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**What controls algal greening of  
sandstone heritage?  
An experimental approach**

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# Abstract

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Recent observations have shown that many sandstone buildings, including important components of the UK's cultural heritage, are becoming covered with green algal growths. This is likely to result from recent changes in air quality and the impacts of a changing climate. The northern regions of the UK in particular have an abundance of sandstone heritage and, given the likelihood of warmer, wetter winters here, algal growth on vulnerable monuments is likely to become a primary conservation concern over the next 50 years. Observations of sandstone monuments in the northern regions of the UK, in particular in Belfast (Northern Ireland), Sheffield and Edinburgh have highlighted that algal greening is notably patchy. This is likely due to the array of factors which affect the bioreceptivity of host substrates such as sandstone. The bioreceptivity of a substrate (its ability to become colonised by microbes such as green algae) is dependent on inherent, external and architectural factors. The role of these factors and the interrelationships between them requires further study.

This thesis aims to investigate the inherent, external and architectural factors which encourage colonisation of sandstone by green algae through an integrated programme of laboratory and field experimentation. The primary objectives of this study are: to develop improved laboratory experimental methods to control and monitor algal growth, to investigate the role of external, inherent and architectural factors and to explore the fundamental role of moisture in the development of algal greening.

In order to address these objectives, laboratory and field experiments have been linked within an integrated overall methodology. Short-term laboratory experiments have

investigated the bioreceptivity of four British sandstones (Peak Moor, Dungannon, St Bees and ‘baluster stone’) to single and mixed green algal treatment with *Stichococcus bacillaris*, *Chlorella vulgaris* and *Desmococcus olivaceus*, under controlled conditions. Two field experiments have also been conducted. The first exposed unweathered blocks of Dungannon sandstone in the wet environment of Derrygonnelly, Northern Ireland for 30 months. The second exposed reclaimed sandstone balusters in a shaded and exposed site in central Oxford for 12 months. The laboratory and field experiments presented utilise a range of simple and accessible methods to monitor biofilm development (for example novel methods to map biomass) and changes in substrate condition (such as monitoring surface moisture movements with weight change and hand-held moisture meters, and using light microscopy to help visualise the impact of green algal biofilms).

The results presented in this thesis confirm that moisture plays a fundamental role in the development of green algal biofilms. In laboratory experiments, colonisation often occurred within a consistent moisture zone and preferential greening in field experiments was observed in areas of frequent moisture movement. External factors have been shown to have a strong influence, in laboratory experiments where marine salts were applied, these were found to delay colonisation by around seven days. Furthermore, salts resulted in inhomogeneous patterns of colonisation, similar to those observed in scoping studies conducted in Sheffield. Laboratory experiments have also demonstrated that inherent substrate factors such as high porosity and presence of certain minerals (such as clay laminations in Dungannon) can increase the primary bioreceptivity of sandstone surfaces.

Field experiments have demonstrated that architectural factors such as aspect and geometry can increase the bioreceptivity of exposed samples. In particular, preferential

greening was observed on the dynamically wetted south west facing blocks in Derrygonnelly and on exposed compared with shaded balusters in Oxford. Greening was also concentrated in areas of rainwater flows and stores.

Investigation of the role of external, inherent and architectural factors in the development of algal greening as provided by this project, supplies useful information for those managing our sandstone cultural heritage. This will enable more informed decisions to be made over appropriate management and conservation strategies for the future.

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# Chapter 1:

## Introduction and literature review

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The primary aim of the experiments presented in this thesis is to investigate the inherent, external and architectural factors which encourage colonisation of sandstone by green algae. This chapter introduces the thesis title, aim and objectives in the context of current research and findings in the field of heritage conservation.

### 1.1 Sandstone heritage: Nature and threats

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Sandstones are defined as clastic, sedimentary rocks, formed of sand-sized grains (Young, 2004) and dominated by silica (Pettijohn et al., 1987). Due to the shape and distribution of constituent sand grains, sandstones have a relatively higher porosity than other major building stone types (Pettijohn et al., 1987), making them particularly prone to many sorts of deterioration. Despite their worldwide abundance being similar to that of limestones and granites (Meybeck, 1987), and despite the fact that they are found in much of the world's cultural heritage, sandstones and how they weather and deteriorate have received less scientific attention (Turkington et al., 2003).

Quartz-rich sandstones are some of the most abundant examples of sandstone around the world and are extensively found in the UK (Krynine, 1948). Due to their durability and relative abundance, these sandstones have been used widely for building construction in the UK for centuries (Warke et al., 2006). Reviews of the nature and use of building sandstones in Britain are provided by the British Geological Survey Stone Database (see BGS (2008)), and for Northern Ireland by the Natural Stone Database hosted by Consarc

(see Curran (2008) and Curran et al. (2010)). Notable examples of the use of sandstone in heritage buildings in the UK include: St George's Hall (Liverpool), Nottingham Guildhall, the Royal Exchange Manchester and Sheffield Cathedral.

In comparison to denser stones, the porosity of sandstone makes it particularly vulnerable to a wide range of deteriorative processes, as well as microbial colonisation in fissures, cracks and cavities on or within the surface (Macedo et al., 2009). As McCabe et al. (2011, p. 167) highlight, sandstones are particularly receptive to biocolonisation due to their "open-texture, pore connectivity and potential mineral interactions with colonizers". In addition, microbial organisms such as green algae favour slightly acidic surface pH, which occurs on non-calcareous substrates such as sandstone (Urquhart et al., 1996).

Sandstone deterioration can be conceptualised as including both weathering and soiling processes. Like all sedimentary rocks, sandstone is prone to weathering through any of the main mechanisms individually or in association: chemical, mechanical or biological (Bland and Rolls, 1998). Sandstone weathering can proceed by granular disintegration, as individual sand grains are freed from the matrix, or by exfoliation of surface crusts often developed through case hardening. Such processes lead to loss of architectural detailing, as well as sometimes catastrophic development of hollows. The rate and nature of sandstone weathering is dependent upon a number of factors including the lithology of the substrate and the nature of surrounding environmental conditions. Soiling and discolouration of sandstone surfaces is also a component of deterioration and can influence weathering. Soiling may be caused by chemical transformations of minerals, by deposition of dust and accumulation of pollutant-rich crusts, or by biofouling (the growth of microbial biofilms).

One of the most visible forms of sandstone deterioration is, indeed, soiling, especially that due to microbial biofilms. The past 40 years have seen an increase in the apparent 'greening' of sandstone heritage, particularly across the wetter north-western parts of the UK (Cameron et al., 1997; Smith et al., 2004). Here, greening refers to the development of photosynthetic microbial biofilms, dominated by green algae.

Widespread patchy greening of sandstone facades has been observed in many places across the northern parts of the UK including Sheffield, Belfast and Edinburgh (see Figure 1). The vulnerability of British sandstones to algal greening was first highlighted in Scotland in the late 1980's and early 1990's. Significant greening of buildings was observed following a period of extensive cleaning since the 1960's and is known as the 'clean to green' phenomenon (Cameron et al., 1997). This prompted a number of field studies and research reports commissioned by Historic Scotland (see Urquhart et al. (1996), Young (1998), Young and Urquhart (1998), Young and Urquhart (1999)). The aims of these reports were to explore causes for rapid re-soiling by microorganisms following cleaning, to assess the biodeteriorative implications of microbial growth and to test the efficacy of new biocides for their removal. The key findings are summarised in Figure 2. These and other observations of rapid greening from the late 1990's on both untreated and treated (cleaned) sandstone facades in the north-west of the UK (particularly Northern Ireland) have initiated a renewed interest in the causes and consequences of algal greening of sandstone heritage.

**Figure 1: The variability of sandstone greening across scales and locations**



**a:** Architrave of Lanyon building, Belfast, **b:** Buttress of Fitzroy church, Belfast, **c:** 1960's extension, Sheffield Cathedral, **d:** Front façade, Elmwood Hall, Belfast, **e:** Grave monument, Sheffield Cathedral, **f:** Tron Church, Edinburgh, **g:** Corinthian column capital, Elmwood Hall, Belfast, **h:** Replacement baluster, Waterloo Place, Edinburgh, **i:** Western façade, Sheffield Cathedral

**Figure 2: Summary of research findings from Historic Scotland's investigations into the 'clean to green' phenomenon**

- Re-colonisation of cleaned facades by green algae occurs very quickly, often within weeks.
- Residual chemicals from stone cleaning (particularly phosphates) can enhance re-colonisation by microorganisms particularly in porous stones such as sandstones.
- Stone cleaning enhances light penetration (particularly on light coloured sandstones) at depth, which can encourage colonisation by photosynthetic organisms.
- Colonisation was confined to areas of a building with increased time of wetness.
- Local climate (rainfall and sunshine hours in particular) were found to have a significant influence on levels of colonisation observed.

