' (Van Der Molen at al., 1980, p.71).

Chapter 3: The Methodology of the Study

3.1: Introduction

The methodology of this study required the use of live mould cultures. Because mould is an allergen and potentially dangerous to handle, experts in microbiology and occupational safety were consulted. The use of UV-C light also had safety risks that had to be mitigated to limit danger to students and faculty.

Dr. Joao Inacio, a Senior Lecturer at the School of Pharmacy and Biomolecular Science at the University of Brighton was consulted during the planning of the study and helped determine which phases of the study would need to be performed in a microbiology laboratory. A safety induction was given by Dr. Inacio to ensure safety protocols and aseptic techniques[16] were understood and followed, and personal protection equipment (PPE) was provided as needed (*Appendix A*). Dr. Inacio retained the live cultures at the laboratory at University of Brighton, monitored the procedures that took place in his laboratory, and completed specific tasks that required the expertise of a microbiologist or laboratory equipment beyond the capabilities of West Dean College.

In addition to recommendations made by tutors, Jasper Richmond, Safety Technician for West Dean College, was consulted regarding policy and procedures for conducting experiments on campus. Recommendations were made and followed as to the location, set-up, and PPE required to safely conduct experiments with mould and UV-C usage. Arrangements were made to dispose of the mould properly once the experiments were finished. Risk Assessments and Control of Substances Hazardous to Health (COSHH) forms were completed for the equipment and materials used in the study (*Appendices B – D*).

Although the steps of the methodology will be explained in detail, please see *Figure 6* for a flowchart of the process.

[16] Refers to 'a set of routine measures that are taken to prevent cultures, sterile media stocks, and other solutions from being contaminated by unwanted microorganisms (i.e., sepsis)' (Bykowski and Stevenson, 2008).

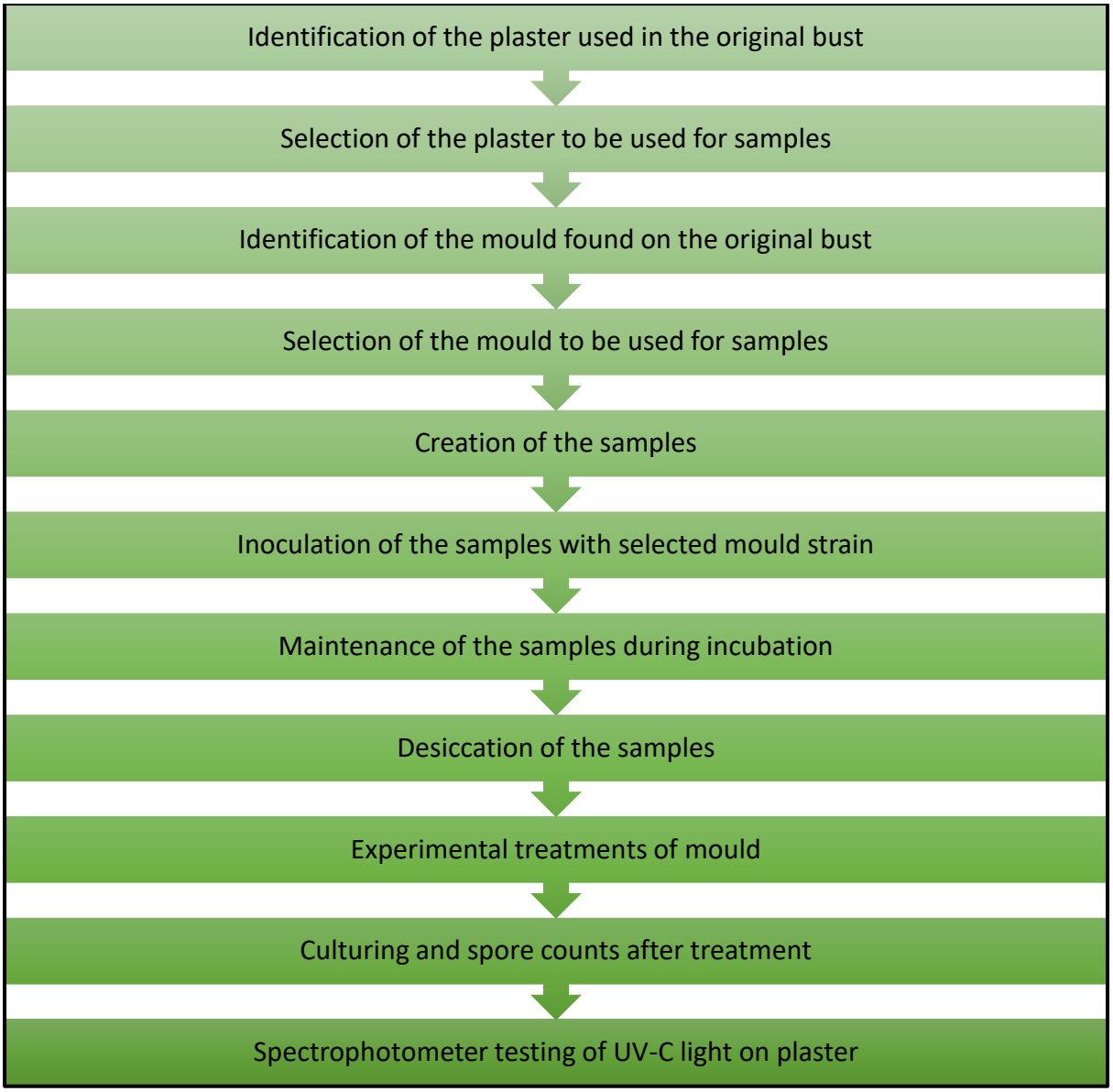

Figure 6, Flowchart of the different steps in the methodology of the study.

3.2: The identification of the original plaster

Because the Livingstone bust belongs to a collection, the study could not be conducted on the original object, but on samples created to mimic the material and conditions that generated mould growth. Although Plaster of Paris, named for the large deposits of gypsum[17] found near Montmartre, France, is the most common material used for casting (Sharma and Prabu, 2013), it was important to determine if this were the casting material used in the bust. Other forms of plaster, such as lime plaster, are made from ground limestone, or calcium carbonate. This material is frequently used in making mortar or wall

[17] A mineral composed mostly of calcium sulphate.

coatings and differs from gypsum-based plaster in that it is harder, less brittle, and dries slowly (Ratcliffe, 1997). It was essential to distinguish between the types of plaster as they have different water absorption rates that could affect the way the mould behaved in the experiment.

To determine the type of plaster, non-destructive X-ray fluorescence (XRF) testing was performed on small pieces of the Livingstone bust. Results revealed that the material contained calcium and sulphur (*Figure 7*), an indication the plaster bust was made of calcium sulphate. Sulphates are compounds of one sulphur atom surrounded by four oxygen atoms (PubChem, 2019), but according to Rigaku, a leading XRF manufacturer, 'Elemental analysis techniques such as ICP[18] and XRF, only detect Ca and S[19], but cannot detect the correct form or phase of the compounds' (Rigaku, 2019).

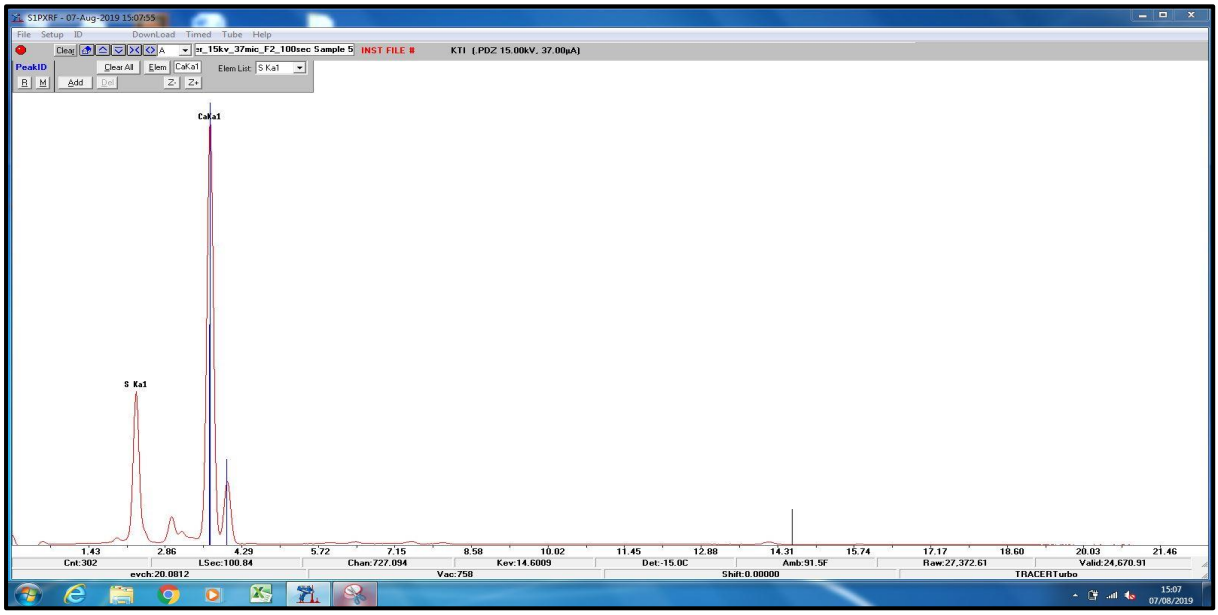

Figure 7, XRF spectrum showing calcium and sulphur elements in the Livingstone bust. For a larger image of the XRF spectrum, please see Appendix E.

XRF testing was also performed on the paint layer of the Livingstone bust (*Figure 8*). Tests showed that the paint had a high copper content as well as zinc, iron, and lead - all commonly used in historical plaster objects (Megens et al., 2011).

[18] Inductively Coupled Plasma.

[19] Calcium and sulphur.

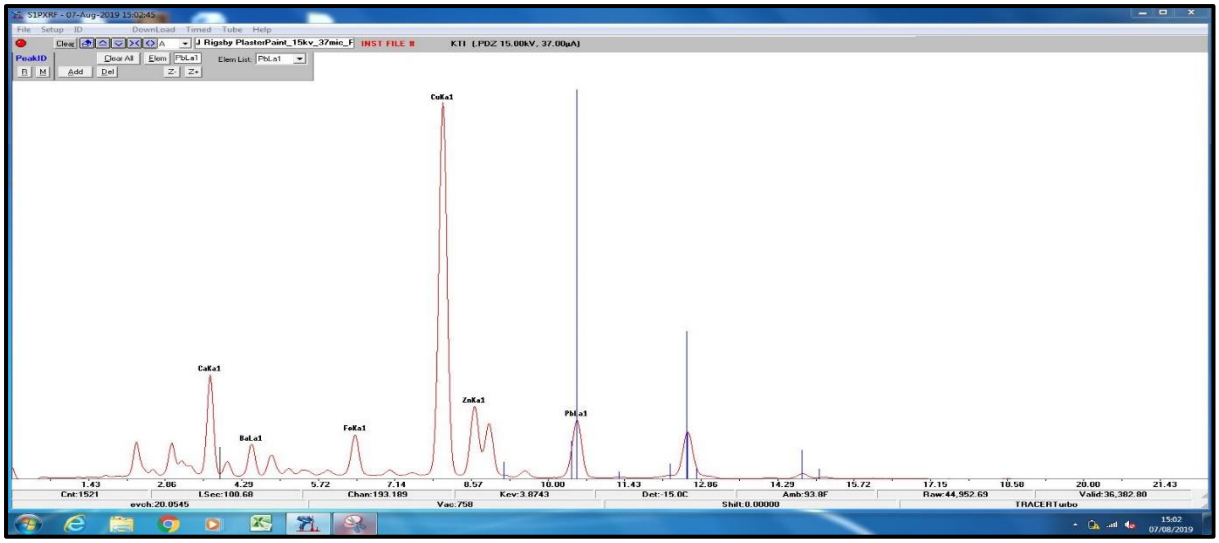

Figure 8, XRF spectrum showing elemental composition of the paint layer of the bust. For a larger version of the spectrum, please see Appendix F.

3.3: The selection of plaster mixture for experimentation

Although XRF testing could not emphatically determine the full composition of the bust, further testing with Fourier-transform infrared spectroscopy (FTIR) also indicated the original plaster was made of gypsum-based calcium sulphate. The FTIR database showed a 99.27% match to modern Basic Alpha plaster, a commercial calcium sulphate-based mixture (*Figure 9*). Basic Alpha was selected to make the samples. For a List of Suppliers, including the Basic Alpha plaster, please see page 93.

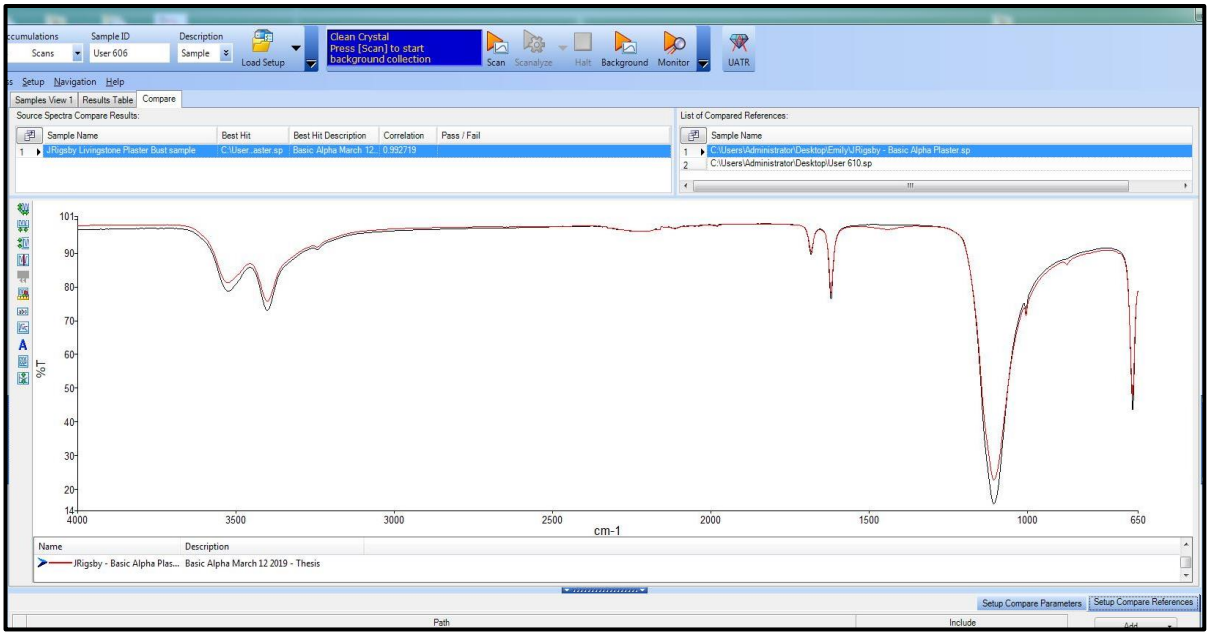

Figure 9, FTIR spectrum showing a 99.27% match between the plaster bust and Basic Alpha plaster. For a larger image of the spectrum, see Appendix G.

With selection of the plaster complete, it was important to consider if any additives should be included in the mixture. In case studies examining mould on gypsum-based materials, researchers mixed nutrient broth into the test samples to ensure propagation (van Laarhoven et al., 2016). Although the addition of growth media was considered, it was decided the samples should resemble the original bust as well as possible. Organic materials such as watered-down rabbit skin glue, called size, were historically added to plaster to retard setting, and size or milk could be brushed onto uncoated plaster to 'close the pores' (Wager, 1970, p.87). These ingredients could be the reason mould attacked the plaster bust. Further testing was needed to determine if these ingredients were present in the sculpture.

Animal and rabbit skin glues rely on a protein called collagen for their adhesion properties (Horie, 2010). A Biuret test[20] was performed to detect the presence of proteins in the plaster and paint of the bust. Small samples of animal and rabbit skin glue, Basic Alpha plaster mixed with both adhesives, and small paint and plaster particles[21] of the bust were created. Nine samples were tested by pulverising a small piece of each item and placing it in a test tube with a solution of deionised water and Biuret reagent (*Figure 10*). For a description of how the Biuret reagent was created, please see *Appendix H*. Three controls were included in the test: deionised water (negative control) and pieces of fish and chicken (positive controls), to ensure the reagent solution was working properly. A violet colour indicates proteins are present, blue or clear indicates no proteins are present (Brilliant Biology Student, 2016).

The results (*Figure 8*) indicated Sample A - Animal Glue and Sample B - Rabbit Skin Glue possessed enough proteins to react with the reagent, but when the same adhesives were added to the Basic Alpha plaster mixtures (Samples C and D), it diminished the response and was not detectable. If a protein-based additive were added to the original plaster bust (Sample E), it was not detectable in this experiment. Sample F containing the paint layer from the plaster bust was inconclusive and turned green, a possible reaction with the copper in the paint (Rising et al., 1930).

[20] The Biuret test is a 'biochemical test to detect proteins in solution, named after the substance biuret, which is formed when urea is heated' (Martin and Hine, 2014).

[21] The samples from the original bust were particulates that were too small to be used in reconstruction of the bust.

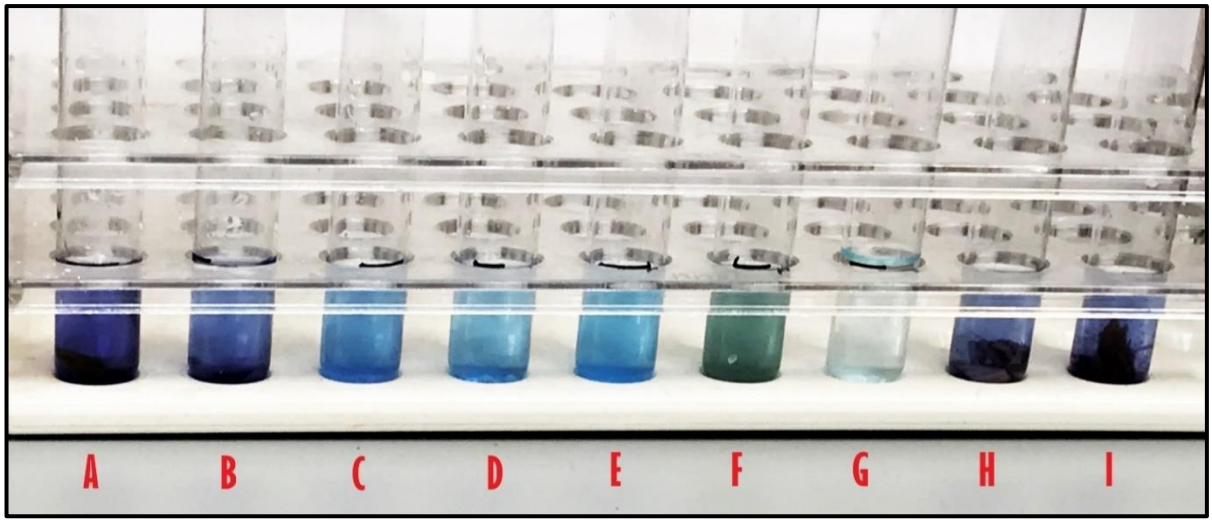

Figure 10, Test tubes with pulverised samples and Biuret/deionised water solution. The colour of the solution indicates if proteins are present. See Table 1 for corresponding results.

Sample	Ingredients	Result
A	Animal Glue	Violet – Proteins present
B	Rabbit Skin Glue	Violet – Proteins present
C	Basic Alpha Plaster with Animal Glue	Blue – No protein detected
D	Basic Alpha Plaster with Rabbit Skin Glue	Blue – No protein detected
E	Plaster from Bust	Blue – No protein detected
F	Paint from Bust	Green – chemical interference, no protein detected
G	Deionised water (Control)	Clear – No protein detected
H	Chicken (Control)	Violet – Proteins present
I	Fish (Control)	Violet – Proteins present

Table 1, Corresponding results of Biuret tested samples.

Testing failed to reveal if the original plaster bust or its paint layer contained proteins, but historical recipes used animal and rabbit skin glues in many applications from sculpture (Wager, 1970) to the manufacture of scagliola[22] and gesso (Rivers and Umney, 2003). It is possible the bust contains one or more of these additives, but the available methods of testing have been exhausted. However, other plaster sculpture from this era used protein-based additives (Megens et al., 2011), thereby making it an option for preparing the plaster samples for the study.

[22] 'Plaster-work of Italian origin, designed to imitate kinds of stone' (OED Online, 2019).

3.4: Identification of the mould

Identifying the mould on the plaster bust was necessary to understand the types of mould that attack plaster. The porosity of gypsum-based plasterboard, a modern building material, is highly susceptible to mould growth in wet climates or flood-damaged areas. A study on the absorbency of gypsum substrates conducted by a Dutch team of scientists found 'that a higher moisture content leads to earlier colonization and higher hyphal extension rates' of mould (van Laarhoven et al., 2015, p.5089). If plasterboard is more susceptible to mould growth than other materials because of the absorbency of gypsum, then art objects made of gypsum-based plaster may also be at higher risk for mould growth than other objects with lower absorbency. The difference in moisture absorption rates among museum objects could be an important factor to consider in their storage, display, and care.