For example, exposure trials conducted between 1999-2001 in Belfast (Smith et al., 2004) noted much more extensive colonisation of sandstone by algal and fungal species than similar trials conducted between 1993-1999 (Turkington et al., 2003). Rapid greening of sandstone facades in Northern Ireland has been associated with an increase in winter rainfall and a decrease in sulphur dioxide emissions over the past two decades (McCabe et al., 2011). More detailed observations of patchy colonisation on sandstone facades were made during pilot site visits at the start of this DPhil project to Belfast, Edinburgh and Sheffield (see Figure 3). The recurrent finding from all of these visits was a concentration of green algal colonisation in areas of moisture accumulation and run-off (see Figure 1c and e). In order to better understand the role of microclimate and local effects (such as historic sulphur pollution) detailed observations across all cardinal aspects of Sheffield Cathedral were carried out and are presented in Figure 3. In particular, green algal colonisation was most apparent on the eastern façade and concentrated around architectural detailing such as string- courses and windowsills. In contrast, the southern façade was comparatively free of colonisation whereas the northern façade was dominated by dark-coloured soiling.

Figure 3: Site observations from Sheffield Cathedral, Sheffield

The recent extension to the building (2004) constructed of Stanton Moor sandstone hosts significantly less biofilm matter than older parts of the building, despite being north-facing and wetted quite continuously.



The picture on the right was taken at the time of construction. The figure on the left however, taken in March 2010 makes for an interesting comparison. There appear to be clear lines of rainwater run-off at either side on the bay window. Contributing factors to this patchy development may include the slope of the roof, and the 45° angle wall capping.



Of all the aspect faces, the eastern facade had the most visually prominent green biofilm development. Interesting patchy formation was noted around buttresses and bay windows (see photo above). This seems to be due to angle of rainfall incidence and frequency of wetting.



The south face had significantly less obvious biofilm development than the north, eastern or western faces of the Cathedral, as has shown to be the case at St. Mary's Church, Lisbellaw also. The facade is generally drier and more exposed to wind vortexing-possible inhibiting factors for growth of chlorophyta.

The 1960's extension to the south-west of the Cathedral would make an interesting point of comparison as facades do not have string courses

There is clear evidence of dark surface crust formation. In areas repeatedly wetted, there is some evidence of gypsum crust formation and spalling off of crusts on the north-western facade. This appears to be the legacy of previous pollution (memory effect)



There is clear evidence of colonisation by green algae on the eastern facade. Colonisation is patchy, but concentrated around architectural detailing such as the low-level string course. There appears to be little colonisation directly below this feature, but heavy soiling occurs at the joint between the feature and the wall itself



The complexity of greening patterns observed at Sheffield Cathedral highlights that a range of inherent, external and architectural factors determines the spatial distribution of colonisation. Together, these factors can be encapsulated in the concept of ‘bioreceptivity’ which this thesis investigates, building on the pioneering work of Guillitte (1995).

Worries about the scale, causes and consequences of algal greening prompted an interdisciplinary EPSRC - funded study which began in 2009 (grant number EP/G011338/1) linking geomorphology, microbiology, climatology and civil engineering. This thesis (which was funded as part of this larger EPSRC project) addresses the need for linked laboratory and field experiments testing the bioreceptivity of British sandstones, which was highlighted as a key gap in current scientific research by the EPSRC project team.

## 1.2 Algal greening: nature and consequences

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Algal greening is a specific manifestation of a more general phenomenon, i.e. the growth of microbial biofilms. Microbial biofilms grow at interfaces – such as between rock and air, or between sediment and water. These interfacial zones are characterised by harsh environmental conditions (Gorbushina, 2007). This is particularly the case for biofilms on building stone where microorganisms are subject to extremes of temperature, wind speed and ventilation, high levels of UV coupled with shading and dynamic patterns of wetting and drying. The degree to which a biofilm will be affected by these extremes depends on the building orientation, latitude, degree of shading and slope (Cutler and Viles, 2010). The stone substrate itself is often devoid of nutrition. Consequently,

organisms that occur on building stone are highly tolerant (capable of surviving in low water and nutrient conditions and able to cope with highly variable conditions) and highly specialised (Gorbushina and Krumbein, 2000; Miller et al., 2012).

Lithobiotic (stone-dwelling) biofilms can consist of bacteria, algae, fungi, lichen and higher organisms such as mosses and liverworts. The microbial constituents of a biofilm are usually the primary colonisers. These improve the bioreceptivity of the surface paving the way for more nutritionally demanding and physically damaging, higher organisms (Welton et al., 2003; Bartoli et al., 2014).

In a microbial biofilm, a wide range of organisms often co-exists in different niches on the stone surface (epilithic niche) and within the sub-surface (endolithic niche). The simultaneous effects of these organisms on the substrate act synergistically to cause deterioration of the stone (Gómez-Alarcón et al., 1994). Research on the characteristics and implications of microbial biofilms on stone has been conducted for over a century, focusing heavily on potentially biodeteriorative impacts and usually only on one or two types of organisms. Only recently, with the advent of culture-independent identification techniques, has it been feasible to study whole biofilm communities and their interconnected impacts on stone algae (Rindi et al., 2011). Cyanobacteria, formerly known confusingly as blue-green algae, are the most widely studied and perhaps understood of biofilm organisms and much is now known about their ecology and activities (Gleeson et al., 2005). Cyanobacteria dominate stone surface biofilms in tropical regions whereas green algae (chlorophyta) are the primary constituents of many stone biofilms in temperate regions such as the United Kingdom (Fritsch, 1907; Urzi and Krumbein, 1992; Gaylarde and Gaylarde, 2005).

The nature and impacts of eukaryotic organisms, such as algae and fungi, within biofilms on stone are less well understood (Cutler and Viles, 2010). After bacteria, algae are the most abundant microorganisms occurring on stone surfaces in the UK and Europe and of these, the Chlorophyta and Bacillariophyta (diatoms) are the most common (Ortega-Calvo et al., 1995; Young and Wakefield, 1995). Chlorophyta are the subject of this thesis.

### *Chlorophyta*

Green algae or Chlorophyta are as phototrophic eukaryotes, distinguished from other algae because they hold chloroplasts containing the chlorophylls *a* and *b*, giving them a characteristic green colour (Madigan et al., 2009a). They occur prolifically in freshwater, marine and soil environments and usually spread to stone surfaces from the latter. Their morphology varies from unicellular, filamentous (cells arranged from end to end in filaments) or sarcinoid (colonial- aggregated cells) in nature (Lewis and McCourt, 2004).

Chlorophyta occur unified in organic layers on the surface of stone as a dominant component of many biofilms. This unification comes about through physical embedding in the colloidal gelatinous layer (Warscheid, 1999) made up of extracellular polymeric substances (EPS) secreted through microbial action. EPS excretion enables attachment of microbial cells to the substrate whilst providing a buffer to environmental stress, enabling the development of competitive strategies and facilitating the uptake of nutrients (Wakefield et al., 1996b).

A wide range of green algal species is known to dwell on stone surfaces across the world, depending on local ambient conditions and substrate characteristics. The diversity of algal

communities on building stone surfaces is often lower than that of bacterial and fungal communities (Cutler et al., 2015). However, some algal genera occur prolifically across continents and substrates. In particular, the chlorophytes *Chlorella*, *Stichococcus* and *Desmococcus* are widely distributed across rock types and geographical areas (Rindi et al., 2011). Species within these genera have been recorded on limestones, sandstones and granites in the Mediterranean (Tomaselli et al., 2000; Rifón-Lastra and Nogueroles-Seoane, 2001; Macedo et al., 2009; Miller et al., 2009), central Europe (Sigler et al., 2003), Ireland (Schlichting, 1975; Rindi and Guiry, 2003) and Scotland (Urquhart et al., 1996).