Figure 11, Vials containing particles of the bust sent to SanAir Technologies.

To test the mould on the bust, dry cotton wool swabs were used to collect particles from areas with the highest mould concentration. The cotton wool was placed in a plastic vial. Small particles of the plaster that could not be reconstructed were collected and placed in a separate vial (*Figure 11*). The vials were sent to SanAir Technologies Laboratory in Powhatan, Virginia, USA, specialists in environmental microbial analysis.

Through microscopic evaluation of the material provided, SanAir Technologies reported a light amount of *Aspergillus* or *Penicillium* spores present as well as *Gliomastix* species (SanAir, 2019), see (*Appendix I*). The technicians could not determine if the sample was *Aspergillus* or *Penicillium* species because the spores are too similar in shape and colour. The only way to determine the species was to culture the mould. Permission was granted for culturing, but no mould grew, indicating the fungal material from the bust was dead. It is possible all the mould growth on the bust is dead, a good outcome for Dr. Livingstone's likeness. However, without knowing when the mould grew or if it experienced any subsequent regeneration due to RH fluctuations, it is not possible to know if all of it is dead.

3.5: Selection of the mould culture for experimentation

The three species in the sample were *Aspergillus*, *Penicillium*, and *Gliomastix*. *Gliomastix,* a small group of fungi found in soil, feed on other fungi (SanAir, 2019). It was discounted for the experiment because it needs another fungus to grow. Of the remaining species, *Aspergillus* and *Penicillium*, several factors were considered. While both species are common around the world, it was important to select a mould that met the needs of the project:

1) It must be a common species that conservators might encounter
2) It must grow quickly enough to fit within the timeline of the study
3) It must thrive in set conditions that could be monitored and manipulated
4) It must be a low safety risk for use in the West Dean laboratory.

In 2009, the World Health Organization (WHO) released a list of primary colonisers found in homes around the world. Of the 17 moulds listed, eight were *Aspergillus* species and six were *Penicillium* species (Heseltine et al., 2009). According to the United States National Center for Biotechnology Information, '*Aspergillus* produces some of the most significant known mycotoxins including aflatoxin, gliotoxin, and ochratoxin A' and can cause a disease called aspergillosis (Mousavi et al., 2016, p.37).

Conversely, the P*enicillium* strains on the WHO list were classified as weak pathogens (Heseltine et al., 2009) or common in the decay of fruits, cheese, and grain (Pitt, 2006). The subspecies *Penicillium chrysogenum (P. chrysogenum)* is also known for growing on building materials. This strain was used by the van Laarhoven study that determined gypsum-based building materials were prime breeding grounds for mould because of high water absorption rates (van Laarhoven et al., 2015).

P. chrysogenum is also used to create the antibiotic penicillin (Houbraken et al., 2011), and as a common household mould, it is unlikely to cause major health problems in people with healthy immune systems (Barcus et al., 2005). The biological resource centre that provides living cultures lists *P. chrysogenum* as 'Group 1', a designation of the European Parliament Directive 2000/54/EC, meaning that it is 'unlikely to cause human disease' (European Parliament, 2000, p. L 262/22). These characteristics, along with the knowledge that the *P. chrysogenum* strain to be purchased was harvested from wallpaper and showed an affinity for adhesive and building material, indicated it was the most appropriate choice for this study.

3.6: Creation of the samples

It was decided that 30 samples would be made of Basic Alpha plaster as well as 30 samples of Basic Alpha plaster mixed with rabbit skin glue. It is unknown if a proteinaceous material in the plaster bust provided a food source for the mould, so providing a likely food source in one set of samples enhanced the chances of mould growth within the timeframe.

For the 60 plaster samples, plastic vented petri dishes (90mm x 15mm) were used as they provided a relatively large area for testing and could be covered to prevent contamination.

To avoid cross-contamination from any impurities in the plaster, a new container of Basic Alpha plaster was purchased and the powdered plaster was sterilised in an oven for 15 minutes at 121°C (Kumar C et al., 2013). The plaster was then placed in sterile plastic bags.

Calcium sulphate plaster sets quickly through exothermic chemical reaction (Royal Society of Chemistry, 2019) requiring the samples be made in small batches of six. The materials required for mixing the plaster[23] were sterilised with isopropyl alcohol on cotton wool before use.

The plaster was mixed according to the manufacturer's instructions[24] using pre-packaged sterile water to prevent cross-contamination. Each petri dish was marked at 5mm to indicate the level at which the plaster should be poured. The mixture was spooned into the petri dishes and gently agitated to level the contents. The lid was immediately replaced to protect the sample from contaminants in the air. Leaving the samples uncovered to dry would have been desirable, but it was thought that mitigating the resulting condensation was preferable to the possible introduction of other strains of mould or bacteria. The condensation was drained by tilting the closed dishes until the water ran off the lid and out of the vents. This process was repeated several times until the dishes were dry.

[23] A rubber mixing bowl, metal spoon, and glass beakers.

[24] 2.8kg plaster to 1L of water, or approximately 28g plaster to 10mL of water per dish and 168g of plaster to 60mL of water per batch of six samples.

For the plaster and rabbit skin glue samples, a new package of rabbit skin glue was purchased and mixed to the manufacturer's specifications[25]. It was not possible to sterilise the rabbit skin glue prior to mixing as the recommended preparation temperature is 55-63°C, and temperatures above 90°C can weaken the collagen (Schellmann, 2007). Heated sterilisation requires temperatures at or over 100°C to kill microbes (General Bacteriology, 2019). However, sterile water was used to prepare the adhesive.

Wager recommends 'a tablespoon of thin size to three pints of water' to slow down the cure time of plaster (Wager, 1970, p.4). With conversions to millilitres and the ratio of plaster to water, the mixture for a batch of six samples was approximately 168g of plaster to 60mL of water with an addition of .625mL of rabbit skin glue. As with the plain plaster samples, the petri dishes were filled to approximately 5mm and covered immediately. The sets were distinguished from each other by the tape used to secure the lids: cellotape for the plain plaster samples and masking tape for the plaster with rabbit skin glue (*Figure 12*).

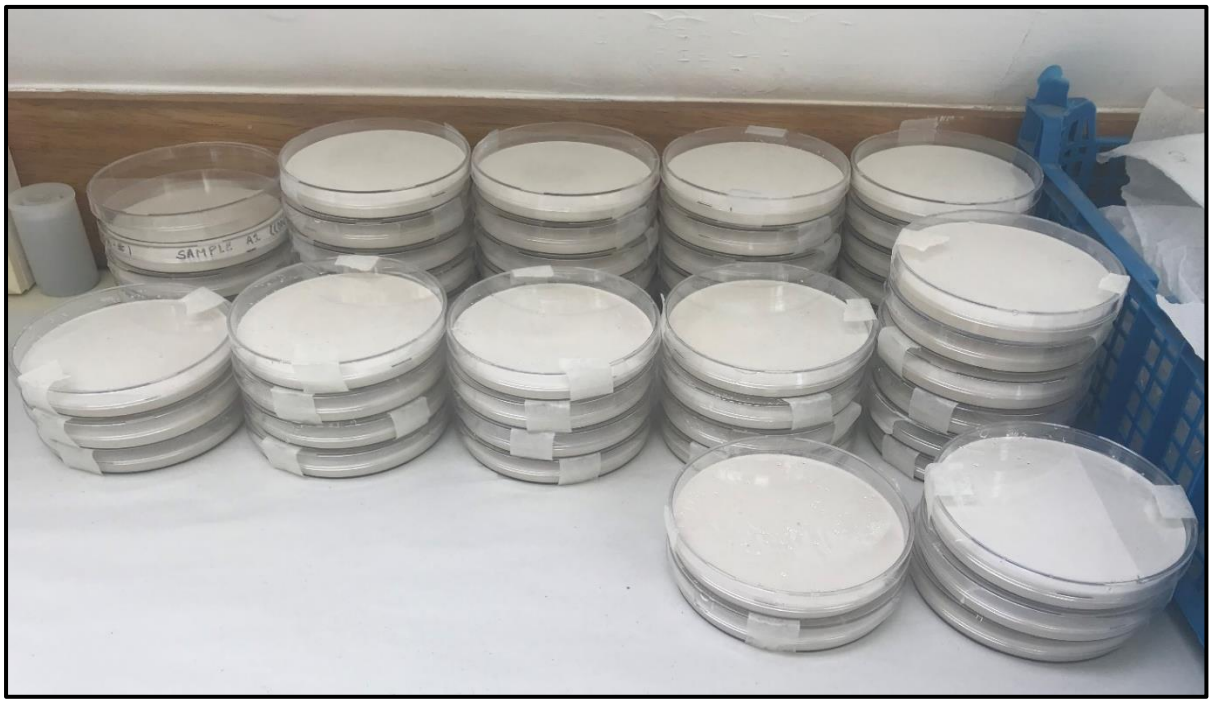

Figure 12, Photograph of both sets of samples in petri dishes after completion.

[25] 13:1 water to granules.

3.7: Inoculation of the plaster samples

The live culture of *P. chrysogenum* was sent from the distributor to the laboratory at the University of Brighton where it was refreshed by removing some of the mould and adding it to new agar[26] plates for propagation. The new plates were incubated at 25°C for two weeks to generate enough growth for inoculation.

Figure 13, Photograph of agar plate containing the refreshed P. chrysogenum *strain. Photograph taken by Dr. Joao Inacio.*

The plaster samples were taken to the laboratory at the University of Brighton. Sterile water was added to two agar plates containing new mould growth. The dishes were shaken to mix the conidia with the water. The water was poured into a beaker and more sterile water added to make a 100mL suspension. The beaker was placed on a magnetic stirrer to evenly distribute the conidia.

The cover of each sample was removed and 1mL of suspension was pipetted onto the surface. A sterile plastic spreader was used to distribute the suspension evenly and the lid was replaced. This process continued for all 60 samples. One mL of the suspension was placed in a Neubauer Chamber[27]. The procedure indicated each sample received an average of $1x10^6$ conidia/mL or 1,000,000 conidia per dish.

[26] 'A gelatinous substance obtained from certain red seaweeds and used in biological culture media and as a thickener in foods' (OED Online, 2019).

[27] A device used under a microscope to count the number of conidia in a given area to determine the average number of conidia contained in a suspension (LaboratoryInfo.com, 2019).

The samples were brought back to West Dean College and placed in an incubator[28] at 30°C. The optimum environment for growing *P. chrysogenum* is 25-30°C with at least 90% RH (Singh and Chauhan, 2012). To achieve the RH, a solution of sodium carbonate in deionised water[29] was placed in the incubator (Lide et al., 2005). A datalogger was placed with the samples to record environmental conditions (*Figure 14*).

Figure 14, Incubator set-up with inoculated petri dishes, glass beaker of sodium carbonate solution, and datalogger.

3.8: Incubation and maintenance of the samples

The samples and environment were monitored daily. Mould needs oxygen to grow, so the incubator was opened each day to provide an air exchange and rotate the dishes for even growth. Because the door was opened often and the incubator thermostat had a ±4°C gradient (Lucky Reptile, 2019), the targeted temperature range was 25-30°C with 90-100% RH for the incubation period. Environmental data was collected every ten minutes throughout this time. For a graph of the datalogger readings for this period, please see *Appendix J*.

It was originally estimated that the incubation period would take approximately two weeks with extra time added as a contingency. In reality, the incubation period lasted 40 days, from 20/05/2019 – 29/06/2019, due to complications with getting sufficient mould growth.

[28] Lucky Reptile II Incubator.

[29] 57.4g to 200mL of water.

By Day 7 of incubation, small spots of mould appeared on both sets of dishes, but by Day 19 there was no discernible progress. Research indicated *P. chrysogenum* grows best at 25-30°C, but it was thought 30°C might be too warm with the ±4°C gradient, and the temperature was lowered by 1°C.

Although the project timeline allowed for a generous growth period, the mould growth was not sufficient to meet deadlines. At this point, the application of a liquid accelerant[30] was considered. Research indicated *P. chrysogenum* grows better when given sufficient amounts of phosphorus, sulphur, iron, potassium, magnesium, zinc, and copper (Jarvis and Johnson, 1950). In a laboratory, these elements are usually delivered by nutrient broth containing sugars[31] and peptone[32] (Aryal, 2019), but for this study, it would take a great deal of time and expense to acquire it.

However, two liquids with significant nutritional content were readily available: plant fertiliser and UHT[33] milk. Both delivery systems were rich in phosphorous, iron, and potassium (*Table 2*), and both liquids could be sprayed for application. The Doff® Growmore 777 fertiliser contained many elements that could encourage growth (Doff Portland Ltd., 2014), and the UHT milk was sterilised, meaning a lower likelihood additional mould or bacteria would be introduced to the samples.

Nutrients in Experimental Accelerants									
	P	S	Fe	K	Zn	Mg	Cu	Sugars	Peptone
Fertiliser	✓		✓	✓	✓		✓		
UHT Milk	✓		✓	✓	✓	✓		✓	✓

Table 2, Nutrients contained in each of the experimental accelerants. (P) = Phosphorus, (S) = Sulphur, (Fe) = Iron, (K) = Potassium, (Zn) = Zinc, (Cu) = Copper.

[30] Refers to a chemical solution to be applied to the mould that can accelerate growth.

[31] Refers to simple sugars such as dextrose, glucose, lactose, and sucrose. These carbohydrates provide the energy needed by mould to reproduce (Aryal, 2019).

[32] Peptones are the enzymatic digest of casein and animal tissue that provide nutrients needed for mould to reproduce (Aryal, 2019).

[33] Ultra-High Temperature processed milk.

On Day 23, fertiliser was diluted with sterile water according to the manufacturer's instructions (Doff Portland Ltd., 2014) and placed in a sterilised spray bottle. The milk was placed in another sterilised spray bottle. Two of the plain plaster samples were marked 'M' for milk and 'F' for fertiliser and photographed. Approximately .5mL of accelerant was added to each sample. The lids were replaced, and the samples returned to the incubator.

By Day 26, there was some mould growth. The M dish had more mould, but there was insufficient growth for testing. Further measures would have to be taken to stay on schedule. The recommended growth media for this study was Sabouraud Dextrose Agar (SDA) (Inacio, 2019a). Ready-to-use SDA plates were already purchased for the culture stage of the study. Sterile water was added to a plate and agitated. Approximately 2mL of the water was poured onto a plain plaster sample. The plate was marked 'S' for Sabouraud and placed back in the incubator. Within three days, the mould growth was significant. It was decided Sabouraud accelerant would be applied to the remaining plain plaster samples. Two samples were left out as examples of mould growth with no accelerant.

A 50% solution of Sabouraud agar in water was created by adding the agar to sterile water in a sterilised spray bottle. The bottle was placed in a double boiler of hot water for five minutes and shaken. Photographs of the samples were taken in a custom light box (*Figure 15*) to show the status of the mould before the added accelerant was added.

Figure 15, Custom enclosed light box for photography. Contains marked template to hold petri dishes in place, neutral coloured lighting with remote control, and a hole in the top for the camera lens.

See *Figure 16* for a 'Before Application' photograph of Sample A3. After the photographs were taken, approximately .5mL of the solution was sprayed on the surface of the sample. The dishes were closed and placed back into the incubator. On Day 6 of the Sabouraud application, 21 of the samples showed adequate mould growth. Photographs were taken of the samples. See *Figure 17* for an example of 'Day 6' Sample A3.

Figure 16, Sample A3 before Sabouraud accelerant applied.

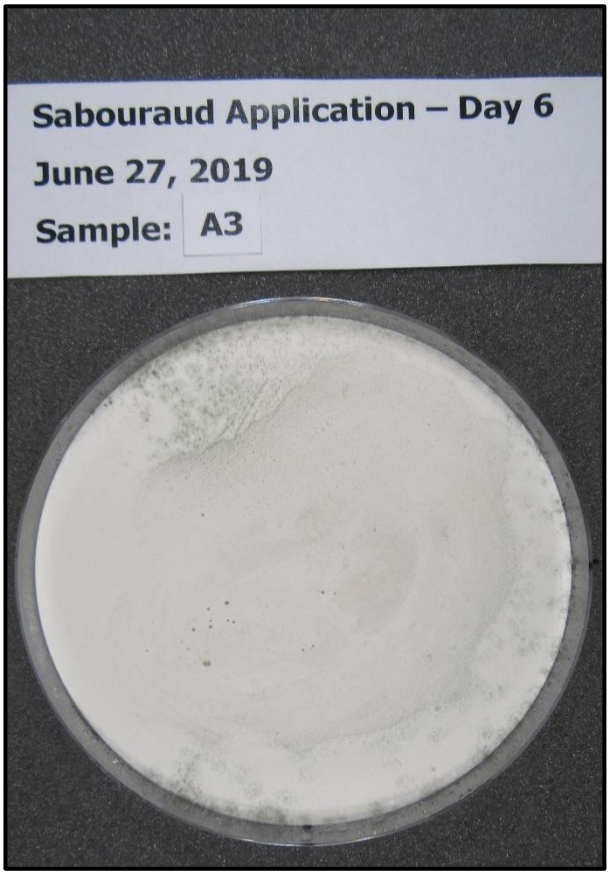

Figure 17, Sample A3 six days after Sabouraud accelerant applied.

Achieving an acceptable amount of mould growth absorbed the entirety of the contingency time allowed for the project. To maintain the timeline for the project, the samples with the Sabouraud accelerant were given eight days of incubation. The decision was made to proceed to the desiccation phase of the project with the 21 viable samples. For a visual timeline of the incubation period, see *Figure 18*.

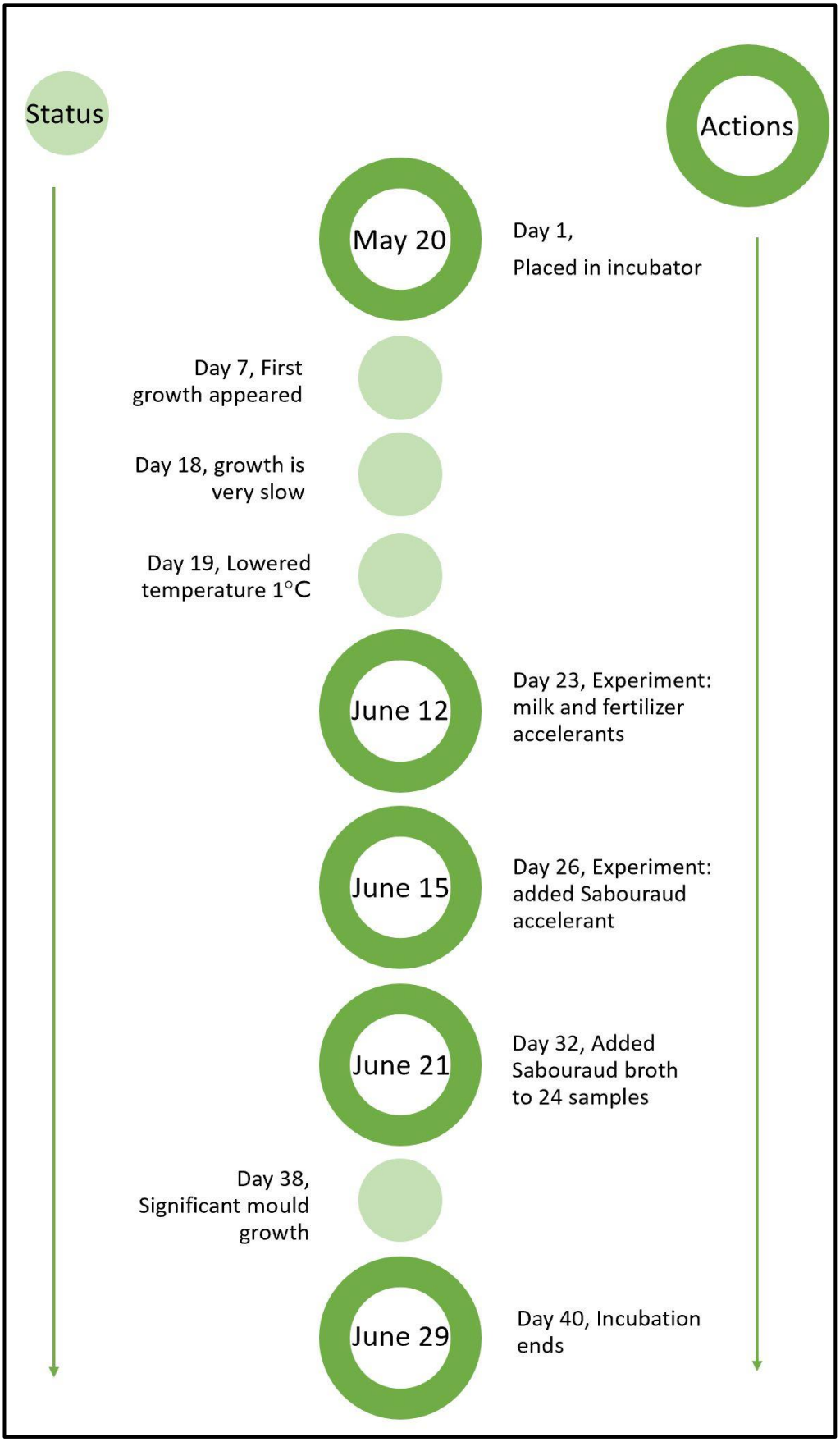

Figure 18, Timeline of the incubation period with major methodology shifts regarding accelerants from inoculation to desiccation stage, a total of 40 days.