### 1.3 The impact of microbial biofilms on stone

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Wilimzig (1996) suggests that approximately 20-30 percent of stone deterioration results from biological activity. Biodeterioration refers to the undesirable impacts that biological growths may have on the underlying stone substrate they colonise including impacts on both weathering and soiling (Griffin et al., 1991). These processes may be accelerated or even initiated by microorganisms and other biological growth (Crispim et al., 2003), but on the other hand microbial biofilms can also have a bioprotective effect (through retarding or halting other deterioration processes). Gaylarde *et al* (2003) term the influence of microbial biofilms on stone deterioration as an *assimilative process* where biodeterioration is encouraged by microorganisms using the host substrate as a nutrient. Interestingly, Dornieden *et al* (2000) note that microorganisms may exhibit brief, but particularly virulent outbursts of biodeteriorative activity.

The biodeteriorative roles of prokaryotic microorganisms (unicellular organisms devoid of a nucleus; Madigan et al. (2009b)) have received considerable attention in the literature (Gleeson et al., 2006; López-Bautista et al., 2007; Miller et al., 2009). However,

there is much less research on the role of eukaryotic organisms (single or multi-cellular organisms distinctly containing a membrane-enclosed nucleus; Madigan *et al.*, 2009), in particular fungi and green algae. Welton *et al.* (2003) carried out preliminary experimental studies which demonstrated potentially important deteriorative capabilities of some green algal species. The following section reviews the state of the art of knowledge on the role of green algae in soiling (biofouling) and weathering (bioweathering and bioprotection).

### *Biofouling*

Biofouling, or staining of surfaces through biological growths, is the most visible form of biodeterioration. Algal greening is one particularly obvious form of biofouling. Whilst ‘greening’ is a generic term used to describe growth of photosynthetic organisms, in reality, algal biofilms range widely in colour. Chlorophyll, carotenoids and melanins produced by metabolising microbes are all associated with surface staining. For instance, dark brown and bright red surface growths are often characterised by the presence of species from the green algal genus *Trentepohlia* and other species with brightly coloured mucilage-sheaths (Wakefield *et al.*, 1996a; Urzi and Realini, 1998). Dark brown and black stains are often attributed to deposition of fly ash, but may also be a result of cyanobacterial, fungal or algal growths (Young *et al.*, 2008). For instance, in their study of stone tablets exposed in Oxford, Viles and Gorbushina (2003) found that fungi and other microorganisms contributed greatly to the dark- coloured soiling they observed.

The aesthetic appearance of these pigment- rich microorganisms is often undesirable on monuments of significant cultural heritage and/ or beauty (Nugari *et al.*, 2009). In addition, the appearance of a biofilm may acquire a ‘dirty’ look through adhesion of atmospheric pollutants and soot particles (Ortega-Calvo *et al.*, 1995; Urquhart *et al.*,

1996). Moreover, entrapped particles and carbon fixed by biofouling epilithic algae may have a nutritive effect, encouraging further biofouling and or bioweathering (Saiz-Jimenez, 1997).

The appearance of a microbial biofilm may change over time in line with changes in ambient conditions and levels of pollution causing changes to the patterns of surface staining. Lithobiontic organisms are also known to multiply quickly and cause rapid surface blooms which can be unsightly (Bland and Rolls, 1998). Some prominent heritage buildings such as Fitzroy Church, Belfast (which is constructed of light-coloured Scrabo sandstone) have suffered rapid and extensive algal greening, which in this case was exacerbated by chemical stone cleaning (Warke et al., 1999).

### *Bioweathering*

Bioweathering of stone can take a number of forms, notably biophysical and biochemical processes. Biophysical weathering refers to mechanical damage to the stone through the physical growth activity of biological species (Griffin et al., 1991). Conversely, biochemical weathering refers to dissolution and decay of the stone substrate caused by the direct and indirect action of the products of microbial metabolism (Surdam et al., 1989). The degree of weathering produced is dependent upon the species present, the lithology of the substrate and surrounding environmental conditions (Dornieden et al., 2000).

With regards to biophysical damage, it has been argued that the expansion and contraction of the gelatinous cell wall of a number of algal species (through uptake and loss of moisture) can cause physical stress within the substrate (Ortega-Calvo et al., 1995; Miller et al., 2010). Similarly, biophysical damage has been noted through hygroscopic

expansion of EPS deposited by cyanobacteria (Ortega-Morales et al., 2005). Furthermore, the often vigorous nature in which chasmoendoliths (organisms which colonise pores on the stone surface – these can be bacteria, fungi or algae) colonise surface and sub-surface pore-spaces and micro-fissures may result in mechanical stress within the stone (Viles, 1987; Wakefield et al., 1996a). For example, Bartoli et al. (2014) found that *Trentepohlia* cells penetrated between sandstone grains to an average depth of 0.26 mm on the ruins of temples at Angkor Wat, Cambodia.

Biochemical damage to stone surfaces from microbial biofilms has been noted through secretion of oxalic acids which can lead to dissolution (Gómez-Alarcón et al., 1994; Bland and Rolls, 1998; Gorbushina, 2007). For example, damage to White Stone in Russia has been attributed to dissolution by acids of microbial origin (e.g. nitrous, nitric and sulphuric) produced by chemolithotrophic bacteria (Lyalikova and Petushkova, 1991). In addition, select species such as the chlorophytic genus *Ostreobium* have demonstrated their distinct ability to bore into calcified structures through organic-acid dissolution (Highsmith, 1981; Viles, 1987). Damage can occur to actual grains or to cementing minerals in the interstitial matrix. Either way, dissolution leads to disaggregation of grains and an increase in porosity, which can further encourage surface and sub-surface colonisation. However, the overall role of organic acids in the bioweathering of stone is much debated (Ashurst and Dimes, 1998). In particular, the chemical impacts of chlorophyta remain relatively understudied apart from the experimental work of Welton et al. (2003).

Biofilms may also cause indirect weathering to underlying substrates by influencing moisture movements. Complex feedback loops are created whereby algal growth

encourages retention of surface moisture and hence further greening (McCabe et al., 2007). EPS and other metabolic products excreted by algae may have the effect of reducing evaporation (Häubner et al., 2006) and improving the water retention capacity of surfaces. Consequently as biofilms retain surface moisture, they increase the reaction time of soluble salts which could lead to direct dissolution of minerals or physical breakdown due to crystallisation of salts (Ortega-Morales et al., 2005).

Furthermore, microbial cells may block stone pores thereby reducing their water absorption capacity (Webster and May, 2006), which could have the effect of trapping harmful salts at depth. For example, in their study of sandstone heritage structures in Belfast, Cutler et al. (2013b) found some evidence of lower surface infiltration rates on colonised green patches compared to uncolonised sections. Existing research demonstrates that overall, the role of biofilms in influencing moisture movements on building facades is not well understood.

### *Bioprotective or benign?*

In some cases it is difficult to establish whether a biofilm causes deterioration or not and the net effects may be bioprotective at least in some places and on some timescales. For instance, Mottershead et al. (2003) noted that patchy colonisation (by a mixed microbial community, predominated by green algae) on stone surfaces led to increased weathering whereas abundant growth protected the substrate beneath.

There is a growing body of evidence citing the protective role microorganisms have on stone cultural heritage. Processes such as these are grouped under the term *bioprotection* (Carter and Viles, 2005). For instance, some fungal communities have been observed to

bind together mineral grains with networks of hyphae (Sterflinger, 2000). Biotic colonies may also have the ability to harden and bind friable rock surfaces (Pope et al., 2002). For example, in their study of sandstone outcrops in Utah, USA, Kurtz Jr and Netoff (2001) found biofilms dominated by cyanobacteria to have a stabilising effect on friable surfaces through excretion of EPS resulting in a reduction in surface porosity. Similarly, in their study of heritage structures in Northern Ireland Cutler et al. (2013b) found evidence to suggest that green algal patches were associated with less weathered and harder sandstone surfaces. Biofilms may also have a regulatory effect by reducing temperature fluctuations at the stone surface and thereby slowing down mechanical breakdown (McCabe et al., 2015). Organisms known to be useful have been consciously used to create bioprotective effects. For example, bacterial strains isolated in the BIOBRUSH project have been used in the bioremediation of harmful salt crusts (May et al., 2005).

The above review demonstrates many gaps in knowledge, and suggests that further interdisciplinary studies looking at both environmental influences and substrate properties would allow a more holistic approach to biofilm study, resulting in more practical and adequate action (Warscheid, 1999). In particular, little is known about the direct impact of ‘greening’ on culturally sensitive monuments in the UK and the ‘bioreceptivity’ of vulnerable British building sandstones. This thesis aims to address these gaps.

## 1.4 Controls on algal greening

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### *Environmental change*

Increasing interest in algal greening, particularly in the north- west of the UK (including Northern Ireland and Scotland) has been enhanced given projections for future environmental change. Environmental change is likely to have important implications on biofilm development due to changes in atmospheric pollutant concentrations, precipitation, temperature and the frequency of extreme events (Viles, 2002). The noticeable greening of sandstone surfaces over the past 30 years has in part been attributed to a reduction in atmospheric sulphur dioxide, which may previously have inhibited colonisation and an increase in nitrogen oxides (of vehicular origin), which have a fertilising effect (Smith et al., 2011; McCabe et al., 2015). Whilst the impacts of changing carbon dioxide levels on algal greening are underexplored in the literature, enhanced growth rates in higher plants have in many cases been attributed to a rise in atmospheric carbon dioxide levels in the twentieth century (Cure and Acock, 1986; Liu et al., 2016).

Future climatic projections for the north- west of the UK (UKCP09) highlight a trend towards uncertainty in daily conditions and towards wetter winters and drier summers (Smith et al., 2011). In particular, an increase in winter rainfall of between 10-20 % is projected over the following 50 years (McAllister et al., 2011). It is these enhanced levels of winter rainfall and prolonged, warmer, wetter winter conditions that are likely to encourage the growth of moisture and warm-loving chlorophyta (Smith et al., 2009). Furthermore, it is the associated increased ‘time-of-wetness’ and ‘deep wetness’ of stone which will be conducive to biological colonisation (Smith et al., 2004). In this context ‘time-of-wetness’ refers to the length of time for which the stone surface remains wet and

‘deep wetness’ the extent to which the interior of the stone remains wet (Smith et al., 2011).

In Northern Ireland it is likely that temperature conditions predicted under future climate scenarios will favour biofilm development. By 2080, we can expect an increase in summer mean temperatures of between 1-3.5° C and an increase in winter mean temperatures of 1-2.5° C from baseline 2007 figures (Arkell et al., 2007). Of particular relevance in the growth of surface biofilms will be the increase in winter temperatures. This will in effect increase the length of the optimum growing season of spring (Harding et al., 2015).

In order to better predict the implications of future environmental change on green algal biofilms, it is necessary to better understand the mechanisms of their growth and the bioreceptivity of vulnerable British sandstones.

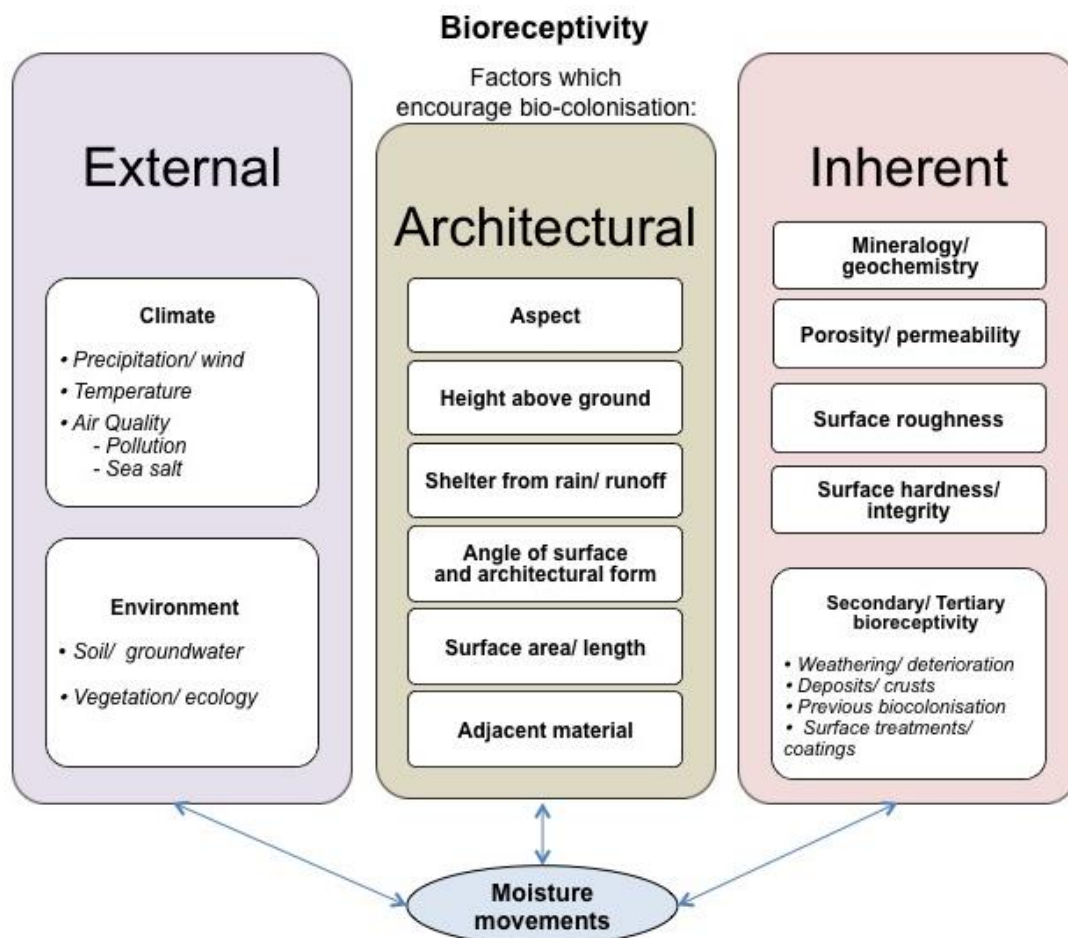
## 1.5 Defining bioreceptivity

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The term ‘bioreceptivity’ was first comprehensively defined by Guillitte (1995) as the relative ability or aptitude of a material to become colonised by microbial organisms. Under this conceptualisation, bioreceptivity mainly reflected material and surface characteristics. Material properties that contribute to establishment of microbial colonies include chemical composition, porosity, permeability, pH and surface roughness. Where these properties are similar to the initial state of the lithotype (i.e. unweathered or freshly quarried stone), their influence on biocolonisation is referred to as ‘primary bioreceptivity’. Where material properties change over time due to exposure to the

environment and or action of microbial communities themselves, this is referred to as ‘secondary bioreceptivity’. Finally, any human activity that has affected the material, (such as surface dressing or application of biocides or consolidants) results in ‘tertiary bioreceptivity’. This thesis builds on Guillitte’s original concept, and groups primary, secondary and tertiary factors affecting bioreceptivity as ‘*inherent factors*’ as they relate to the intrinsic properties of the substrate (see Figure 4).

**Figure 4: A holistic approach to bioreceptivity analysis**



Conversely, where exogenous particulates such as salt, dust or fly- ash are deposited on the surface and affect the material’s ability to become colonised, Guillitte (1995) terms this ‘extrinsic’ bioreceptivity. In this thesis such extrinsic factors are considered alongside

the influence of other climatic factors, such as precipitation and temperature as well as environmental interactions, such as groundwater and vegetation with host substrates. These are grouped as ‘*external factors*’ controlling bioreceptivity (see Figure 4).

The role of inherent and external factors has been considered extensively in both laboratory and field studies, as reviewed in the following sections. Hitherto, models of bioreceptivity have not, however, incorporated the role of architectural factors in encouraging bio- colonisation in addition to inherent and external factors. This thesis also addresses these factors and regards them as central (see Figure 4). Consequently, there is a need for a holistic approach when assessing the bioreceptivity of any given lithotype or stone structure incorporating consideration of all three groups of factors as presented in Figure 4. The current state of knowledge on the individual factors which encourage bio-colonisation is reviewed below.

## 1.6 Factors which encourage biocolonisation

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### *1.6.1 The fundamental role of moisture*

Many have argued that the most influential factor controlling the ecology of lithobiontic algae and the degree of biological soiling is *moisture availability* (Hoffmann, 1989; Crispim et al., 2003; Gorbushina, 2007). This may impact and accumulate on the substrate through relative humidity or directly through precipitation and wind-driven rain. Whilst water is required directly for healthy metabolic activity of green algae, it also acts to transport soluble nutrients and often aids in the dispersal of algal propagules across stone surfaces. Therefore, the *movement of moisture* on the stone surface and within its sub-surface is also likely to impact its bioreceptivity. For example, biological colonisation is

often concentrated in dynamic moisture zones on building facades characterised by frequent rain-washing (Turkington et al., 2003). Indeed, we observed abundant colonisation on rain-washed surfaces on the eastern façade of Sheffield Cathedral (see Figure 3). Miller et al. (2012) note that the petrophysical properties related to the movement of moisture on and within a substrate in addition to its chemical composition are the key determinants of its bioreceptivity.