3.9: Desiccation of the samples

The desiccation of the samples was an important delineation in the study because it signified the end of the microbiological growth phase and aligned with the point where a conservator might be requested to examine a plaster artefact that had mould present.

Because most mould growth occurs above 60-65% RH, the first element in inhibiting growth is to control the environment and lower the RH to 45-55% (United States National Park Service, 2007). In a severe mould outbreak, most conservators would move an object to a lower RH environment. However, large variations in temperature and RH can be dangerous for porous materials like plaster. Dropping the temperature and RH too quickly can result in delamination of surfaces or even cracking of the plaster. In a 2014 Declaration, the IIC[34] listed several guidelines for the maximum fluctuation in temperature and RH for collections. The declaration cited the Bizot Interim Guidelines for Hygroscopic Materials as follows:

> For many classes of object[s] containing hygroscopic material (such as canvas paintings, textiles, ethnographic objects, or animal glue) a stable RH is required in the range of 40–60% and a stable temperature in the range 16–25°C with fluctuations of no more than ±10% RH per 24 hours within this range (Bickersteth, 2016, p.S1-12).

The first step in drying out the environment was to lower the temperature 1°C in the incubator, bringing it to 27°C. The temperature was systematically lowered over the next days to bring the samples into the range of the IIC guidelines. For a condensed version of the temperature and RH recordings of the desiccation period, please see *Table 3*.

To bring down the RH, the sodium carbonate solution was halved and replaced with tap water, and later removed altogether. The RH continued to be high, and it was thought that the plaster samples might be holding moisture. All the unused samples were removed from the incubator, and a pre-conditioned 45% RH Pro Sorb cassette[35] was introduced. Although the cassette was new, and the manufacturer's instructions were followed, the cassette did not properly regulate the incubator space. The samples were moved to a laboratory refrigerator that was recorded at 15°C and 47% RH. The thermostat on the refrigerator

[34] International Institute for Conservation of Historic and Artistic Works.

[35] Pre-packaged containers filled with silica gel used to control humidity within enclosures. The cassette was reported to regulate RH to 45% in a space up to 1m^3 (Preservation Equipment Ltd., 2019).

was raised to bring up the temperature, but instead, the temperature went down. After several attempts, it appeared that the refrigerator could not maintain a consistent temperature and could have been affected by the high temperatures in the laboratory.

An alternative solution was employed in which the samples were placed in a plastic container with 5kg of silica gel desiccant (*Figure 19*). The petri dishes were vented, allowing for air circulation, and the silica gel had an indicator component: it changed from orange to clear when saturated. The edges of the container were sealed with tape. Each day, the datalogger was checked and the silica gel reconditioned by baking it in an oven at 130-140°C for three hours (Merck Millipore, 2019). Rather than a controlled descent of 10% RH each day, the RH with this method was more erratic, with swings of 30% or more each day. This method was less controlled than anticipated, but by Day 7 of desiccation, the mould could be lifted from the surface with a dry brush, indicating that it was sufficiently dry to proceed to the treatment phase. The samples were left in the container with the silica gel until time for treatment. Please see *Table 3* for a timeline of the desiccation process.

Figure 19, Desiccation of samples in small plastic container with indicating silica gel and datalogger.

Date	Time	Action	Temp. °C*	RH %*
29/06/2019	10:55	Lowered temp 1°C	27.8	100
	17:25	Lowered temp 1°C	26.5	97
30/06/2019	13:07	Lowered temp 1°C, removed half of the sodium carbonate solution and replaced it with tap water	25.2	91
01/07/2019	08:24	Lowered temp 1°C	25	88
	09:51	Removed all water from the incubator, placed a pre-conditioned 45% RH ProSorb cassette in the incubator	23.6	87
	16:43	Removed all extra samples that would not be used in the study	23.3	86
02/07/2019	09:11	Moved samples to the top shelf of the incubator (drier air)	22.1	84
	10:50	Moved samples to laboratory refrigerator	18.3	73
	15:10	Temp. low, adjusted refrigerator dial	8.2	57
	17:50	Temp. low, adjusted refrigerator dial	0.3	40
03/07/2019	10:00	Temp. too low, initiated new protocol	6.5	57
	13:20	Moved samples to small container, placed samples on top of silica gel, sealed edges with tape	19.8	59
04/07/2019	09:10	Checked datalogger, top layer of silica gel turning from orange to clear under samples	23	47
	15:05	Reconditioned silica gel	21	30
05/07/2019	09:10	Reconditioned silica gel	20.9	35
06/07/2019	10:50	Reconditioned silica gel, layered the silica gel on top of and around petri dishes	21.2	84
07/07/2019	12:10	Reconditioned silica gel, layered the silica gel on top of and around petri dishes, tested the mould by brushing gently, the mould is dry	23.8	49
08/07/2019	09:14	Desiccation complete	21.1	10

*Table 3, Timeline of desiccation process from 29/06/2019 – 08/07/2019. *The accuracy of the Onset HOBO© Datalogger for temperature is ± 0.35°C from 0° to 50°C and the RH is ± 2.5% from 10% to 90% below 10% and above 90% ±5% typical (Onset, 2019).*

3.10: Mould treatments

The purpose of the study was to determine if the use of UV-C light is more effective at reducing the conidia on the surface of uncoated plaster than dry brushing alone. The use of the UV-C light would not be a replacement for dry brushing – it is in addition. Once an object is stable and the mould has dried out, it may be necessary to remove the visible surface debris. Dry brushing is an effective way of achieving this if the surface can withstand the treatment. Then, by exposing the uncoated plaster to the UV-C light, it is thought that the chances of regrowth may be further diminished.

To demonstrate this point, the 21 dishes with dried mould were subjected to different treatments. Because the dishes displayed varying levels of mould growth, they were divided evenly to ensure fairness in testing. The 21 dishes were subdivided into three groups: those with low mould growth (5), medium mould growth (7), and high mould growth (9).

Three new groups of seven dishes each were created to ensure that examples of every growth level were included, i.e. each new group received three dishes with high mould growth, two with medium mould growth, and one with low growth. The remainders were randomly placed in groups. Another student was asked to randomly assign letters A, B, and C to the three groups with no knowledge of what the letters would represent.

These three groups represented the types of treatment to be examined in this study:

A) Control – No Treatment
B) Dry Brushing Treatment Only
C) Dry Brushing with UV-C Treatment.

Each dish was assigned a group letter, A, B, or C, and given a number, 1-7, and photographed before treatment. Group A was placed back into the container with the silica gel.

Groups B and C were taken outside to undergo dry brushing treatment. All PPE requirements were met: a well-ventilated area, and particulate mask and gloves were worn. Each dish was brushed with a No. 6 hog bristle brush working in a counterclockwise motion towards the centre of the dish until all visible mould was removed (*Figure 20*). A new brush was used for each dish to avoid cross-contamination. The lids were replaced immediately

upon completion of the dry brushing. Group B was photographed and placed back into the container with the silica gel.

Figure 20, Dry brushing plaster samples with mould growth.

Group C was taken to the analytical laboratory for UV-C treatment[36]. The lamp used for this project was the Analytik Jena 95-0016-15 Shortwave UV lamp, 4 watts, 230 VAC/50 Hz (*Figure 21*).

Figure 21, The Analytik Jena 95-0016-15 handheld UV-C lamp. The lamp is often used for sterilisation in small applications as well as forensic document examination and DNA analysis. The lamp was selected for the study because of the strength of the bulb, its portability, and relatively low cost (Analytik Jena, 2019).

[36] A 'No Entry' sign was placed on the door warning of the danger of exposure to UV light and appropriate PPE was worn throughout the testing.

The lamp emits ultraviolet intensity at 1120µW/cm^2 at 3" distance (Cole-Parmer, 2019). The following is a breakdown of the exposure time for Group C:

Conversion of microwatts to milliwatts: 1120µW/cm^2 = 1.12mW/cm^2

Converted lamp intensity = 1.12mW/cm^2 at 3"

The 99.9% kill rate, or dosage[37], for *P. chrysogenum* is 150mWsec/cm^2, (Renzel, 2016). Therefore, the correct UV-C exposure time for Group C is:

$$\frac{150\text{mWsec}}{\text{cm}^2} \div \frac{1.12\text{mW}}{\text{cm}^2}$$ or 133.9 seconds (2:14 minutes) at 3"

The lamp was placed in its stand and sheets of polyethylene foam were layered to raise the height of the samples to 3" distance from the lamp (*Figure 22*). Each sample in Group C was placed under the UV-C light for 2:14 minutes[38]. Group C was photographed and placed back into the container with the silica gel.

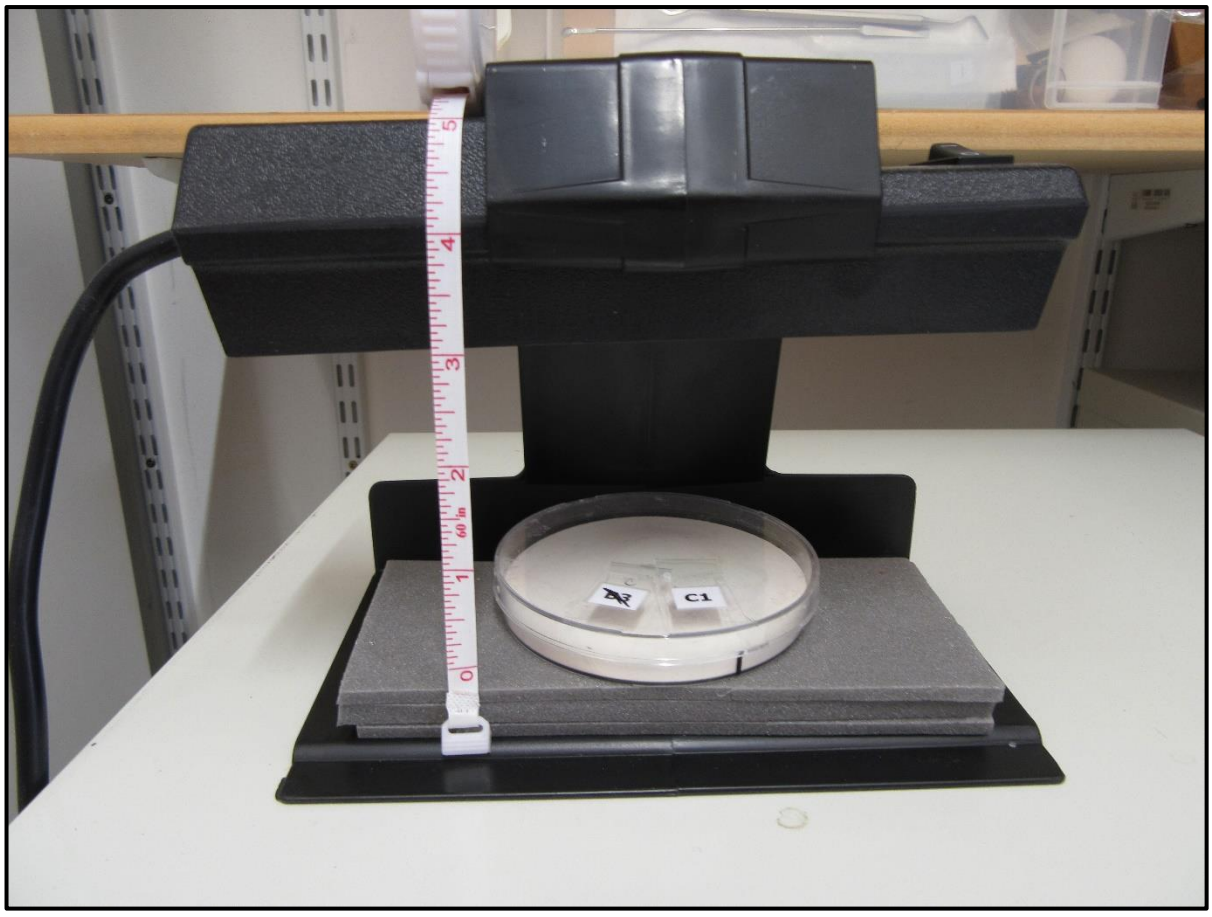

Figure 22, Configuration of UV-C lamp, and sample placement for treatment. The bulb was placed at a 90° angle and 3" to the surface of the sample.

[37] The required amount of UV light intensity and exposure time to kill microorganisms (DaRo UV, 2019).

[38] A timer application on a mobile phone was used to measure the time under the UV-C lamp.

3.11: Culturing of the samples

To determine if the treatments were successful, a culture was taken from each sample. Due to the technical nature of this phase, the samples were processed at the University of Brighton microbiology laboratory. Under the direction of Dr. Joao Inacio, each sample underwent a serial dilution[39] with five levels: 1:1, 1:10, 1:100, 1:1000, and 1:10000. See *Figure 23* for an explanation of the serial dilution process.

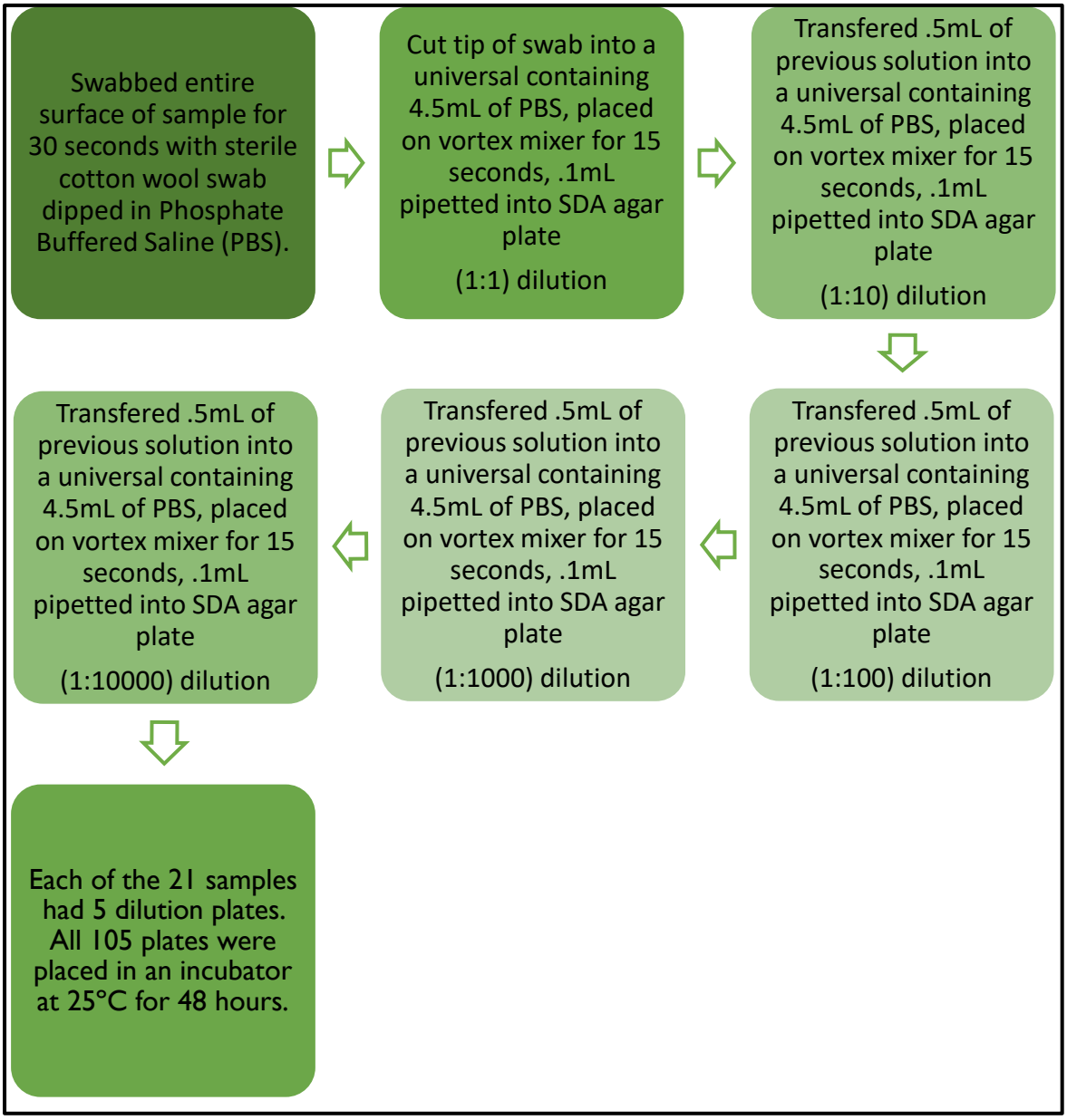

Figure 23, Flowchart of serial dilution process for this study.

[39] 'A series of sequential dilutions used to reduce a dense culture of cells to a more usable concentration' (Study.com, 2019).

In the inoculation phase, approximately 1,000,000 conidia were introduced to each dish. The high inoculation count furthered the chances that mould would grow. The purpose of swabbing the surface for the culture was to pick up as many of the living conidia as possible and deposit them in the 1:1 PBS[40]solution for the highest level of mould concentration. A portion of that suspension, .1mL, was placed in a prepared SDA[41] agar plate, but at that strength, it would grow too many colony forming units, or CFUs, so .1mL of the highly concentrated solution was diluted by placing it into the next universal[42] with fresh PBS. That solution was mixed, and then .1mL was added to a new plate and another .1mL added to the next universal with fresh PBS. The process was continued until there were five plates with differing dilution levels. In this way, one plate would have a reasonable number of colonies to count.

With 21 plaster samples and five dishes of dilutions for each sample, there were 105 total plates created from the culturing process. Due to the equipment and technical expertise required to count the cultures, and the necessity to correctly dispose of so many live cultures, it was decided that the plates would remain at the University of Brighton for incubation, counting, and disposal. The plates were placed in the incubator in the microbiology laboratory at 25°C for 48 hours. For a full reading of the protocol set forth for this study by Dr. Inacio, please see *Appendix K*.

3.12: Testing the effects of UV-C light on calcium sulphate plaster substrates

Testing the efficacy of UV-C light in the treatment of mould on calcium sulphate plaster substrates was the main goal of the study, but the use of UV-C light would be irrelevant if it caused harm to plaster. Although plaster is considered 'white', inert, and lacking in pigmentation, no research could be found to verify that UV-C would be safe to use. A basic test was devised to detect changes in colour to plaster after UV-C light exposure.

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[40] Phosphate Buffered Saline, or PBS, is a common solution used in microbiology for washing cells or creating dilution. The solution is non-toxic, pH neutral and does not affect the osmolarity of a cell. Osmolarity refers to the shrinkage or expansion of the cell wall due to available water content (Oklahoma University, 2019).

[41] Sabouraud Dextrose Agar, the same medium used as an accelerant during the growth phase of the study.

[42] Containers typically used in a scientific or medical laboratory for the 'collection and storage of biological matter' (Alpha Laboratories, 2018).

The Konica Minolta CM-2300d spectrophotometer[43] was chosen as the method for detecting colour changes for this study. The test relies on CIE[44] L*a*b theory, a standardised method of characterising colour by three values:

> L* indicates lightness, a* is the red/green coordinate, and b* is the yellow/blue coordinate. Deltas for L* (ΔL*), a* (Δa*) and b* (Δb*) may be positive (+) or negative (-). The total difference, Delta E (ΔE*), however, is always positive (Konica Minolta, 2019a).

In this way, a target recording of an area can be taken and compared to a sample, for example, after exposure to UV-C light.

The plaster samples had to remain in an incubator for most of the study and the risk of contamination was too great to use those samples. Twenty tiles were made of the sterilised Basic Alpha mixture with sterile water at the same ratio as the other samples. The tiles were allowed to dry for two days, but moisture could still be felt in the surface of the samples, so they were given three more days to dry. On the fifth day, the samples were dry to the touch and ready for testing. The tiles were arranged into four categories (*Figure 24*):

A) Control – no UV-C exposure
B) Underexposure to UV-C, 1:07 minutes
C) Correct exposure to UV-C, 2:14 minutes
D) Overexposure to UV-C, 4:28 minutes.