In comparison to bacteria, fungi and lichen, chlorophyta require a relatively high amount of water for microbial growth. However, once a colony is established, production of EPS can have a buffering effect allowing algae to survive in drier conditions (Urzi and Krumbein, 1992). Indeed, some genera of chlorophyta such as *Chlorella* have demonstrated the ability to lay dormant in periods of desiccation and to quickly resume metabolic activity on wetting (Klochkova and Kim, 2005).

As moisture is essential to metabolic growth, the primary bioreceptivity of a stone surface is largely dependent on factors which control its moisture content. The majority of the factors depicted in Figure 4 enhance bioreceptivity by increasing the moisture content of the stone surface or by increasing the time for which it remains moist. These factors can be differentiated as *external*, *inherent* and *architectural*.

### **1.6.2 External factors**

#### **Precipitation**

Precipitation contributes largely to the volume of moisture available to epilithic algae to metabolise and grow (Cameron et al., 1997). Precipitation is variable across scales, for instance results from exposure trials conducted in Northern Ireland by Adamson et al. (2010) demonstrate that biological colonisation on stone surfaces is strongly influenced

by regional precipitation patterns. They found greater biological soiling in the wetter north-west in comparison to the rain-sheltered south-east. As discussed earlier, Northern Ireland and other northern regions can expect an increase in winter and decrease in summer rainfall over the next 50 years under future climate projections. This is likely to lead to a general prevalence of drought- tolerant chlorophyta.

However, localised features may override the effects of ambient conditions at smaller scales (McCabe et al., 2011). Indeed, at the micro level one part of a building may receive more direct rainfall than another due to shading by protruding walls or due to building orientation.

#### *Air quality: pollution and sea salt*

In general algal diversity is greater in rural areas away from direct sources of pollution. In urban areas, growth is dominated by toxi-tolerant species, which produce uniform, widespread surface biofilms (Cameron et al., 1997). Pilot observations revealed noticeably less green algal colonisation on north- facing walls at Sheffield Cathedral (see Figure 3). These walls tended to exhibit widespread surface soiling and crust formation likely to be associated with historic sulphur pollution. Thus, while the climatic and microclimatic setting was favourable for algal greening, the residual effects of long-term air pollution reduced colonisation.

Water availability depends not only on absolute levels of moisture available, but is also a function of minerals and salts dissolved in the water as high solute concentrations can impede microbiological growth (Madigan et al., 2009b). High levels of sea salt in coastal areas may thus reduce the bioreceptivity of stone surfaces.

In addition to atmospheric pollution and the influence of marine salts, there may be additional, locally important growth factors related to inputs of elements and chemical compounds from animal and other sources. For instance, some buildings are particularly prone to pigeon activity where faecal nutrient enrichment is argued to be conducive to biological colonisation (Agrawal et al., 1988; Garg et al., 1995; Haag-Wackernagel and Geigenfeind, 2008).

### *1.6.3 Inherent factors*

The nature of the stone substrate is important in influencing greening due to the close relationship biological growths have with the host substrate. Indeed, substrate factors such as texture, porosity, pH and mineralogy determine the kinds of biological growths which will colonise the surface (Tiano et al., 1995). In turn, these growths may impact the stone substrate through bioweathering or bioprotection, as discussed earlier, causing changes to bioreceptivity over time.

#### *Mineralogy/ geochemistry/ pH*

Some stone minerals are known to encourage biocolonisation. Stone types rich in clay and feldspars are likely to have a higher bioreceptivity (Warscheid et al., 1991). These minerals offer microorganisms a greater surface area to adhere to as well as a ready supply of retained moisture. In their study of Portuguese building stone substrates, Miller et al. (2006) reported that differences in bioreceptivity were mainly attributed to the contrasting chemical compositions of the substrates rather than due to their physical characteristics.

Conversely, in their study of granites Prieto and Silva (2005) note that pH has more of an influence on bioreceptivity than the chemical composition of the substrate. As discussed, green algae favour conditions of lower pH (Urquhart et al., 1996).

### *Porosity and permeability*

Stones with fine pores have the ability to retain greater volumes of water and are therefore more likely to become colonised by green algae. In their exposure study of a range of Scottish sandstones, Urquhart et al. (1996) found a good correlation between porosity and colour change, where higher porosity sandstones became greener over time. Furthermore, in laboratory studies, substrates characterised by high porosity and associated high capillary absorption have been shown to be more bioreceptive to a range of microbial species when compared with substrates with lower porosities (Guillitte and Dreesen, 1995; Miller et al., 2006; Miller et al., 2010). As discussed at the beginning of this chapter, the relatively high porosity of sandstones makes them particularly receptive to microbial colonisation. Micro- fissures occur at the grain boundaries of porous sandstones, which are often rapidly colonised. Microorganisms may then go on to remove or dissolve the interstitial matrix through physical and chemical action (Hoppert et al., 2004). In effect, this increases the porosity or roughness of the stone surface, enhancing its secondary bioreceptivity.

### *Surface roughness*

Rough or uneven porous surfaces favour algal colonisation (Barberousse et al., 2006). Shear forces are reduced on rough surfaces resulting in easier attachment of microbial propagules (Morton et al., 1998). Rough surfaces also offer a greater surface area and an abundance of microscopic surface niches for algal colonies to congregate around

(Urquhart et al., 1996; Warscheid and Braams, 2000). These micro-refuges offer a degree of protection at the otherwise harsh stone-atmosphere interface by trapping moisture and nutrients and protecting cells from hydrodynamic forces (Miller et al., 2012). For instance, in their study of limestone surfaces in Madrid, Flores et al. (1997) found microalgae to grow preferentially in surface cavities and fissures which retained moisture for longer. This was due to relatively low evaporation and protection from wind and direct solar radiation in these micro-refuges.

#### *Secondary/ tertiary bioreceptivity*

Previous biocolonisation is likely to increase the bioreceptivity of a surface. Existing microbial biomass will have the effect of retaining moisture, reducing run-off and trapping soot particles which will promote subsequent colonisation (Urquhart et al., 1996). Furthermore, algal colonisation may often enhance surface roughness, change the pH, and provide more nutrients. There are also examples whereby management practices themselves become conducive to biological weathering; for instance, the application of polymers or resins as waterproofing agents on stone surfaces. It has been argued that these treatments may provide a food source for microbes, thus encouraging their growth and contributing to surface and sub-surface biodeterioration (Santoro and Koestler, 1991; Koestler, 1999).

#### *1.6.4 Architectural factors*

##### *Aspect*

Whilst the absolute level of moisture a façade receives is important, the aspect or orientation of a building affects the level of moisture its walls receive and the period for which they remain moist (time-of-wetness). In terms of encouraging biocolonisation by

green algae, Cameron et al. (1997) highlight that the period for which a stone remains moist is more important than the frequency of wetting.

In temperate locations such as the UK, north-facing walls should be the most bioreceptive as they receive the least direct sunlight and are consequently dried out least frequently. In particular, damp loving algae such as *Trentepohlia* thrive in these locations (Rindi and Guiry, 2002). Whilst east- and west-facing walls receive similar absolute levels of solar radiation, east-facing walls receive early sunshine following cooler nights whereas west-facing walls receive sunshine later, allowing them to warm up and dry out during the day (Cameron et al., 1997). Therefore, east-facing walls remain moist for longer and are more likely to support microbial colonisation. At Sheffield Cathedral, pilot observations confirmed a greater degree of green colonisation on east-facing walls in comparison to south and west-facing walls (see Figure 3). As discussed, darker soiling was observed on northern facades.

South-facing walls in the UK are often the most hostile habitats for microbial colonisation as a result of dynamic patterns of wetting and drying due to prevailing rain and higher levels of direct solar incidence. For instance, Mottershead et al. (2003) found patchier biofilm development (dominated by green algae) on more hostile south-facing walls of Fort Perch Rock, New Brighton, whereas north-facing walls were covered with a protective, dense and homogeneous biofilm. They noted that colonisation on southern facades of the sandstone (Runcorn Stone) monument was limited to surface depressions which trap moisture, thereby ameliorating otherwise hostile conditions.

However, smaller scale factors of building layout and architecture may confound these aspect-related trends. For example, Jones et al. (1999) observed colonisation of the southern face of Hermitage Castle by the moisture-loving green algae *Trentepohlia aurea*. They suggested that as Hermitage Water (a local river) was located on the southern side of the Castle, this created a moist microclimate around the south face, enabling colonisation of the otherwise drier and more dynamic face. Similarly, in studies of biocolonisation of north and south-facing limestone walls in Oxford, aspect could not be identified as the primary controlling variable as the influence of microclimatic variables (such as local variations in temperature and wind due to shading) could not be excluded (Thornbush, 2013). This demonstrates that a range of microclimatic factors at different scales controls surface bioreceptivity.

#### *Height above ground*

Preferential greening of building facades often occurs towards the base of walls (Cutler et al., 2013b). Moisture is drawn up from the ground through capillarity driven by surface evaporation and moisture accumulates at the base as a result of runoff from upper regions of the wall (Cameron et al., 1997). In addition, lower regions are wetted frequently or remain moist due to splash-back from the ground. Splash-back can also supply nutrients from soil in a fertilising effect, explaining why a higher diversity of organisms is often found at the base of walls (Rindi and Guiry, 2004).

#### *Angle of surface and architectural form*

Architectural details such as mouldings, string courses and ledges are likely to have a higher bioreceptivity than vertical surfaces as they retain moisture for longer (Young and Urquhart, 1998). Such features receive run-off from larger overlying areas and are often

partially shaded from direct solar incidence, which prevents drying out. Fine details can also accumulate soot and dust particles, which may have a nutritive effect, encouraging colonisation. Furthermore, architectural details can provide micro- habitats for colonising microorganisms. These are often sheltered from direct solar incidence, wind and rainfall but fed by run-off from adjacent areas, providing very suitable niches for algal colonisation.

Until now only very limited studies have been conducted to analyse the influence of architectural features on green algal colonisation of sandstone. Where studies have been conducted, these have involved exposing angled prisms or blocks in exposure trials. Alternatively they have consisted of surveying morphological features on building facades in situ. This gap in current knowledge is directly addressed in this thesis.

## 1.7 Studying algal greening: methods and approaches

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### *Laboratory investigations of stone colonisation*

As Guillitte (1995) suggests, it is possible to assess the bioreceptivity of different substrates by artificially inoculating test materials with microbial cultures and incubating under ideal or optimum environmental conditions. Laboratory simulations enable study of stone- dwelling biofilms under controlled conditions without the need for invasive field sampling which can damage culturally sensitive monuments (Miller et al., 2012). The literature documents a range of laboratory studies, which have aimed to simulate epilithic biofilms. These are compared and summarised in Table 1 and critically reviewed below.

Simulation studies can be an effective method by which to test the influence of intrinsic stone properties on bioreceptivity. For instance, in an early study, Guillitte and Dreesen (1995) inoculated natural Belgian limestone and sandstone cubes as well as three man-made materials (two cements and brick) with a mixed microbial population (MMP). The mixed inoculant consisting of cyanobacteria, mosses and algae was applied to substrates intermittently over 6 months using an innovative sprinkler system. They found chlorophyte species were the first to colonise all substrates and concluded that aerated concrete had the highest bioreceptivity due to its high macroporosity. Their study aimed to obtain maximum microbial biomass under accelerated environmental conditions. Whilst the resultant biomass may have allowed easier analysis of the impacts of colonisation on the substrates tested, the conditions created and the biofilms produced were not necessarily representative of those occurring in the natural environment.

Table 1: Comparison of laboratory simulation studies

	Reference	Stone types used	Sample size and shape	Number of replicates	Microbial treatment	Inoculation method	Incubation conditions	Methods for monitoring colonisation/ substrate change
Laboratory simulations	Guillitte and Dreesen (1995)	5 Belgian lithotypes (including sandstone, limestone, brick and cement)	50 x 50 x 50 mm cubes	Not specified	Mixed Microbial Population (MMP) of cyanobacteria, mosses and algae	Intermittent sprinkling in a conditioned environmental chamber	20- 30° C, 80- 90% RH for 9 months	Visual observations, thin section microscopy and automated image analysis
	Tiano et al. (1995)	12 Italian lithotypes (including marbles, sandstones, limestones, brick and granite)	50 x 50 x 5 mm slabs	3 replicates for each lithotype (36 total)	A cyanobacterium ( <i>Lynbgya</i> ) and a green alga ( <i>Pleurococcus</i> ) isolated from a natural biofilm	Cultures applied directly to the surface at beginning of experiment and spread manually	20, 30, 40° C, pH 5, 7, 9 for 28 days	Image analysis of chlorophyll fluorescence
	Papida et al. (2000)	2 Greek limestones and a dolomite from the UK	38 x 10 mm discs	Not specified	MMP from a natural biofilm	Not specified	27° C for 21 days, followed by variable weathering cycles for 100 days	Bacterial counts, dry weight, dry density, porosity, water content and surface hardness
	Miller et al. (2006)	4 Portuguese lithotypes (including limestones, granite and marble)	44 x 10 mm cylindrical probes	4 replicates for each lithotype (16 total)	A cyanobacteria ( <i>Gloeocapsa alpini</i> ) and a green alga ( <i>Stichococcus bacillaris</i> ) isolated from a natural biofilm	Cultures applied directly to the surface at beginning of experiment	20 ± 2° C near a window for 4 months	Chlorophyll <i>a</i> fluorescence measured using a spectrofluorometer
	Miller et al. (2008)	Anca Limestone (sourced from Portugal)	40 x 10 x 40 parallelepiped probes	6 replicates	Mixed Microbial Population (MMP) of algae and fungi from a natural biofilm	Intermittent sprinkling in a non- commercial unit	Not specified, 3 month duration	Denaturing Gradient Gel Electrophoresis (DGGE)
	Jain et al. (2009)	Weathered and colonised sandstone from Agra, India	Not specified	Not specified	None (samples with a naturally occurring biofilm were used)	None	Desiccators conditioned at a range of humidity's (52- 96%), 2 year duration	SEM, EDX analysis
	Manso et al. (2014)	Man-made substrates (ordinary Portland cement, magnesium phosphate cement)	80 x 80 x 20 mm	3 replicates for each of the 6 cement mixtures tested (18 total)	Axenic culture of <i>Chlorella vulgaris</i>	Intermittent sprinkling in a conditioned environmental chamber	22- 25° C, 85- 90 % RH, for 10 weeks	Colorimetry and Chlorophyll <i>a</i> fluorescence measured using a fluorometer

Furthermore, due to the sprinkling of enriched water throughout the experiment, it was not possible to determine whether the biofilms produced were a result of the intrinsic nutritive properties of the stone or due to calcium carbonate enrichment. This highlights that caution is required when evaluating the inherent bioreceptivity of materials where nutrients are applied throughout an experimental run.

In a similar study, Tiano et al. (1995) inoculated samples of a range of Italian limestones, granites, marbles and sandstones with cultures of *Pleurococcus* (a chlorophyte) or *Lyngbya* (a cyanobacterium). They found that preferential colonisation of substrates was dependent on inherent physical stone properties such as porosity, surface roughness and pH rather than chemical composition. However, as the study used non-axenic cultures, it was not possible to relate bioreceptivity solely to colonisation patterns of individual species. This is a key point as inoculation under laboratory conditions with a natural microbial community (or isolates from a natural biofilm) has the advantage of being representative of real epilithic biofilms. Indeed, in this case the authors isolated the selected two strains from a natural biofilm occurring on a marble statue. On the other hand, whilst axenic cultures may over represent the abundance of particular organisms occurring in natural epilithic biofilms, inoculation with single species makes observations of the individual metabolic properties of that strain and its individual relationship with the host substrate possible.

Miller et al. (2006) also found inherent stone properties to be important in their inoculation study of Portuguese lithotypes (including two limestones, a granite and a marble). They concluded that Anca limestone, the substrate with the highest porosity and capillary absorption capacity, was the most bioreceptive. In a subsequent laboratory

simulation using only Anca limestone inoculated with a natural MMP, Miller et al. (2008) were able to reproduce the kind of patchy and temporally variable colonisation as observed on natural stone monuments. However, their experiment focused on determining the abundance of different microbial genera through molecular methods such as Denaturing Gradient Gel Electrophoresis (DGGE) and did not consider the morphological characteristics of the biofilms produced or the subsequent impacts (biodeteriorative/ bioprotective) on the limestone substrate tested.