The times for exposure were based on the same reported kill rate for *P. chrysogenum* that was used on the previous samples, 2:14 minutes at 3". If a conservator were trying to treat that particular mould, 2:14 minutes is the length of time the plaster would have also been exposed to the light. By including underexposure and overexposure rates, the testing would determine if a smaller amount of light could do harm or if a larger exposure would have similar or more impact than the recommended dosage.

[43] 'An instrument designed to measure the relative intensity of light (usually transmitted or emitted by a substance under study) at different wavelengths in a particular region of the spectrum' (OED Online, 2019).

[44] Commission Internationale de l'Eclairage.

Figure 24, Uncoated plaster tiles prepared for spectrophotometer testing.

To ensure the 'before' and 'after' readings were taken from approximately the same area, a circle slightly larger than the reading area of the spectrophotometer was drawn on the back of each tile (*Figure 25*). The backs of the tiles were chosen for testing because they were the smoothest surface and could remain face-down in their casting containers to avoid any external light exposure. An arrow was added to the back of the tile to ensure the tile was being placed in the same direction each time to minimise differences in the reading area.

Figure 25, Detail of back of plaster tile used in spectrophotometer testing.

A single target recording was taken from the centre of all 20 tiles. Group A, the control, received no light exposure. Groups B, C, and D were exposed to the UV-C light for the prescribed amounts of time using the same lamp set-up that was used in the treatment of the mould samples: the tiles were placed on layers of polyethylene foam at a 90°angle, approximately 3" distance from the lamp. A single sample recording of each tile was taken and compared to the original target sample taken prior to UV-C exposure.

Chapter 4: Results and Limitations

This chapter examines the results that were gathered from the testing phase of the study. The results from the treatment of the mould samples will be discussed as well as the outcome of the spectrophotometer testing on plaster tiles. The limitations of the study will also be examined.

4.1: Results from mould cultures

After a 48-hour incubation period at the University of Brighton, the 105 plates were removed from the incubator and the colonies counted by Dr. Inacio.

The purpose in making five plates with different dilution ratios was to select the dish with 30-300 CFUs[45]. This range of CFUs is standard in the microbiology field and the basis for the counts used in this study. Below 30 CFUs, there are more variables to consider that create discrepancies, whereas counts above 300 CFUs tend to overlap and can be difficult to count (Sutton, 2012).

In counting the CFUs, the lower the dilution of the plate with the appropriate CFU range, the more successful the treatment. For example, to find the right range in the cultures from sample C6, one of the plates that received UV-C treatment, the very first dish in the serial dilution, 1:1 was counted (*Figure 26*). The 1:1 dilution had the highest concentration of living conidia. Conversely, to find the same range in the cultures from sample A2, one of the control plates that received no treatment, the last dish in the serial dilution was counted, 1:10000, (*Figure 27*), what should have been the lowest concentration of living conidia.

For photographs of all the cultures, please see *Appendix L*.

[45] Colony forming units.

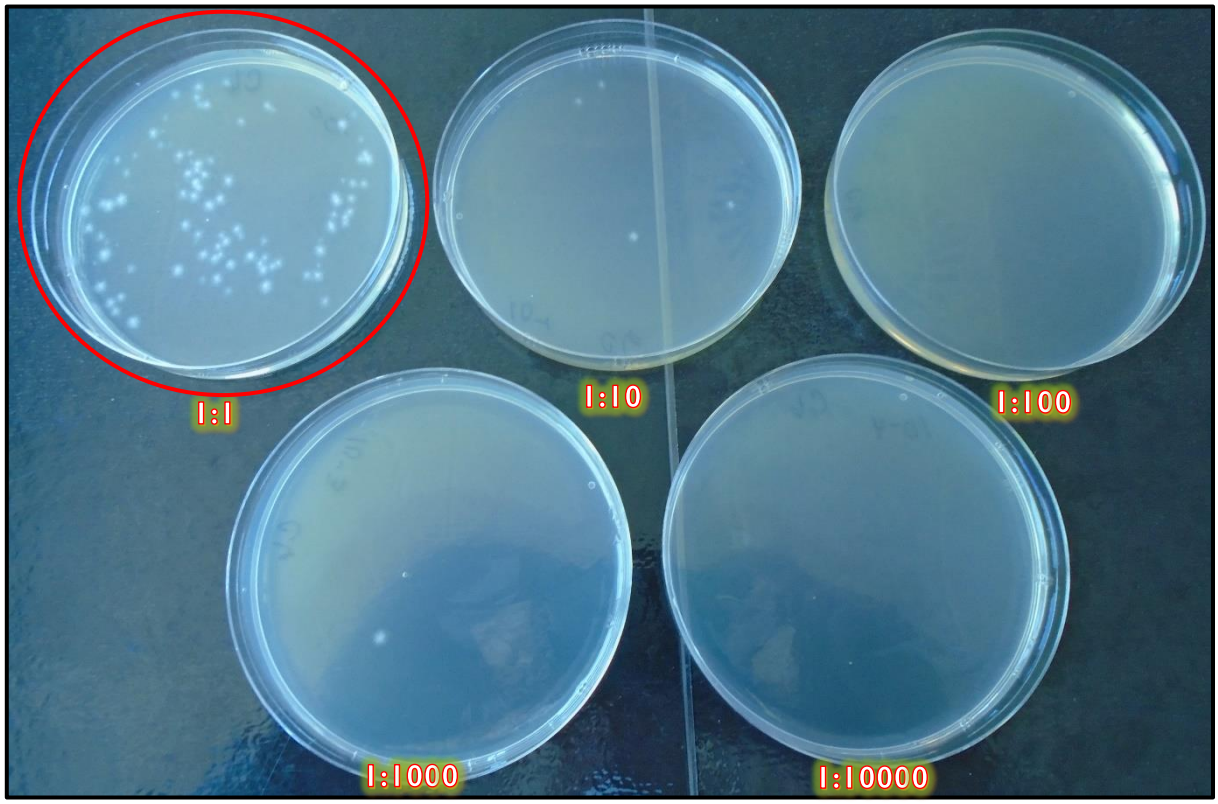

Figure 26, The cultures from sample C6 (UV-C treated), showing the 1:1 ratio dish (circled in red) as having the appropriate range for counting CFUs. There were 86 CFUs counted.

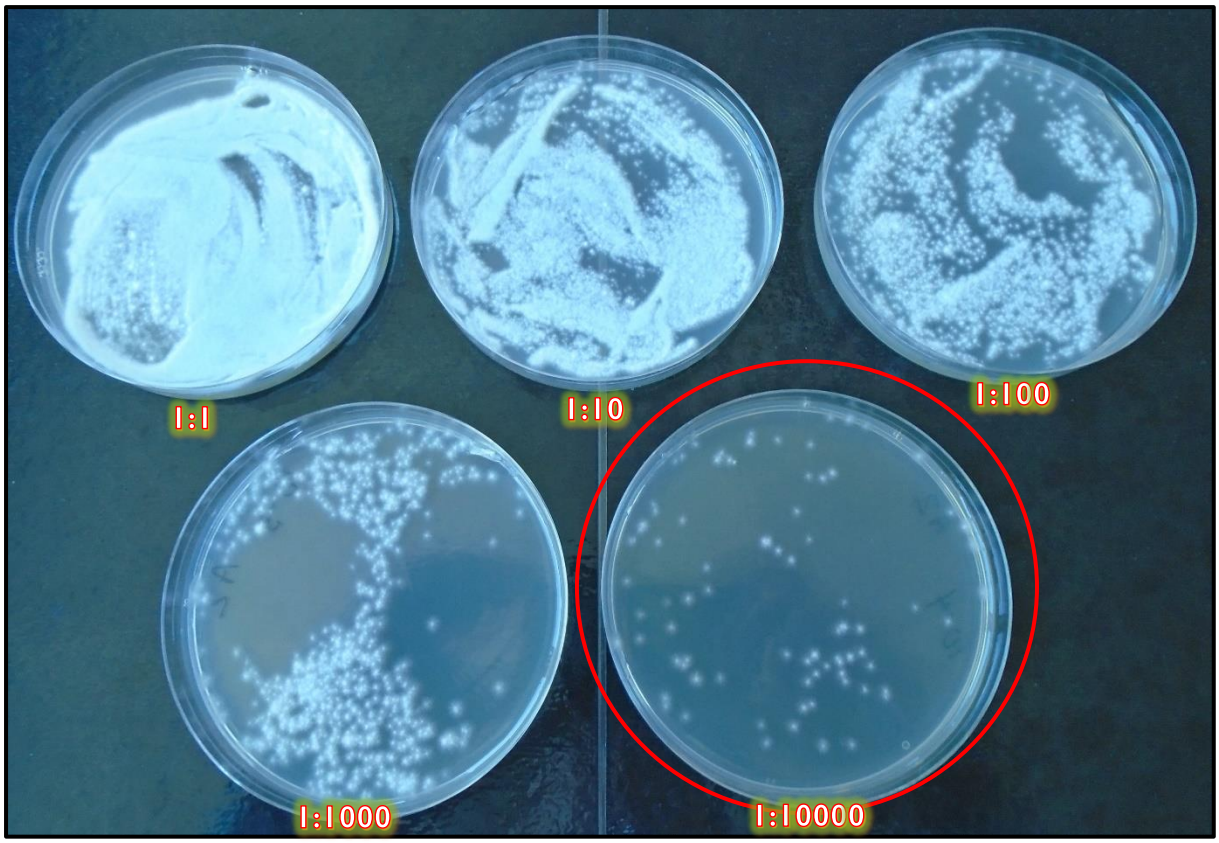

Figure 27, The cultures from sample A2 (no treatment), showing the 1:10000 ratio dish (circled in red) as having the appropriate range for counting CFUs. There were 78 CFUs counted.

The fact that the 1:1 plate from sample C6 only had 86 CFUs means that it had fewer viable conidia collected from the surface of the plaster with which to make the 1:1 solution, the highest concentration of all the dilutions. Of the three groups: A) Control – No Treatment, B) Dry Brushing Treatment Only, and C) Dry Brushing and UV-C Treatment, Group C had the lowest countable dilutions. Please see *Table 4* for a full accounting of the dilution levels.

Group	Sample	Dilution	CFUs on plate	CFUs/cm^2 on the plaster plate*
Group A, No Treatment	**A1**	1:10000	39	2.8×10^5
	A2	1:10000	78	5.5×10^5
	A3	1:10000	49	3.5×10^5
	A4	1:1000	106	7.5×10^4
	A5	1:10000	44	3.1×10^5
	A6	1:10000	68	4.8×10^5
	A7	1:10000	31	2.2×10^5
Group B, Dry Brushing Only	**B1**	1:1000	115	8.1×10^4
	B2	1:1000	66	4.7×10^4
	B3	1:1000	95	6.7×10^4
	B4	1:100	129	9.1×10^3
	B5	1:1000	111	7.9×10^4
	B6	1:1000	102	7.2×10^4
	B7	1:1000	61	4.3×10^4
Group C, Dry Brushing and UV-C	**C1**	1:10	104	7.4×10^2
	C2	1:10	51	3.6×10^2
	C3	1:100	37	2.6×10^3
	C4	1:100	210	1.5×10^4
	C5	1:1	71	5.0×10^1
	C6	1:1	86	6.1×10^1
	C7	1:1	4	2.8×10^0

Table 4, The CFU counts taken and calculated by Dr. Inacio. The lower the dilution count, the lower the amount of living conidia in the original suspension. The last column indicates the number of CFUs/cm^2 that were on the surface of the plaster at the time of culture (Inacio, 2019c). See Appendix M for full results and calculations of the CFUs/cm^2 on the plaster plate.

The results indicate the samples in Group C, except for C3 and C4, had fewer CFUs than any of the other samples. The samples C3 and C4 were both in the 1:100 dilution level, the same as only one sample in Group B, classified at the same dilution level. Please see *Figure 28* for a chart of the results.

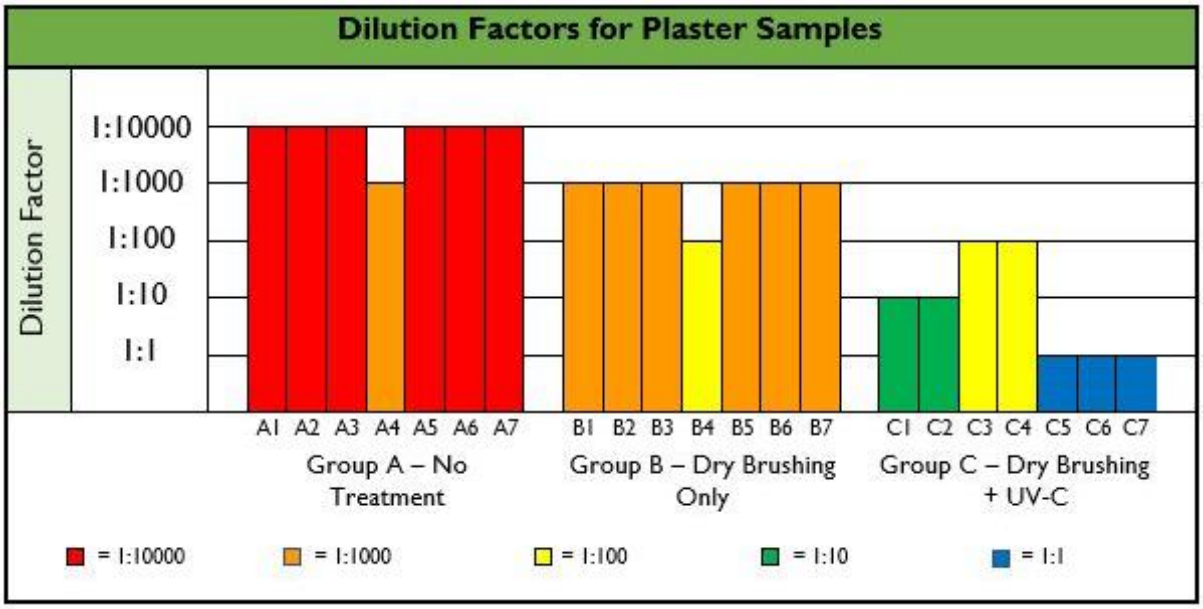

Figure 28, The dilution factors of the plates with the countable CFUs. The lower the dilution factor, the more successful the treatment. Group C, dry brushing with UV-C, had the lowest dilution factor of all the groups.

Based on the CFUs/cm^2 calculations, Group C, dry brushing with UV-C treatment, had the fewest living conidia on the surface at the time of culture. The difference between the groups varies widely, as seen in *Figure 29*.

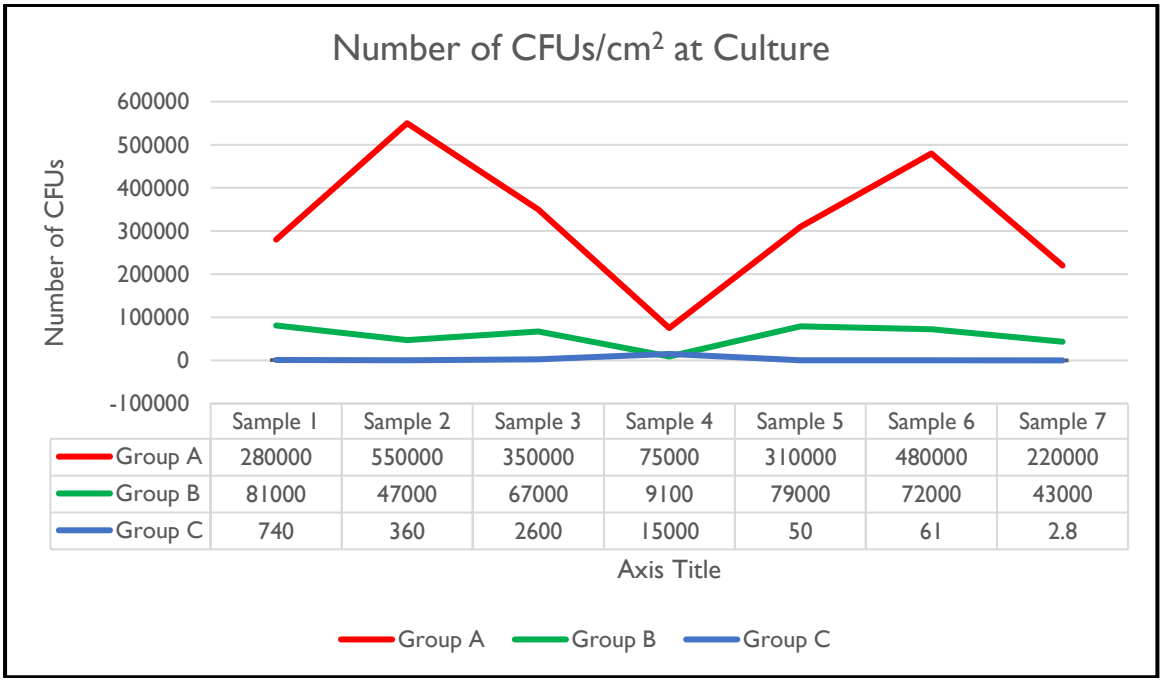

Figure 29, Chart with the total number of CFUs/cm^2 at the time of culture.

A comparison of the CFUs/cm^2 among the three groups indicates a substantial difference in the final counts. For instance, the counts in Group A (no treatment) show an average of 323,571 CFUs/cm^2, whereas Group C (dry brushing and UV-C treatment) averaged at 2,688 CFUs/cm^2. Group C and Group B (dry brushing only) were the closest in numbers, but the average count of Group B was still significantly higher than Group C at more than 56,000 CFUs/cm^2. For total counts and averages of CFUS, please see *Table 5*.

	Total CFUs/cm^2 for all samples in the group	**Average CFUs/cm^2 per plate in the group**	**Percent of decrease of average CFUs/cm^2 per treatment compared to Group A, no treatment**
Group A, No Treatment	2,265,000	323,571	*
Group B, Dry Brushing Only	398,100	56,871	82.4%
Group C, Dry Brushing and UV-C	18,814	2,688	99.2%

Table 5, Total number of CFUs/cm^2 for all samples and the average CFUs/cm^2 per plate in each group.

Dry brushing alone did appear to reduce the average number of CFUs/cm^2 that remained on the surface by 82.4%, from 323,571 down to 56,871. However, the treatment is much more effective when combined with UV-C exposure, giving a reduction of 99.2% of CFUs/cm^2 on the surface. Additionally, when comparing the average number of CFUs/cm^2 remaining on the dry brushed samples (56,871) to those left on the samples treated with UV-C (2,688), there were approximately 95.3% fewer CFUs.

4.2: Results from spectrophotometer testing

The results from the spectrophotometer testing did not show a significant difference in the colour of uncoated plaster tiles that were subjected to UV-C light. The four groups that were tested included:

A) Control – no UV-C exposure
B) Underexposure to UV-C, 1:07 minutes
C) Correct exposure to UV-C, 2:14 minutes
D) Overexposure to UV-C, 4:28 minutes.

The measurement by which colour changes are measured is the Delta E, or ΔE*. The CIE 1976 standard held that any changes below ΔE* of 1.0 were not detectable by the human eye, and although there have been advances in the evaluation of colour, such as the recognition that some highly trained individuals have the ability to discern colour better than the average person, the 1976 standard is still commonly used (Habekost, 2013) and was the basis for interpreting the spectrophotometer results (*Table 6*).

ΔE* Change in Plaster Colour after UV-C Exposure				
	Group A – No UV-C Exposure	**Group B – Underexposure 1:07 minutes**	**Group C – Correct exposure 2:14 minutes**	**Group D – Overexposure 4:28 minutes**
Sample 1	0.04	0.04	0.04	0.04
Sample 2	0.04	0.08	0.02	0.03
Sample 3	0.03	0.01	0.02	0.06
Sample 4	0.02	0.05	0.06	0.09
Sample 5	0.04	0.04	0.02	0.02
Average ΔE* of all samples in each group	.034	.044	.032	.048
Standard Deviation between all groups = .007724				

Table 6, ΔE results for each of the 21 plaster tiles and the averages within each group. The standard deviation amongst the groups is .007724.*

If a ΔE*<1.0 indicates that there is no visible[46] change in the colour of a surface, then all the results from the spectrophotometer testing fell well below that mark. It is interesting to note that the recordings from Group C (correct exposure) had less change than Group A that received no UV-C light. The result could be due to the manufacturer's specifications that the readings can have up to a .08 standard deviation (Konica Minolta, 2019b), or it could be due to variations in the mixing of the plaster that read differently once the spectrophotometer was repositioned for the sample reading. Even with the added .08 variation in the readings, none of the results exceeded the standard of ΔE*<1.0 and no visual change in the tiles was observed.

[46] As in detectable by the average human observer.