In a more recent study of cements, Manso et al. (2014) conducted an accelerated algal fouling test on ordinary Portland cement (OPC) compared with magnesium phosphate cement (MPC). Samples were intermittently inoculated with an axenic culture of *Chlorella vulgaris* by means of a sprinkler system and subjected to a 12 hour day/ night regime. They found that MPC had a higher bioreceptivity to *C. vulgaris*, attributed to its lower pH and higher porosity. This is an interesting study as it focuses on the potentially positive impacts of biofouling. That is, the ability to encourage substrates to green as a way to integrate nature into urban landscapes. However, as only one algal species was used for inoculation, the results obtained are only indicative of the potential bioreceptivity of the materials tested to green algal growth.

In contrast to the studies discussed above, some authors used experiments to focus on the environmental factors driving bioreceptivity. For instance, in their study of fungal communities, Jain et al. (2009) tested the influence of relative humidity on naturally colonised sandstone samples collected from an historic temple in Agra, India. These samples were placed in desiccators and conditioned at different relative humidities ranging from 52-96.6 %. They found that fungi proliferated in conditions of high RH

between 80-90 % resulting in surface deterioration through mineral disaggregation, as observed through SEM observations. Whilst use of naturally colonised samples is representative of the diversity of microbial communities present on historic monuments, it must be noted that organisms will grow at different rates and some will proliferate whilst others fail to thrive under laboratory conditions. Therefore, the fungal biofilms produced by Jain et al. (2009) under different levels of humidity were not necessarily representative of the natural biofilms that occur on monuments in Agra.

Laboratory simulations have also aimed at assessing the impact of microbial biofilms on underlying substrates, that is, whether they are bioprotective or biodeteriorative. For example, Papida et al. (2000) inoculated discs of limestone and dolomite with a natural MMP of bacteria isolated from a coastal location. A key strength of this study was the attempt to maintain natural nutritive conditions on the stone substrates by using a naturally recovered microbial community for inoculation as well as avoiding re-inoculation or application of additional nutrients during the experimental run. Samples were initially incubated at 21° C for 21 days and then treated with distilled water, sodium chloride solution or sodium sulphate solution and subjected to weathering treatments. They found bacterial- induced decay was enhanced under the presence of salts, sodium sulphate in particular. However, the study was solely focused on the role of bacteria in biodeterioration without consideration of other organisms likely to be present in naturally occurring biofilms on stone monuments.

Whilst several interesting laboratory simulations have been conducted, Tiano (2002, p. 2) stresses that “the results reported in few lab experiments can be considered pertinent only for the stones used, the organisms tested and the incubation conditions applied”.

Because of the nature of simulation methodologies, namely involving one or two comparative stone types or cultures, results cannot always be generalised to a host of substrates and or microbial communities. However, inoculation with single species makes individual observations of the metabolic properties of that strain and its relationship with the host substrate possible. Therefore to account for this, it is necessary to conduct a wide range of simulations to assess the bioreceptivity of individual materials to different microbial organisms. In addition, in the studies reviewed above the methods used to monitor colonisation and impacts on host substrates are often highly specialised and or expensive (e.g. molecular methods to measure species abundance, measurement of chlorophyll *a* fluorescence, SEM and EDX analysis). Overall, there appears to be a lack of reproducible studies with multiple replicates, a lack of simple, easily accessible methods to monitor colonisation and decay patterns as well as a general lack of study of the bioreceptivity of British building sandstones to green algal colonisation (see Table 1).

### *Field investigations of stone colonisation*

Whilst laboratory studies allow for controlled analysis of microbial communities on stone substrates, not all microbes can be cultured in the laboratory and laboratory growth does not necessarily mimic conditions in the external environment (Miller et al., 2012; Mihajlovski et al., 2014). Therefore, field investigations are required to monitor biofilm development in the natural environment as well as to contextualise findings from laboratory studies. Field investigations of green algal colonisation on sandstone heritage have included observations and monitoring of biofilms on heritage monuments in situ or pared- down exposure trials.

Surveys of green algal colonisation on sandstone buildings and monuments in situ have been conducted across the north of the UK. For example, Jones et al. (1999) correlated patterns of *Trentepohlia* colonisation with variations in microclimatic conditions across different facades of Hermitage Castle, Scotland. Warke et al. (1999) surveyed Fitzroy Church, Belfast and found extensive green algal colonisation on cleaned facades, in comparison to uncleaned facades which were dominated by gypsum crusts. More recently, Cutler et al. (2013b) conducted a field study of four heritage sandstone structures in Belfast, Northern Ireland, and correlated the spatial distribution of water absorption, inferred surface moisture and surface temperature patterns with surface greening. Whilst surveys of historic structures in situ allow for real-time analysis of the relationship between microenvironmental conditions and biocolonisation, they are hampered by the difficulty of untangling the complex interactions of current and past conditions in influencing algal greening and weathering.

As an alternative, field exposure trials allow focused study of a combination of variables (such as stone type, aspect, microtopography, or surface treatment) on bioreceptivity and associated biodeterioration or protection processes. They can also better reflect natural environmental and climatic conditions in comparison to laboratory experiments.

In early studies assessing the efficacy of biocides, Urquhart et al. (1996) exposed six sandstone lithotypes on a specially designed test-rig in Scotland. Samples were cut in prisms (50 x 50 x 15 mm) and exposed at an angle of 60° (to represent sloping masonry) facing north and south for 100 weeks. The study exposed a good number of replicates for each lithotype. Twelve replicate prisms of each stone type were positioned on each side of the test rig (72 replicates on each of the north and south sides; 144 in total). The study

found noticeably lower colour change (greening) on lithotypes with lower porosities. The influence of porosity was not significant on samples treated with biocides.

In a later study, Turkington et al. (2003) exposed tablets (70 x 50 x 10 mm) of Baumberger and Dunhouse sandstones in exposed and sheltered conditions in Belfast for a maximum duration of six years. Tablets were hung vertically to represent vertical walls. They assessed the relative abundance of different decay features on the samples through SEM observation and Energy Dispersive X-Ray Analysis (EDS) at 30 pre-defined points on the surface of each sample. These results were tabulated and the percentage abundance of each feature calculated. They found preferential biological colonisation of exposed and therefore rain- washed samples, whereas sheltered samples were dominated by gypsum deposition. Whilst the authors did not attempt detailed assessment of biological colonisation, which would be required to accurately assess the bioreceptivity of the test substrates, they were able to identify the presence of green algae on all surfaces through SEM observations.

Most recently, Adamson et al. (2013a, 2013b) exposed sandstone blocks in a range of nine locations across Northern Ireland for a total of 21 months to assess the influence of aspect and surface roughness (respectively) on algal greening. Blocks of Peak Moor sandstone were exposed at 45° on purpose-built test rigs to represent buttress capstones. Similarly to Urquhart et al. (1996), they used measurements of colour (greening and darkening) to monitor algal colonisation. They found that north-facing and rougher samples became greener than south-facing and smoother samples. Whilst the majority of the studies discussed made an attempt to simulate architectural features by angling

samples in the field, in reality historic building facades comprise of a complex combination of angled, vertical and horizontal surfaces.

## 1.8 Summary and gaps in knowledge

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- There is a need for accessible and non- specialist methods to assess the bioreceptivity of vulnerable building sandstones.
- There is a need for more detailed studies of the external, inherent and architectural factors that affect sandstone bioreceptivity to green algal colonisation.
- There is a need for comparative studies using a range of sandstones and algal species, as most studies of eukaryotic biodeterioration have focused on a single stone type, type of microbial species or location (Cutler and Viles, 2010).
- Inoculation studies in the laboratory have not compared the relative bioreceptivity of a range of representative British building sandstones to green algal colonisation.
- Field exposure trials have primarily focused on single influential variables, such as aspect or stone type. There is a need for exposure trials that address the complex geometry of heritage buildings and how this affects their bioreceptivity.

These gaps in knowledge have informed the direction and structure of research in this thesis (see Section 1.9 below). This thesis aims to address the gaps in knowledge identified in Section 1.8 by investigating the factors that encourage colonisation of sandstone by green algae, through an integrated programme of laboratory and field- based experimentation. This involves developing improved laboratory methods to control and monitor algal growth, using accessible and non- specialist methods (Objective 1).