4.3: Limitations

The purpose of the study was to determine if the use of UV-C light could effectively limit regrowth of mould on plaster, but much time was spent working to understand how and why mould grows on plaster. Working with a microbiologist was vital to the project because it provided access to advanced knowledge, equipment, and scientific protocols. However, the specialised nature of elements of the work had to be performed by an experienced microbiologist. Certain tasks could not be completed in-house for safety reasons or lack of technical equipment to perform the work. Although the concept of the project was conceived and developed independently, the execution of the plan required considerable input from experts outside the field of conservation.

Growing mould on plaster was an exploratory process that required flexibility with a living organism and corresponding methodological adaptations to continue the study. In the beginning, adding a nutrient broth to the plaster mixture was discounted because it was a foreign substance that would not have been historically introduced to plaster. However, mould has evolved to survive under varied conditions, living on the nutrients found in common dust. It is a natural process that unfolds as the mould adapts to its surroundings or lies dormant waiting for more favourable conditions. Paradoxically, choosing one mould and its characteristics was necessary for the study to be reproduceable, but the aseptic techniques needed to exclude outside contaminants and control the parameters also deprived the mould of normal conditions that would help it thrive. In the end, the focus of the study was to examine UV-C light as a possible treatment for preventing mould regrowth on plaster. It was decided that obtaining sufficient growth for testing was more important than maintaining the original protocol, and the concession was made to add the Sabouraud broth to the samples. Including it in the mixture at the start might have yielded better results and ensured there would have been more samples to include in the study and more data with which to understand and compare the treatments.

Furthermore, *P. chrysogenum* was selected for this study because it had known parameters, is common in all parts of the world, and was a relatively low safety risk. The results of the study can only recommend that the UV-C light was more effective than dry brushing in this instance and cannot measure how well it would work for other moulds, nor does it imply that the exposure time for *P. chrysogenum* is appropriate for all mould species.

Although the number of conidia was significantly reduced by exposure to UV-C light, they were not completely eradicated. It is possible that irregularities in the surface of the plaster prevented direct contact of the light necessary to destroy the nucleic acids in the conidia. The angle of the light could also be a factor in the effectiveness of the treatment. The samples were placed under the lamp at a 90° angle, which may not have allowed access to the crevices where the plaster met the sides of the petri dish. It is possible that lighting the dish from multiple angles would have resulted in a higher success rate, but in the case of the Dr. Livingstone bust, it would not be possible to access all of the recesses on the interior, and would most likely reduce the effectiveness of the treatment in this case.

Additionally, the incubation and maintenance of the plaster samples was measured and deliberate, but the desiccation phase was difficult to control. It is unknown if rapid temperature and RH fluctuations had a negative effect on the samples, although no visual damage was noted. The small size and relative thickness of the plaster samples could have been a factor in how well they withstood the RH fluctuations. A larger piece of plaster, especially a bust the size of Dr. Livingstone, would have varying degrees of thickness in the walls of the sculpture. The exothermic reaction in mixing an amount of plaster that size could have changed the structural bonds of the material that could behave differently or be damaged under heavy RH fluctuations.

Additionally, more testing could be conducted regarding the safety of the UV-C light on uncoated plaster. Of all the wavelengths in the UV spectrum, UV-C light has the greatest ability to cause photolytic scission[47], although the occurrence is rare (Feller, 1994). It is possible that using SEM[48] to review the surface of the plaster before and after exposure to UV-C light could determine if there were changes in the crystalline structure (Ingham, 2010). Combining SEM imaging with XRD[49]can magnify a surface up to 100,000x and give further information about the crystalline structures and even the binders used in the plaster (Ellis, 2002). These types of testing could be used to determine if the UV-C exposure caused weaknesses in the structure of the plaster that could lead to powdering or pitting.

[47] A process by which light can break chemical bonds (Feller, 1994).

[48] Scanning electron microscopy.

[49] X-Ray diffraction.

Chapter 5: Conclusion

Treating uncoated plaster artefacts can be a challenge for conservators because the material is highly absorbent and can solubilise with the mildest of solvents. Mould growth on these objects is particularly difficult to treat because the introduction of chemical fungicides, a common way of killing mould in conservation, can stain or disintegrate the surface.

Historically, objects with mould would be moved to lower RH environments to dry out the mould and the remains would be removed by dry brushing, but this treatment does not kill the conidia, the microscopic parts of the mould that can reproduce. With few options, most conservators stop treatment after dry brushing and environmental stabilisation.

The purpose of the study was to determine if UV-C exposure could be used to limit the regrowth of mould after treatment without causing damage to uncoated plaster. Plaster samples were created and inoculated with *P. chrysogenum*. After a period of growth, the samples were treated with the same approach that might be taken by a conservator in the field: they were dried out in a low RH environment and the surface was brushed to remove the visible mould. However, one group of the samples was irradiated under a UV-C lamp for 2:14 minutes, the recommended kill dosage for *P. chrysogenum*. The samples were then cultured to determine how many of the conidia were still living on the surface after treatment.

The results of the study indicate that UV-C exposure is more effective at reducing the number of conidia that could regenerate after treatment than dry brushing alone. The UV-C light killed approximately 95.3% more of the conidia on the surface of the samples. Although the UV-C treatment was not 100% effective, it greatly reduced the chances that mould growth could reoccur. Through spectrophotometer testing, it was discovered that the UV-C light had virtually no visual effect on the colour of the plaster sample. All results from the spectrophotometer showed ΔE^* readings of far less than 1.0, the point at which visual changes can be detected by the human eye.

The findings of this study are a preliminary investigation into the possibility of utilising UV-C technology used in the medical and food industries for the treatment of mould on uncoated

plaster substrates. The kill dosage for other moulds could be examined to determine if there might be a standard dosage that could treat the most commonly found moulds. Other plaster substrates, such as lime-based materials, could be tested to ensure that the technique is safe for other types of uncoated plaster. Given more time, it would have been interesting to compare the efficacy of the UV-C exposure with and without dry brushing to determine if it could still be considered a useful treatment where dry brushing is not appropriate, such as in the case of a friable surface.

Spectrophotometer testing showed that there were no discernible changes in the colour of the plaster after UV-C exposure, but additional SEM testing could determine if there were structural damage to the plaster, further ensuring that UV-C light is safe for use on uncoated plaster.

Currently, the most common approach for treating mould on uncoated plaster, desiccation and dry brushing, does not go far enough to stop the re-emergence of mould if favourable environmental conditions occur. For a solution to be practical for use by conservators, it needed to meet the following criteria:

A) safe for use on plaster
B) safe for use by the conservator
C) affordable
D) reasonably attainable by the average conservator
E) effective in eradicating mould.

With adherence to basic safety guidelines and a relatively small investment in equipment, the addition of a UV-C lamp to the toolkit could be a real-world, practical solution for conservators faced with preventing the regrowth of mould on uncoated plaster objects.

Plaster has long been used as a medium for creating any manner of decorative object from architectural features and embellishments on furniture to sculptures and busts of memorable people, such as Dr. Livingstone. Advancements in treatment options for this material can only help in the maintenance and care of these important heritage artefacts.

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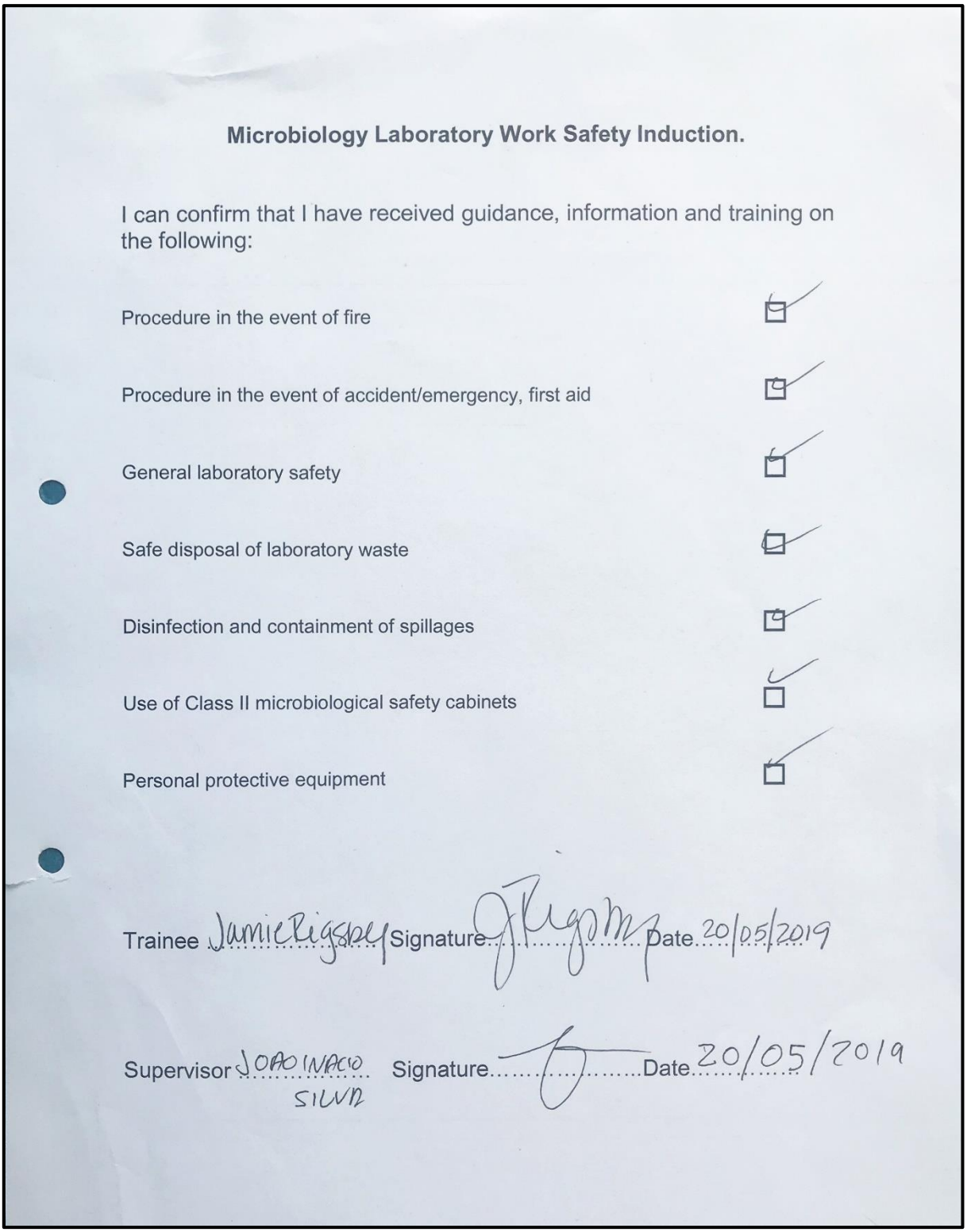
Appendices

Appendix A – University of Brighton Safety Induction Form



Microbiology Laboratory Work Safety Induction.

I can confirm that I have received guidance, information and training on the following:

- Procedure in the event of fire ☑
- Procedure in the event of accident/emergency, first aid ☑
- General laboratory safety ☑
- Safe disposal of laboratory waste ☑
- Disinfection and containment of spillages ☑
- Use of Class II microbiological safety cabinets ☑
- Personal protective equipment ☑

Trainee Jamie Rigsby Signature [signature] Date 20/05/2019

Supervisor JOAO INACIO SILVA Signature [signature] Date 20/05/2019

Form 13 - HAZARD RISK ASSESSMENT FORM

Refer to 102-HSE-SOP – Risk Assessment for guidance on completion

Document Title	MA Thesis Experiment effect of UV light on mould – Ceramics/Analytical Laboratory – Jamie Rigsby
Document Number	JR-MA-001/2019

Hazard	Who could be harmed and how?	Control measures	Risk Rating with controls			Action Required
Inhalation of mould	Students, Staff, or Visitors entering area: Respiratory illness	• Establish a set location for the experiments with Science Tutor and Safety Officer • Schedule off-peak times to do experiments • Use aseptic techniques, sterilized tools • Close doors and windows and reduce air movement • Place 'Do Not Enter' signs on the door during testing • Any people in the room will be wearing N-95 particulate dust mask, gloves, and protective clothing • Wipe down all surfaces with disinfectant after use	1	2	2	
Storage of mould specimens (Accidental opening of containers)	Students, Staff, or Visitors: Respiratory Illness	• Wear N-95 particulate dust masks, protective clothing, and gloves when handling containers • Place signs on incubator/storage area 'DANGER – LIVE MOULD CULTURES – DO NOT OPEN' • Brief other laboratory users • Disinfect incubator and surfaces after use • Properly dispose of specimens after use	1	1	1	
Use of scalpel blades	Student: Cuts	• Training on proper blade replacement techniques • Be aware of surroundings, move slowly • Dispose of used blades properly	1	2	1	
Mixing of plaster	Student, staff, and visitors: Inhalation of powder	• Wear N-95 particulate dust mask when mixing • Wear safety goggles Move with care; stir slowly to avoid distributing powder into the air.	1	1	1	
Mixing of rabbit skin glue	Student:	• Avoid moving or carrying kettle of boiling water across the room (i.e. mix glue at station near kettle)	1	2	2	

Appendix B – Hazard Risk Assessment for Experiments (Continued)

Form 13 - HAZARD RISK ASSESSMENT FORM

Refer to 102-HSE-SOP – Risk Assessment for guidance on completion

Document Title	MA Thesis Experiment effect of UV light on mould – Ceramics/Analytical Laboratory – Jamie Rigsby
Document Number	JR-MA-001/2019

	Burns	• Move slowly when pouring boiling water to make the glue Wear protective gloves and gauntlets				
Use of UV-C Light	Student, Staff, Visitors: Damage to skin and eyes	• Have light inspected by West Dean Safety Officer before use • Inspect light for damage before use • Schedule off-peak times to perform tasks to avoid exposure to other people • Operate light in enclosed room or chamber • Post signage 'DO NOT ENTER – UV LIGHT IN USE' • Wear UV protective eyewear • Wear protective clothing, gloves when moving specimens under light • Store light with lock out/tag out safety device on plug	1	1	1	

Assessment reviewed by:	Name: Jasper Richmond GIFireE	Position: Safety Technician	Signature:

Appendix C - West Dean COSHH Assessment for Mould

Material/s used	**WEL (LTEL/STEL)**	**Hyperlink to SDS**
P. chrysogenum Mould	4 hours @ mg/l	http://tools.thermofisher.com/content/sfs/msds/2016gwi/14-4160-01_MTR-NATS_EN.pdf

Activity/process being assessed
Using live cultures of mould for experimentation

Quantity used g/ml (state a range, eg. "up to 250ml")	**Material form** **(solid, liquid, gas)**	**Temperature** **(Enter if not Room Temp)**	**Duration of activity** **(hours)**	**Frequency of activity** **(daily, weekly, monthly, yearly)**
Up to 1 g/ml	Solid		Up to 4 hours	weekly

Location of activity/process	**No. of operators**	**People at risk**
Analytical Laboratory	1-2	Students, staff, visitors

Hazard Pictogram **Delete those not required**	

Hazard Statements eg. H225 Highly flammable liquid and vapour	**Precautionary Statements** eg.. P210 Keep away from heat/sparks/open flames/hot surfaces. – No smoking
H333 May be harmful if inhaled	P280 Wear protective gloves/protective clothing/eye protection/face protection.
H335 May cause respiratory irritation	P280 Wear protective gloves/protective clothing/eye protection/face protection.

Routes of Entry to the body **Delete those not required**				
Eyes	Inhalation	Ingestion		

Emergency Procedures	
Eye Contact:	Rinse thoroughly with plenty of water, also under the eyelids.
Inhalation:	Remove to fresh air, contact physician if difficulty breathing.
Skin Contact:	Wash off immediately with soap and plenty of water. Remove contaminated clothing.
Ingestion:	Wash mouth out with water and call physician then administer three cups of water. Do not induce vomiting unless instructed to do so by a physician.
Mandatory Safe System of Work Describe the measures that must be used to control the risk, include local exhaust ventilation, LEV and personal and respiratory protective equipment, PPE, necessary to inhibit route of entry into the body and specification where relevant.	
Eye/Face: Wear goggles	
Respiratory: Wear particulate dust mask	

Skin: Wear protective clothing and gloves

PPE Delete those not required										
		Goggles		Dust Mask				Apron		Gloves

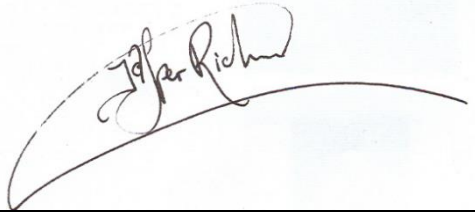
Disposal method **Delete those that do not apply**	• Unwanted/excess material must be collected, clearly and fully labelled, and disposed of via main workshop.	
Is specific training required? **Delete those that do not apply**	• Specialised training required to ensure the activity is carried out safely, given as needed and recorded	
Is supervision required? **Delete those that do not apply**	• Low risk activity: unsupervised working allowed	
Is professional health surveillance required?	Yes/No NO	Details **If "yes", do not use the process**
Additional Information	eg. Environmental monitoring results, possible combination exposure, classification of biological agent	

Following the assessment and complying with the safe system of work, estimate the risk rating. This must be LOW.

Risk Rating	likelihood		severity		**2**	Date of assessment/ review	23/04/2019
	1	X	2	=			

Likelihood		Severity			Risk Rating = likelihood x severity	
Highly likely	5	Death	5		High Risk	15 - 25
Likely	4	Serious Injury	4		Medium Risk	7 - 14
Possible	3	Injury	3		Low Risk	1 - 6
Unlikely	2	Minor Injury	2			
Highly unlikely	1	No injury	1			

One of these must be a member of West Dean staff

Assessor name	**Signature**	**Date**
Jamie Rigsby		23/04/2019
Assessment countersigner name	**Signature**	**Date**
Jasper Richmond GIFireE		27/04/2019

Appendix D – COSHH Assessment for UV-C Lamp

Material/s used	**WEL (LTEL/STEL)**	**Hyperlink to SDS**
UVC Lamp (254 nm)	30J/m² (8 hours)	http://tools.thermofisher.com/content/sfs/msds/2016gwi/14-4160-01_MTR-NATS_EN.pdf

Activity/process being assessed
Whether UV-C light can eradicate mould on plaster surfaces

Quantity used g/ml (state a range, eg. "up to 250ml")	**Material form (solid, liquid, gas)**	**Temperature (Enter if not Room Temp)**	**Duration of activity (hours)**	**Frequency of activity (daily, weekly, monthly, yearly)**
254nm	Light		Up to 4 hours	once

Location of activity/process	**No. of operators**	**People at risk**
Analytical Laboratory	1-2	Students, staff, visitors

Hazard Pictogram Delete those not required	

Hazard Statements eg. H225 Highly flammable liquid and vapour	**Precautionary Statements** eg.. P210 Keep away from heat/sparks/open flames/hot surfaces. – No smoking
H316. Can cause mild skin irritation	P280 Wear protective gloves/protective clothing/eye protection/face protection.
H320. Causes eye irritation (acute photokeratitis)	P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.
	P337 + P313 If eye irritation persists: Get medical advice/ attention.

Routes of Entry to the body Delete those not required				
Eyes	Skin			

Emergency Procedures	
Eye Contact:	Remove from vicinity of light. If eye irritation persists: Get medical advice/ attention.
Inhalation:	
Skin Contact:	Remove from vicinity of light. If skin irritation persists: Get medical advice/ attention.
Ingestion:	
Spill Procedure:	

Mandatory Safe System of Work Describe the measures that must be used to control the risk, include local exhaust ventilation, LEV and personal and respiratory protective equipment, PPE, necessary to inhibit route of entry into the body and specification where relevant.
Eye/Face: Wear UV-filtered goggles and/or face mask

Skin: Wear protective clothing and gloves (pay particular attention to any open wounds)

PPE Delete those not required										
		Goggles						Apron		Gloves

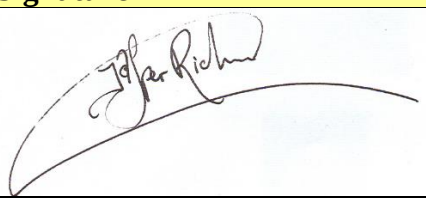
Disposal method **Delete those that do not apply**	• Bulbs to be disposed of through workshop.	
Is specific training required? **Delete those that do not apply**	• Specialised training required to ensure the activity is carried out safely. • Competent person to train and sign as proficient. • Manufacturers guidelines to be followed.	
Is supervision required? **Delete those that do not apply**	• Low risk activity: unsupervised working allowed	
Is professional health surveillance required?	NO	Details **If "yes", do not use the process**
Additional Information	eg. Environmental monitoring results, possible combination exposure, classification of biological agent	

Following the assessment and complying with the safe system of work, estimate the risk rating. This must be LOW.

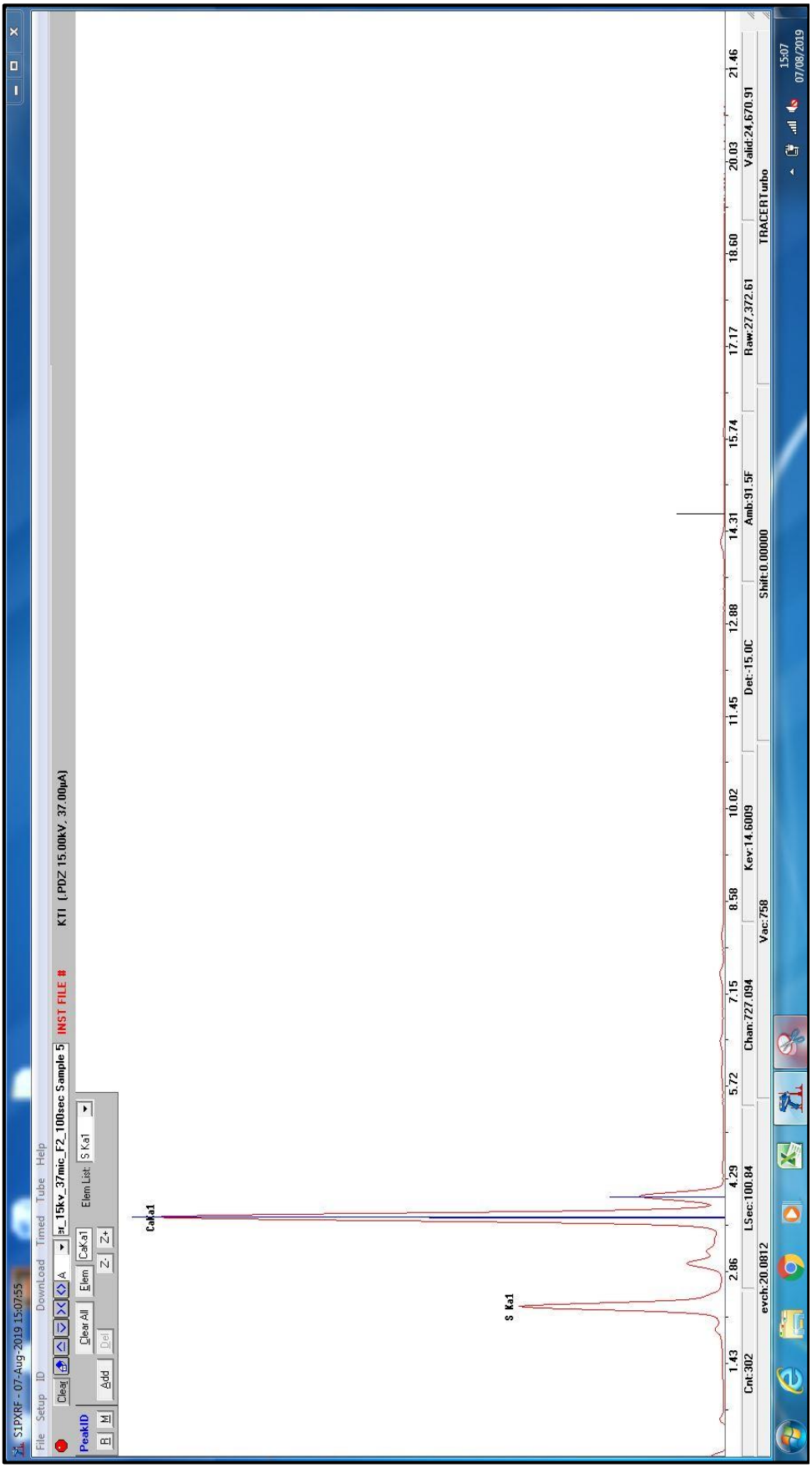
Risk Rating	likelihood		severity		**4**	Date of assessment/ review	23/04/2019
	1	X	4	=			

Likelihood		Severity			Risk Rating = likelihood x severity	
Highly likely	5	Death	5		High Risk	15 - 25
Likely	4	Serious Injury	4		Medium Risk	7 - 14
Possible	3	Injury	3		Low Risk	1 - 6
Unlikely	2	Minor Injury	2			
Highly unlikely	1	No injury	1			

One of these must be a member of West Dean staff

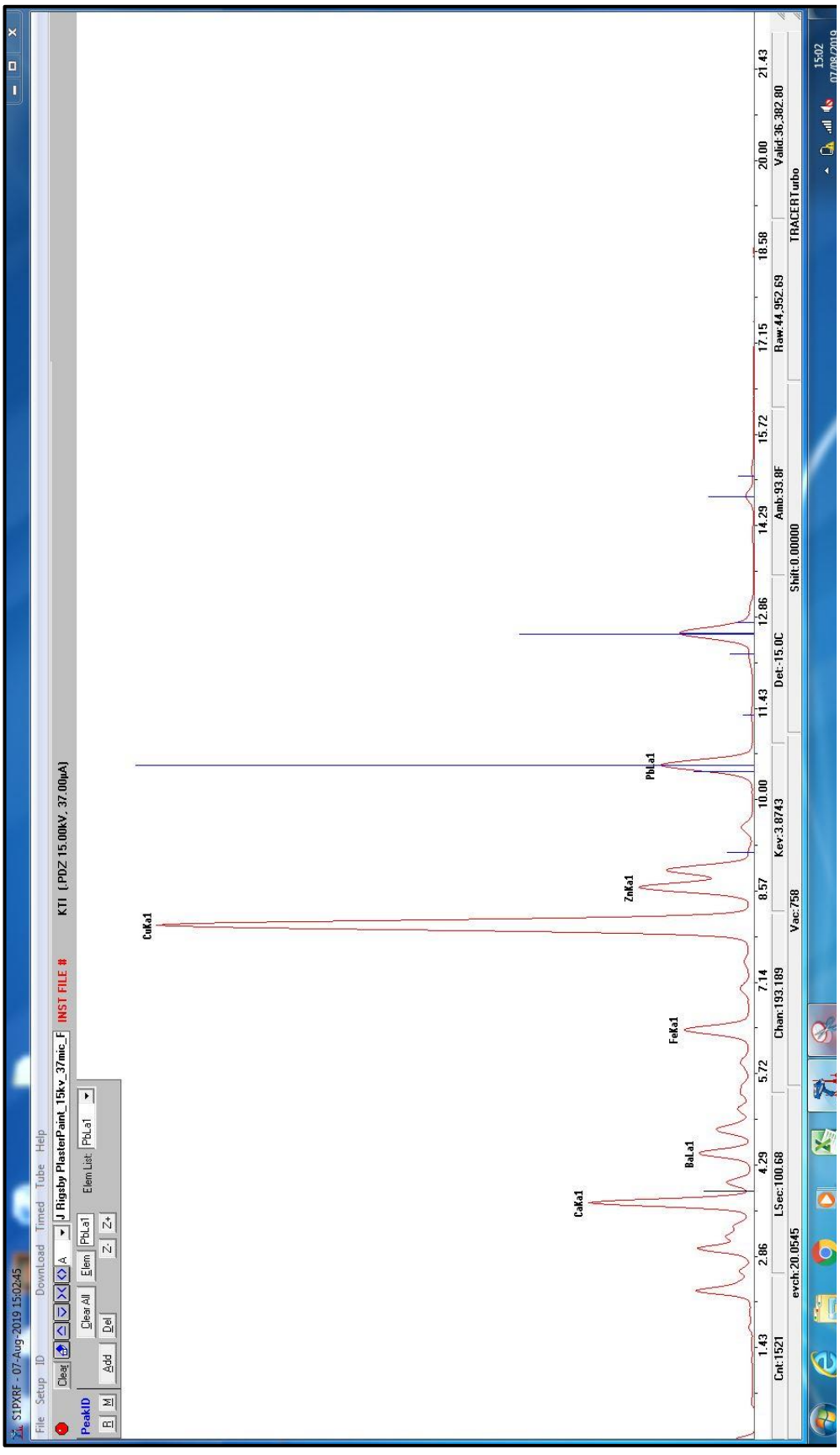
Assessor name	**Signature**	**Date**
Jamie Rigsby		23/04/2019
Assessment countersigner name	**Signature**	**Date**
Jasper Richmond GIFireE		27/04/2019

Appendix E – XRF Spectrum of Plaster Bust Results



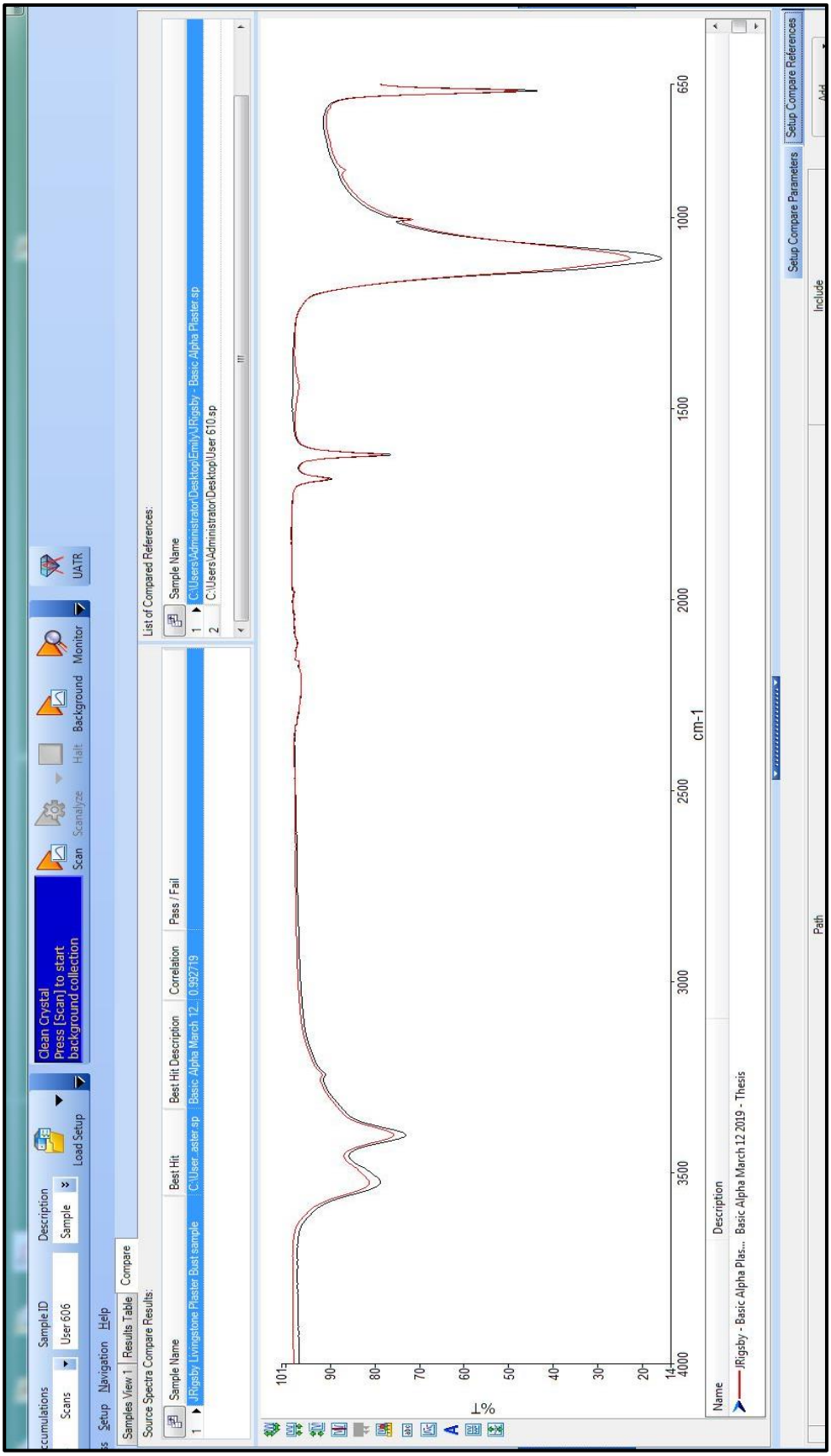
XRF Spectrum of Plaster Bust Results. The test indicated a high amount of calcium (Ca) and sulphur (S) which is consistent with a calcium sulphate-based plaster, however, XRF cannot detect compounds and could not definitively determine that the plaster from the bust was calcium sulphate. Further testing would need to be performed.

Appendix F – XRF Spectrum of Paint Layer of Bust Results



XRF Spectrum of Plaster Bust Paint Layer Results. The test indicated a high amount of copper (Cu), calcium (Ca), zinc (Zn), and lead (Pb), which is consistent with historical ground and paint formulas.

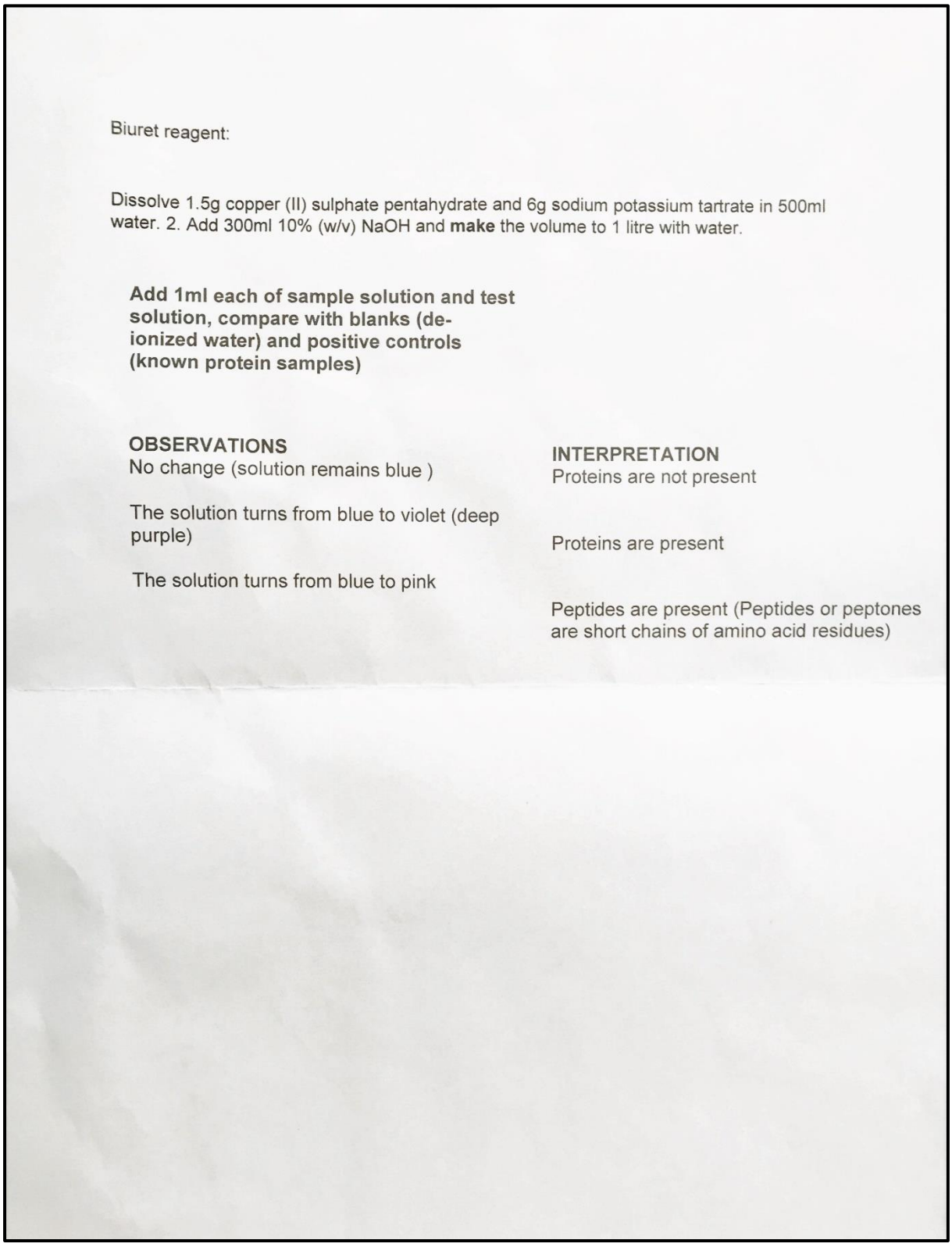
Appendix G – FTIR Spectrum of Plaster Bust Results



FTIR spectrum of test results from plaster bust compared with modern Basic Alpha plaster. The results showed a 99.7% match between the two types of plaster, ensuring that the samples for the study would be similar in composition to the original plaster bust.

Appendix H – Biuret Reagent Preparation

The Biuret reagent was prepared in-house using the following directions from the website Brilliant Biology Student (2019):



Biuret reagent:

Dissolve 1.5g copper (II) sulphate pentahydrate and 6g sodium potassium tartrate in 500ml water. 2. Add 300ml 10% (w/v) NaOH and **make** the volume to 1 litre with water.

Add 1ml each of sample solution and test solution, compare with blanks (de-ionized water) and positive controls (known protein samples)

OBSERVATIONS	INTERPRETATION
No change (solution remains blue)	Proteins are not present
The solution turns from blue to violet (deep purple)	Proteins are present
The solution turns from blue to pink	Peptides are present (Peptides or peptones are short chains of amino acid residues)

Appendix I – SanAir Technologies Laboratory Report

SanAir
Technologies Laboratory

SanAir ID Number
19008597
FINAL REPORT
2/28/2019 9:45:47 AM

Name: Jamie Rigsby
Address: 136 Gas Plant Road
Doyline, LA 71023
Phone:

Project Number:
P.O. Number:
Project Name:
Collected Date: Not Provided on COC
Received Date: 2/25/2019 2:13:00 PM

Analyst: Goodwin, Aaron

Direct Identification Analysis

SanAir ID: 19008597-001 Sample #:1 Sample On Original Substrate (Plaster)

D1 - Direct Identification Analysis on Bulk Material using STL 104

Direct ID of Mold

Fungi	Estimated Amount
Aspergillus/Penicillium	Rare

SanAir ID: 19008597-002 Sample #:2 Sample On Cotton Wool Taken From Surface Of Plaster

D1 - Direct Identification Analysis on Bulk Material using STL 104

Direct ID of Mold

Fungi	Estimated Amount
Ascospores	Rare
Aspergillus/Penicillium	Light
Chaetomium globosum	Rare
Gliomastix species	Light

Other	Estimated Amount
Mycelial Fragments	Rare

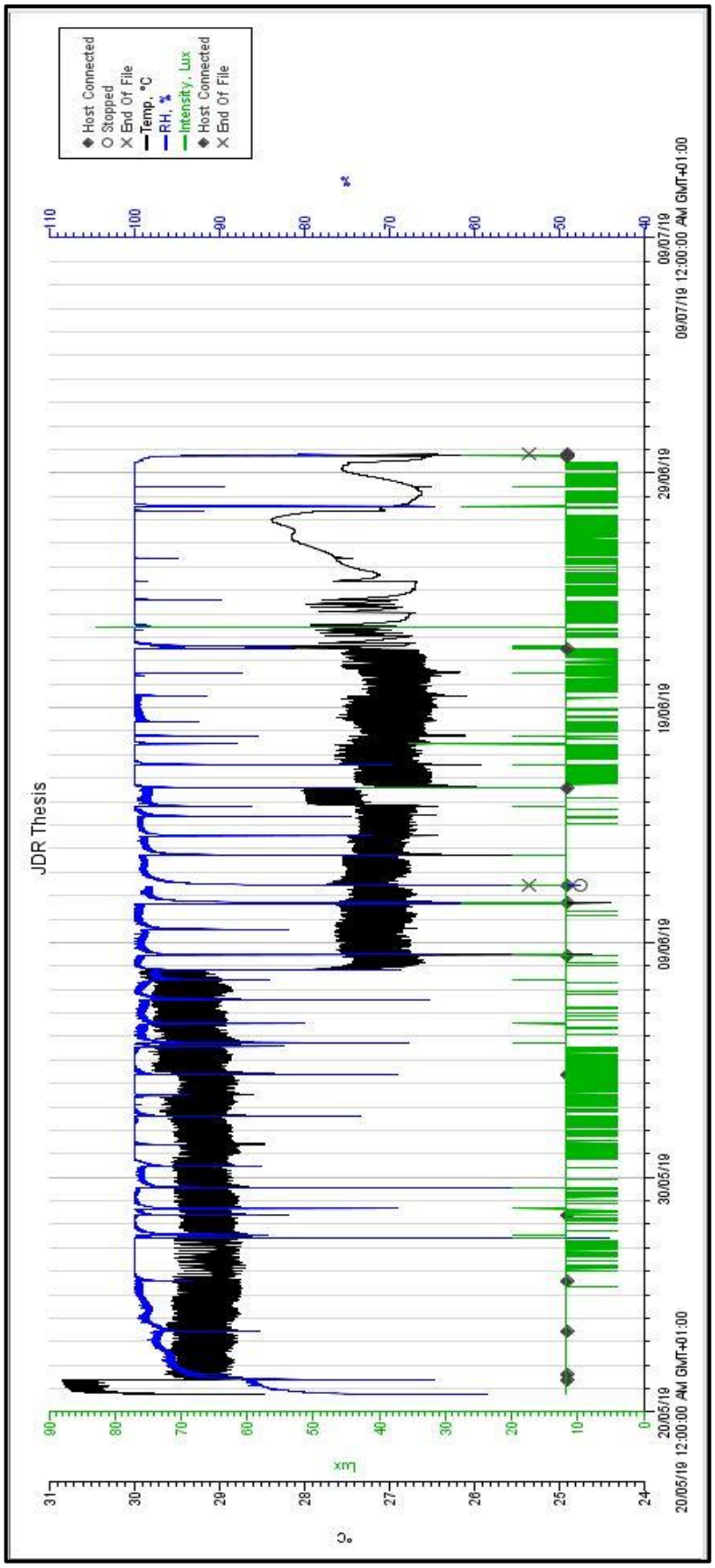
Estimated Amount	Indication of Growth	Evidence of Mycelial Fragments Counted
Rare	Not Likely	None
Light	Possible	Some, 10 to 25% of Tape Covered
Moderate	Probable	Abundant, 25 to 50% of Tape Covered
Heavy	Significant	Throughout, 50 to 100% of Tape Covered

*Refer to additional information page for further details

Signature: Date: 2/28/2019

Reviewed: Date: 2/28/2019

Appendix J – Environmental Monitoring



The environmental monitoring in this study was performed by the Onset HOBO© U12-012 Datalogger. Recordings were made throughout the study, but the most crucial period was during the growth phase of the project, from May 20, 2019 – June 29, 2019. The average temperature during this time was 28°C and the average RH was 98%. The graph represents a timeline of environmental readings from the growth phase. The black numbers and line represent the temperature in °C. The blue numbers and lines represent the RH%. The green numbers and lines represent the light intensity that was recorded during the phase. Low light is preferable for growing P. chrysogenum *although not crucial to this study. The samples were kept below 10 lux for most of the study.*

Appendix K – Microbiological Protocols by Dr. Joao Inacio

DRAFT PROTOCOL – JAMIE

Materials for microbiology work

Note: all these materials may be acquired for example from Sigma-Aldrich (www.sigmaaldrich.com)

- Agar media for fungi (e.g. Sabouraud Dextrose Agar - SDA or Potato Dextrose Agar - PDA; we usually buy the powder and prepare the media ourselves, but you may prefer to buy agar plates already prepared)
- Petri dishes (90 mm)
- Sterile cotton swabs
- Sterile physiological saline solution (0.9% sodium chloride)
- Bunsen burner (or similar something with a flame)
- Scalpel
- Micropipette (200 μL) and respective tips
- Sterile plastic spreaders
- Universals (or test tubes with respective lids)

Preparation of fungal culture (e.g. *Penicillium* sp.)

1. Acquire a strain of the relevant fungi (e.g. from a colleague in some research group, if available; or buy it from some culture collection such as the German DSMZ (www.dsmz.de), the Dutch CBS (www.westerdijkinstitute.nl/collections), or the British NCTC (https://www.phe-culturecollections.org.uk/collections/nctc.aspx))
2. With filamentous fungi, culture collections will probably send you a fresh culture; after receiving it, transfer a bit of the agar containing mycelium onto a new plate (Petri dish) of adequate agar medium (e.g. Sabouraud Dextrose Agar or Potato Dextrose Agar) and incubate at room temperature for about 7 – 10 days. Prepare a few plates so that some plates will be used to inoculate your plaster samples, and you can store a couple of plates at 4 degrees Celsius for a few months (so that you can always prepare fresh cultures from them when needed)

Inoculation of plaster samples

Let's assume you will test each treatment with 5 plaster samples (so that you will need 5 samples as control, 5 samples tested with dry brushing, 5 samples tested with dry brushing and fungicide, and 5 samples tested with dry brushing as UV-C). This way you will need to prepare 20 plaster samples (i.e., 20 Petri dishes containing a thin layer of your plaster samples, with a preferentially a smooth and flat surface)

To prepare a spore suspension

1. With a scalpel, collect fungal spores from the agar plate and suspend in 10 mL of sterile physiological saline solution (homogenise in a vortex for 15 – 30 sec; your suspension will look a bit cloudy)

To inoculate the plaster samples

2. With a micropipette and respective tip, transfer 0.2 mL of the spore suspension onto each plaster sample (briefly vortex the suspension before pipetting to ensure a

homogenous distribution of the spores in the liquid; if the plaster is too dry, the liquid inoculum may be absorbed too quickly, so you will need to rapidly spread the inoculum)
3. Spread the liquid inoculum on each sample using a sterile plastic spreader, and allow to dry

Incubation

Incubate your samples (I am not sure about the conditions you will use for the incubation, but most fungi need a temperature around 20 – 30 degrees Celsius and a high moisture; and will probably need at least one weak incubation; light is not so important, but fungi should prefer low light levels). After enough fungal growth (probably evaluated by the naked eye), you can proceed to the cleaning/disinfection tests. After disinfection, you may proceed to a semi-quantitative determination of the spore survival rates.

Determination of spore survival rates

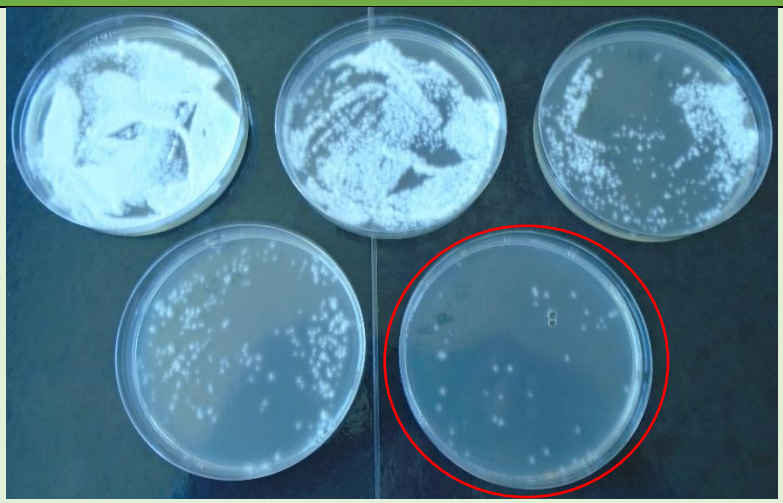
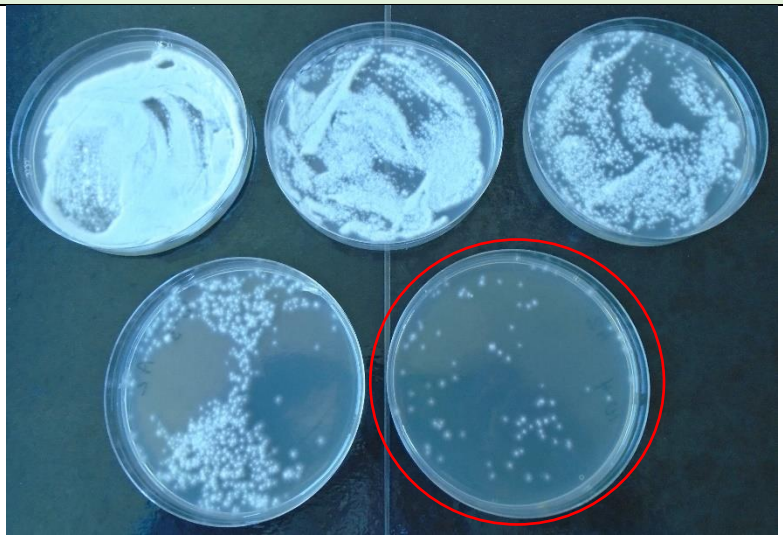
Note: this procedure will be for each plaster sample, including controls

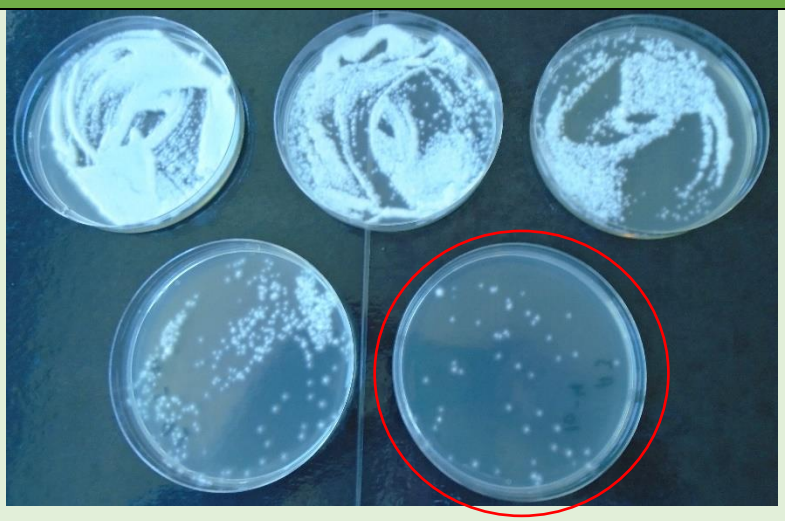
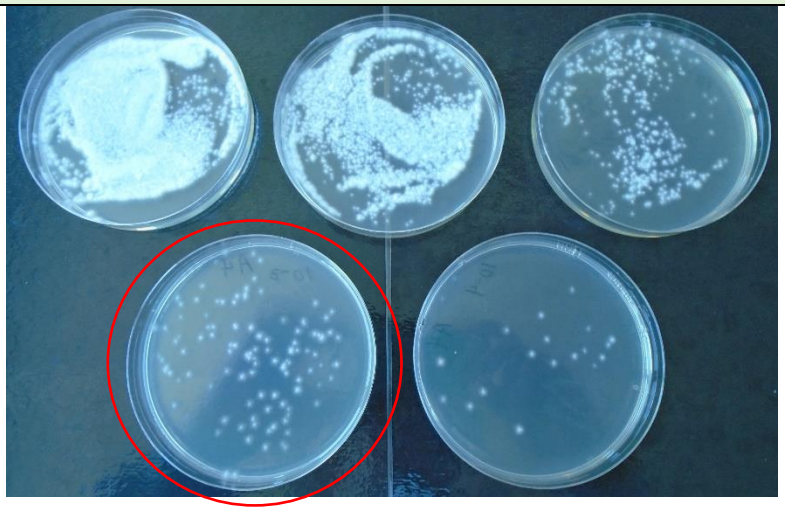
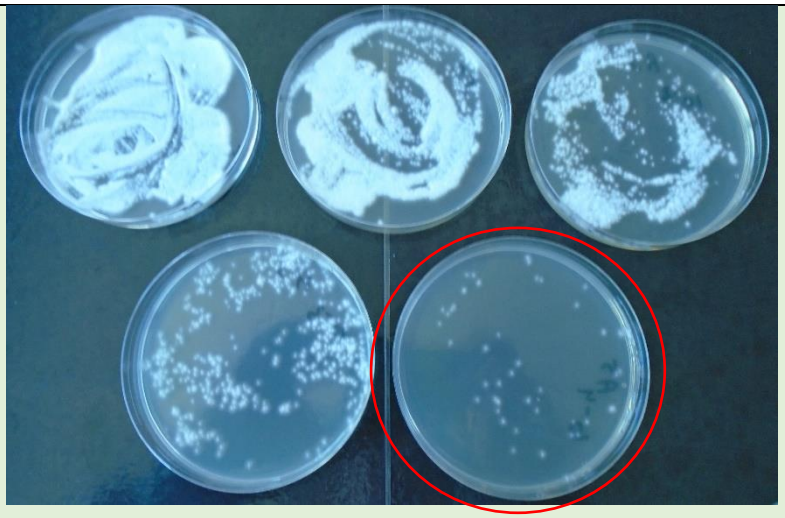
1. Wet a sterile cotton swab in sterile saline solution and remove excess liquid
2. Swab the entire surface of the plaster sample (try to cover all the surface and swab by approximately the same period of time, rotating the swab several times)
3. Cut the tip of the swab into a universal containing 4.5 mL sterile saline solution, and vortex for 3 minutes (label it as suspension A, and proceed to a serial dilution – points 4 to 9; briefly vortex each suspension before each transfer)
4. Transfer 0.5 mL of suspension A into a new universal containing 4.5 mL of saline solution (label it as suspension B)
5. Transfer 0.5 mL of suspension B into a new universal containing 4.5 mL of saline solution (label it as suspension C)
6. Transfer 0.5 mL of suspension C into a new universal containing 4.5 mL of saline solution (label it as suspension D)
7. Transfer 0.5 mL of suspension D into a new universal containing 4.5 mL of saline solution (label it as suspension E)
8. Transfer 0.5 mL of suspension E into a new universal containing 4.5 mL of saline solution (label it as suspension F)
9. Transfer 0.5 mL of suspension F into a new universal containing 4.5 mL of saline solution (label it as suspension G)
10. For each suspension (A to G), transfer 0.1 mL onto two agar plates (e.g. SDA or PDA) (to do duplicates), and spread with sterile plastic spreaders
11. Incubate the plates at room temperature for 5 – 7 days (or until you can see small fungal colonies)
12. After incubation, select the plates with the best dilution factor to count the number of colonies (with something between 30 and 300 colonies)
13. After counting the number of colonies, you can estimate the original number of fungal spores per plaster surface area. And then, you can compare the effectiveness of each treatment by comparing the spore survival rate for each treatment with the baseline (which are the counts for the control plates)

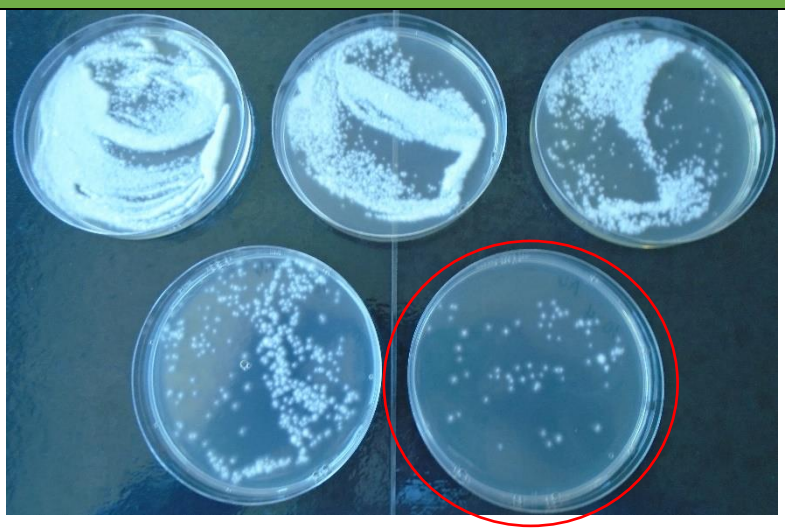
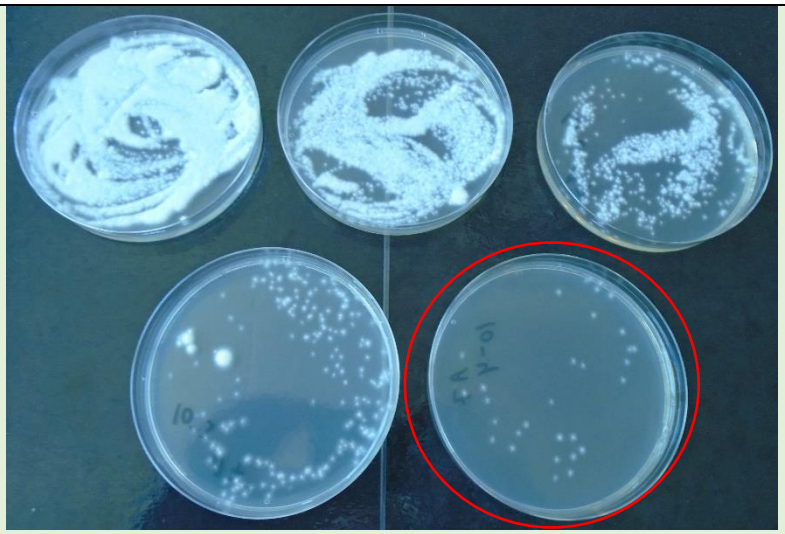
Appendix L – Photographs of Cultures taken from Plaster Samples

The following table includes the photographs for the five dilutions that were taken for each sample from left to right: 1:1, 1:10, 1:100, 1:1000, and 1:10000. The lower the dilution used for counting the 30-300 CFUs, the better the results. The counted dish is circled in red. All photographs in Appendix L were taken by Dr. Joao Inacio (Inacio, 2019c).

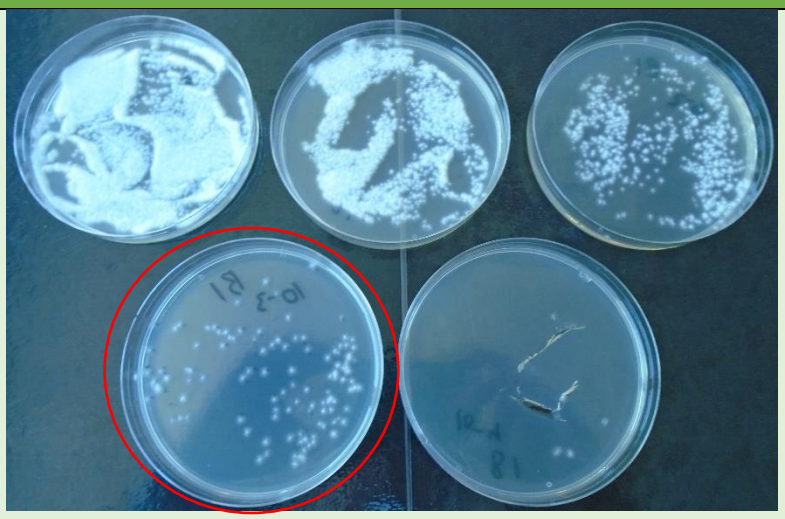
GROUP A – CONTROL, NO TREATMENT

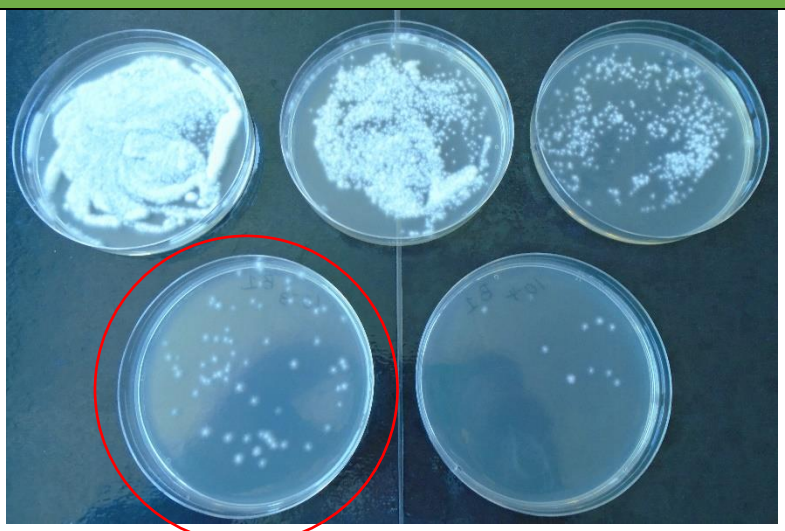
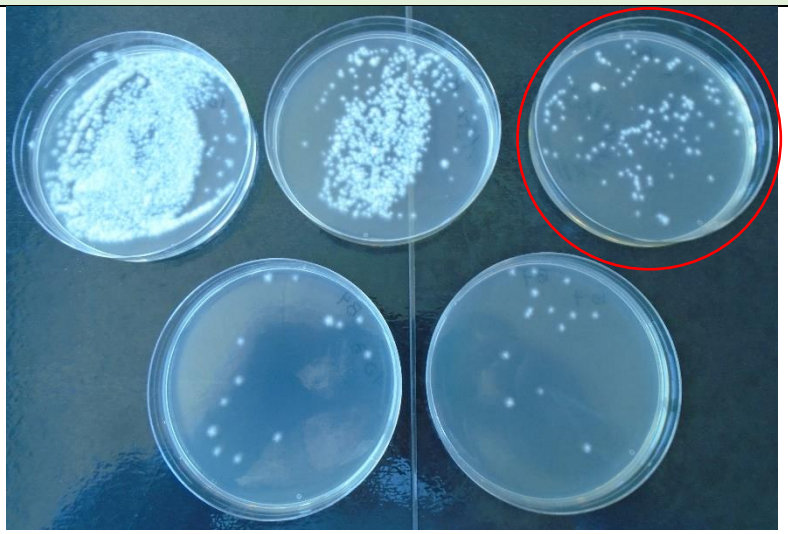
Sample	Dilution	CFUs	Photo
A1	1:10000	39	
A2	1:10000	78	

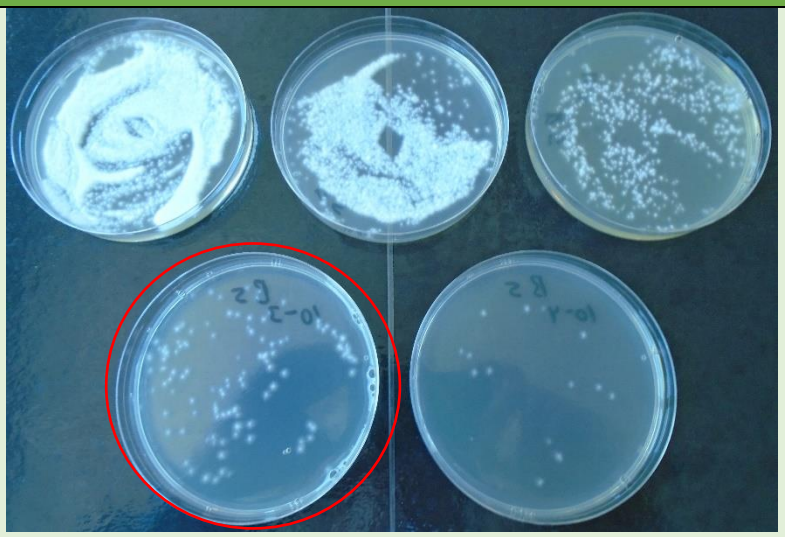
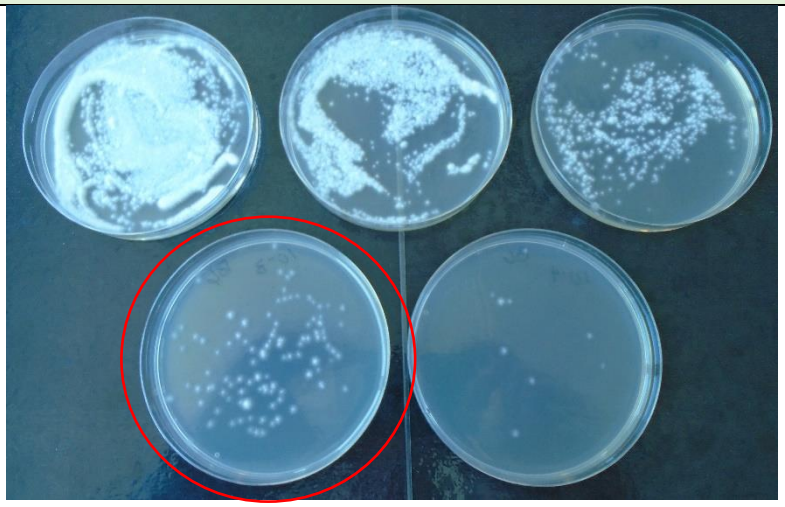
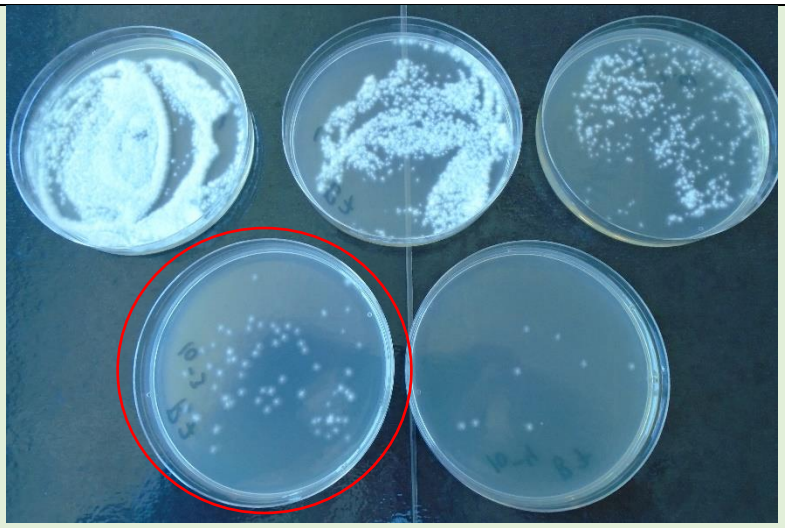
Sample	Dilution	CFUs	Photo
A3	1:10000	49	
A4	1:1000	106	
A5	1:10000	44	

Sample	Dilution	CFUs	Photo
A6	1:10000	68	
A7	1:10000	31	

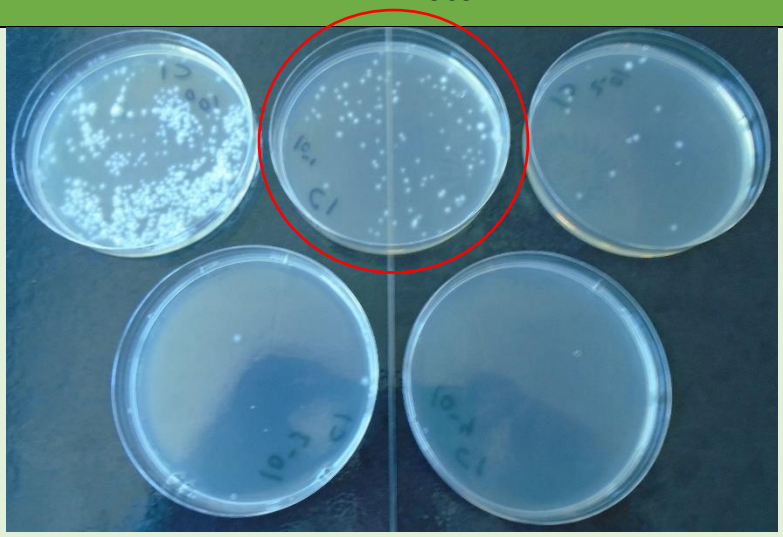
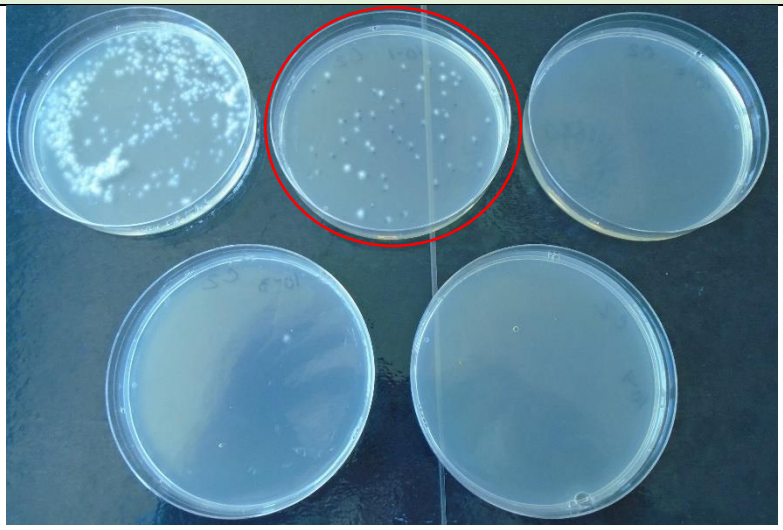
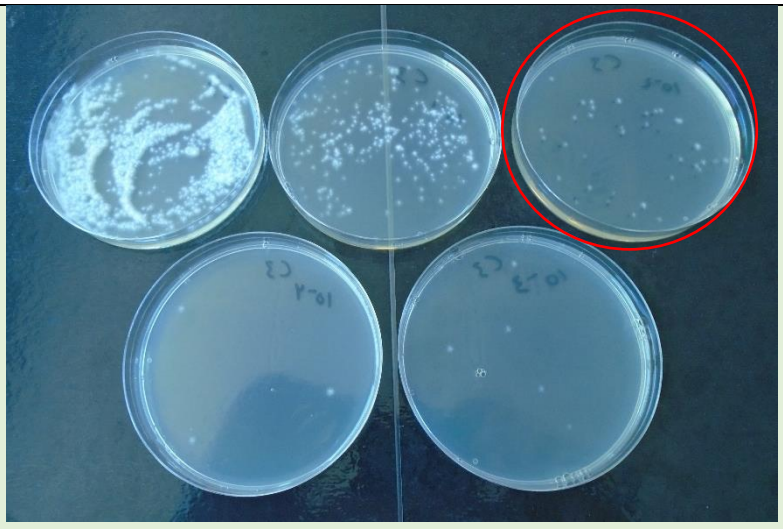
GROUP B – DRY BRUSHING ONLY

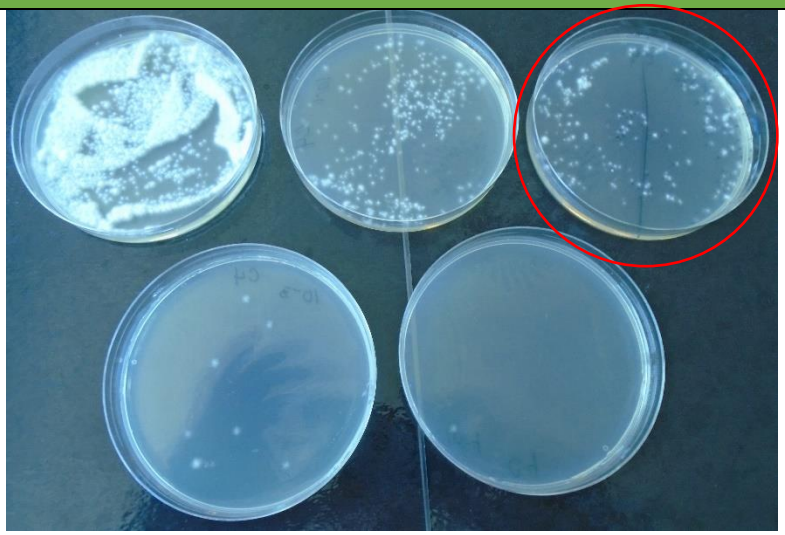
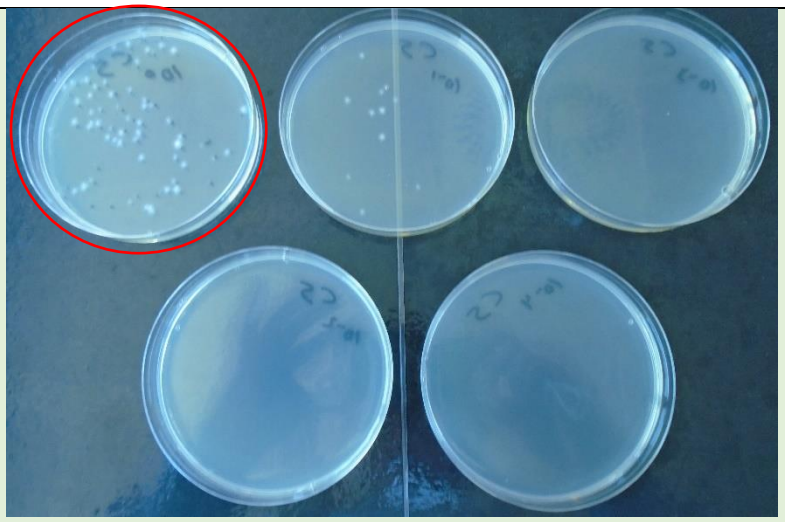
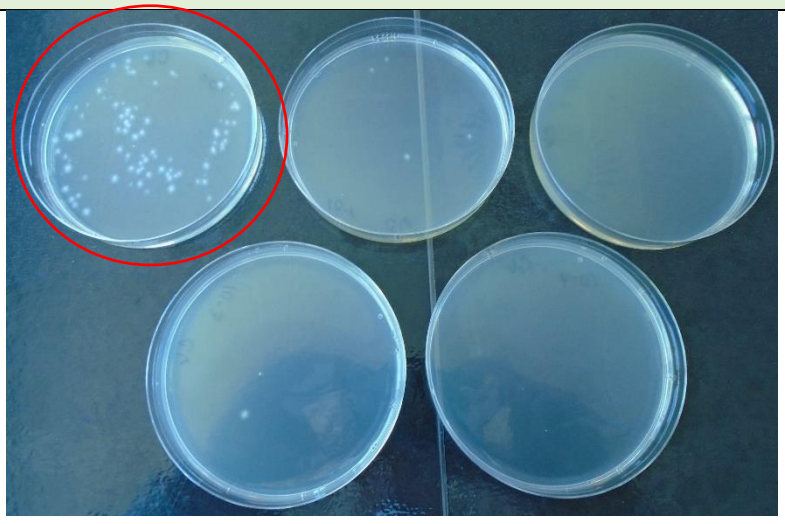
Sample	Dilution	CFUs	Photo
B1	1:1000	115	

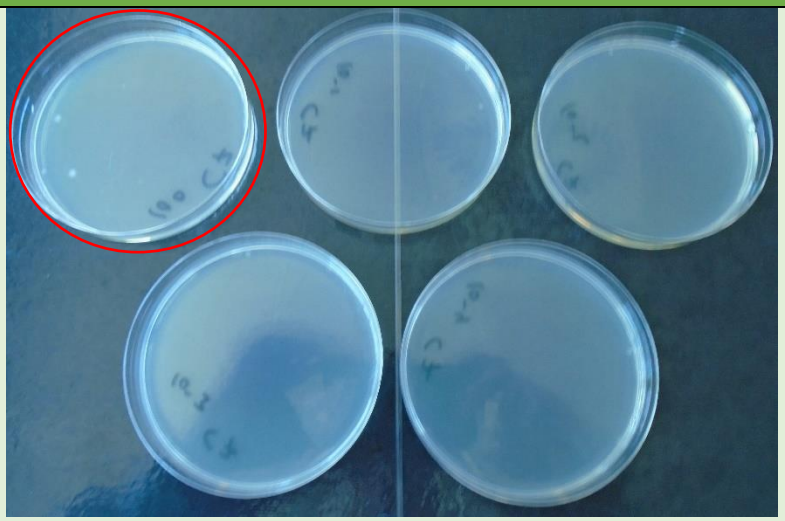
Sample	Dilution	CFUs	Photo
B2	1:1000	66	
B3	1:1000	95	
B4	1:100	129	

Sample	Dilution	CFUs	Photo
B5	1:1000	111	
B6	1:1000	102	
B7	1:1000	61	

GROUP C – DRY BRUSHING AND UV-C

Sample	Dilution	CFUs	Photo
C1	1:10	104	
C2	1:10	51	
C3	1:100	37	

Sample	Dilution	CFUs	Photo
C4	1:100	210	
C5	1:1	71	
C6	1:1	86	

Sample	Dilution	CFUs	Photo
C7	1:1	4	

Appendix M – Microbiology Results by Dr. Joao Inacio

How each plaster plate sample was processed?

1. A sterile cotton swab was soaked with sterile PBS buffer
2. The entire surface of the plaster surface was swabbed for 30 seconds
3. The cotton tip of the swab was cut into a universal containing 4.5 mL of sterile PBS
4. After vortexing for 15 seconds, 0.5 mL of the suspension was transferred to a new universal containing 4.5 mL of sterile PBS (dilution 1:10)
5. The serial dilution was continued in order to obtain dilutions 1:100, 1:100 and 1:10000
6. 0.1 mL of each dilution was transferred onto SDA plates and the suspension was spread with the help of a sterile plastic spreader
7. The plates were incubated 48 hours at 25 degrees Celsius
8. After incubation, the number of CFUs was recorded for the plate/dilution factor containing between 30 and 300 CFUs (see table below)

RESULTS for the enumeration of colony forming units (CFUs)

Sample	Dilution	CFUs on plate	CFUs/cm^2 on the plaster plate*
A1	1:10000	39	2.8×10^5
A2	1:10000	78	5.5×10^5
A3	1:10000	49	3.5×10^5
A4	1:1000	106	7.5×10^4
A5	1:10000	44	3.1×10^5
A6	1:10000	68	4.8×10^5
A7	1:10000	31	2.2×10^5
B1	1:1000	115	8.1×10^4
B2	1:1000	66	4.7×10^4
B3	1:1000	95	6.7×10^4
B4	1:100	129	9.1×10^3
B5	1:1000	111	7.9×10^4
B6	1:1000	102	7.2×10^4
B7	1:1000	61	4.3×10^4
C1	1:10	104	7.4×10^2
C2	1:10	51	3.6×10^2
C3	1:100	37	2.6×10^3
C4	1:100	210	1.5×10^4
C5	1:1	71	5.0×10^1
C6	1:1	86	6.1×10^1
C7	1:1	4	2.8×10^0

*The total number of CFUs in the original/non-diluted 4.5 mL suspension was determined from the number of CFUs growing on plates (taking into consideration the respective dilution factor). This number was then divided by the surface area of the plaster plates (= 63.62 cm^2).

In his email 'Results,' Dr. Inacio further explains the calculation of the CFUs/cm^2 on the plaster plate as follows:

> To calculate these CFU/cm2 we need to know the surface area of the plates. Which have a diameter of 9 cm.
>
> So, the surface of a plate = $\pi \times r^2 = 3.14159 \times 4.5^2 =$ **63.62 cm^2**
>
> For each of your samples, we collected a swab and then prepared a suspension, and then this dilution was serially diluted 1:10, 1:100, 1:1000, etc. An aliquot of **0.1 mL** of each dilution was then plated on the agar plates and, after incubation, colonies were counted. We selected the plates of a certain dilution, containing a number of colonies between 30 and 300.
>
> So, for example, for C4:
>
> There was **210 CFUs** growing on the plate inoculated with the 1:100 dilution **(dilution factor = 100**)
> So, we can calculate the concentration of CFUs in the original non-diluted suspension with the formula:
> CFU/mL (in the non-diluted suspension) = (210 x 100)/0.1 = 2.1 x 10^5 CFU/mL
>
> Now, for your work, I think it may be more useful to represent your results with a concentration by a plaster surface area. So, we can estimate the number of CFUs per square cm of your plaster plates. We assume that all the spores in your original non-diluted suspension came from your plaster sample. And your swab was vortexed into a total volume of 4.5 mL of sterile PBS solution. So, the total number of spores in your swab (and plaster plate) would be 4.5 x 2.1 x 10^5 = 9.45 x 10^5 spores, and all these spores were spread on a surface with 63.62 cm^2
>
> So, the concentration of spore per plaster surface will be 9.45 x 10^5/63.62 = 1.49 x 10^4 CFU/cm^2 (2019).

Supplier List

Basic Alpha Plaster

Alec Tiranti London
27 Warren Street,
Kings Cross, London,
United Kingdom
+44 (0) 20 7380 0808
www.tiranti.co.uk

Culture – *P. chrysogenum*

DSMZ
Inhoffenstraße 7B
38124 Braunschweig
GERMANY
+49 (0)531 2616-0
www.dsmz.de

Isopropanol Alcohol IPA 99.99%

TradeChem/PureChem
www.amazon.co.uk

Onset HOBO© Datalogger

Onset Computer Corporation
470 MacArthur Blvd.
Bourne, MA 02532
+44 1-800-564-4377
www.onsetcomp.com

Petri Dishes

Sterlin
www.amazon.co.uk

Rabbit Skin Glue

Cornellison, Ltd.
105 Great Russell Street
London WC1B 3RY
+44 (0) 20 7636 1045
www.cornellison.com

SDA Plates

Scientific Laboratory Supplies Limited
Wilford Industrial Estate
Ruddington Lane
Wilford
Nottingham NG11 7EP
+44 (0) 11 5982 1111
www.scientificlab.co.uk

Sterile Swabs

neoLab
www.amazon.co.uk

Sterile Water

Baxter
www.amazon.co.uk

List of Abbreviations

AIC – American Institute for Conservation

ASHRAE – American Society of Heating, Refrigerating and Air-Conditioning Engineers

CCI – Canadian Conservation Institute

CFU – Colony Forming Units

CIE – Commission Internationale de l'Eclairage

COSHH – Control of Substances Hazardous to Health

FTIR – Fourier-Transform Infrared Spectroscopy

HVAC – Heating Ventilation and Air Conditioning

IARC – International Agency for Research on Cancer

ICP – Inductively Coupled Plasma

IIC – International Institute for Conservation of Historic and Artistic Works

NM – (nm) Nanometres

NMDC – National Museum Directors' Conference

°C – Celsius

PBS – Phosphate Buffer Solution

PPE – Personal Protection Equipment

RH – Relative Humidity

SDA – Sabouraud Dextrose Agar

SEM – Scanning Electron Microscopy

UV-C – Ultraviolet C

UVGI – Ultraviolet Germicidal Irradiation

WHO – World Health Organization

XRF – X-Ray Fluorescence