A study of the existing literature has identified *moisture* as the primary controlling factor behind patchy algal greening, however, little work has been conducted to compare the role of moisture in both laboratory and field environments using British building sandstones (Objective 2). There is a need for focused field and laboratory experiments encompassing the kind of reality captured in building surveys whilst addressing the influence of the key external, inherent and architectural factors controlling bioreceptivity (Objectives 3, 4 and 5). In particular, little work has hitherto been done to assess the influence of architectural factors such as aspect and geometry (the angle of stone exposure) representative of actual heritage buildings on sandstone greening in the UK (Objective 5).

## 1.9 Thesis aims and objectives

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This section presents the overall thesis aim and objectives informed by field visits to Sheffield, Belfast and Edinburgh as well as an extensive survey of the existing literature on algal greening of building stone. The objectives outlined here are explored in greater detail in subsequent experimental chapters. Each chapter addresses these objectives in greater detail through a set of research questions (see section 3.2 for example).

### *Overall thesis aim*

**To investigate the factors which encourage colonisation of sandstone by green algae, through an integrated programme of laboratory and field- based experimentation**

### *Objectives*

- 1. To develop improved laboratory experimental methods to control and monitor algal growth (chapters 2, 3 and 4)**
- 2. To investigate the fundamental role of moisture in the development of algal greening (all chapters)**
- 3. To investigate the role of *external factors* in the development of algal greening (chapters 5 and 7)**
- 4. To investigate the role of *inherent factors* in the development of algal greening (chapter 6).**
- 5. To investigate the role of *architectural factors* in the development of algal greening (chapters 4.II and 7)**

The following chapter presents and discusses the range of methods used in both laboratory and field experiments to address the objectives above.

# Chapter 2: Methods

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## Chapter Summary

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In order to achieve the objectives of this study a range of methodologies has been used. To reflect the need for a multi disciplinary approach to the study of microbial colonisation on sandstone heritage, the scope of these methods spans across the fields of microbiology, ecology, geomorphology and meteorology.

### 2.1 Introduction to the methods used

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The general approach of this study has been one of experimentation, building on the previous research reviewed in Chapter 1. Therefore, the methods used reflect the nature of experiments conducted in either the laboratory or in the field.

The laboratory experiments in this thesis have focused on ‘simulating’ epilithic green algal biofilms. That is, recreating the kind of stone-dwelling biofilms seen on buildings in the United Kingdom, but under controlled laboratory conditions. The function of these laboratory experiments has been to better understand the conditions required to grow green algae on different sandstone substrates as well as to characterise the nature of algal biofilms, including their morphology and how they change over time.

Field experiments in this thesis have focused on monitoring green algal growth on sandstones under natural conditions. In particular, studies have aimed to replicate more

fully, the complex atmosphere- lithosphere relationship. That is, the relationship stone buildings have with ambient conditions that surround them and how this in turn affects their bioreceptivity. The field experiments have focused on the role of aspect and the interaction of complex architectural features with ambient conditions, whilst monitoring the influence these have on the nature of algal biofilms produced.

In the case of both laboratory and field experiments, this DPhil study has focused on looking at the bioreceptivity of British building sandstones, which have exhibited a particular vulnerability to green algal colonisation in the north-west of the UK, over the past 40 years (as reviewed in Chapter 1).

## 2.2 Selecting stone types

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The nature of the stone substrate is important in influencing greening due to the close relationship biological organisms have with their host substrate. Substrate factors such as texture, porosity, pH and mineralogy determine the kinds of biological growths which will colonise the surface (Tiano et al., 1995).

Sandstones from the Millstone Grit series have a well-established history of use in the north-western parts of the UK and form the main focus for study. Stones from this series are commonly used for building around England and Scotland and are regularly exported to Northern Ireland. They are primarily favoured for their durability (Warke and Smith, 2007). Consequently, they are ideal stone types to study due to their extensive history of use in many towns and cities in the UK. For example, outcrops of Stanton Moor sandstone extend across Derbyshire making it the local building stone for Sheffield. In particular, it

has been used in various stages of building at Sheffield Cathedral, in effect, allowing study of biodeterioration over time. Weathered examples also include the Supreme Courts in Edinburgh and Chethams School of Music, Manchester (Magner, 2006).





Two primary sandstones from the Millstone Grit series have been used for experimentation in this DPhil thesis; Peak Moor and Dungannon, which are both buff in colour. In addition a third (unidentified), buff-coloured and weathered stone from this series has also been used in this thesis. This weathered stone was reclaimed as balusters from a building in Stirling in 2011 and will herein be referred to as 'baluster stone'. In addition, St Bees stone from the New Red Group of sandstones has been used for comparison with the buff- coloured stones discussed. New Red Sandstones also have a long history of use as building stone in northern cities such as Glasgow and Edinburgh and have shown a susceptibility to green algal colonisation (Hyslop et al., 2006). The stones we have selected were also chosen due to their use in other aspects (laboratory testing at Queens University Belfast and exposure trials around Northern Ireland) of the overall EPSRC 'sandstone greening' project, of which this Dphil project is a part.

The focus of the study of these four stone types has been to better understand their intrinsic bioreceptivity; the inherent properties of a stone which control its ability to be colonised by different microbial communities (Gaylarde et al., 2003). The four stone types used are described in more detail in Table 2. They are all quartz- rich and favoured for use in construction due to their durability. The general properties of the stones used in experimentation are detailed in Table 3.

Table 2: Summary of stone types used in laboratory and field experiments

Experiment name	Type and location	Stone type/s used	Fresh or weathered
<b>Chapter 3:</b> Characterising green algal colonisation under ideal laboratory conditions	Laboratory experiment, Oxford	Peak Moor	Fresh
<b>Chapter 4, Part I:</b> Testing the use of spectrophotometric methods to monitor algal biofilms in the laboratory, under controlled conditions	Laboratory experiment, Oxford	Dungannon	Fresh
<b>Chapter 4, Part II:</b> Using spectrophotometry to monitor algal biofilm development in the field environment	Field Experiment, Derrygonnelly, Northern Ireland	Dungannon	Fresh
<b>Chapter 5:</b> The influence of marine salts on green algal biofilm development	Laboratory experiment, Oxford	Dungannon and St Bees	Fresh
<b>Chapter 6:</b> Comparing the inherent bioreceptivity of different sandstones to single and mixed algal cultures	Laboratory experiment, Oxford	Peak Moor, Dungannon and St Bees	Fresh
<b>Chapter 7:</b> The influence of location, aspect and surface geometry on algal greening	Field experiment, Worcester College, Central Oxford	'Baluster stone' Un-named stone from the Mill Stone Grit Series	Weathered- exposed in Stirling for circa 100 years.

Table 3: General stone properties of sandstones used in experimentation

Name	Origin and Age	Petrography	Examples of usage	Susceptibility	Image
<b>Dungannon</b>	Carboniferous Northern Irish (County Tyrone) sandstone from the Millstone Grit series.	<ul style="list-style-type: none"> <li>• Cream, white, grey or yellow in colour,</li> <li>• Fine- medium grained,</li> <li>• Granular texture, bedding, laminations</li> </ul>	Headline building, Belfast	<ul style="list-style-type: none"> <li>• Relatively durable</li> <li>• Vulnerable to surface delamination in polluted environments</li> <li>• Can undergo rapid soiling</li> </ul>	
<b>St Bees</b>	Triassic English (Cumbria) sandstone from the New Red Group of Sandstones. Still actively quarried.	<ul style="list-style-type: none"> <li>• Red to brown in colour,</li> <li>• Uniform texture; granular and fine- grained.</li> <li>• Laminations and bedding.</li> </ul>	Restoration of Liverpool Cathedral	<ul style="list-style-type: none"> <li>• Relatively durable stone,</li> <li>• Susceptible to frost and salt weathering,</li> </ul>	
<b>Peak Moor</b>	Carboniferous English sandstone (Derbyshire) from the Millstone Grit Series. Still actively quarried.	<ul style="list-style-type: none"> <li>• Buff, pink or grey in colour,</li> <li>• Fine- medium grained,</li> </ul>	Used in EPSRC field exposure trials, Derrygonnelly	<ul style="list-style-type: none"> <li>• Durable- weathers gradually through granular disintegration.</li> <li>• 'Free stone'; durable whichever way its bedding is laid,</li> <li>• Little affected by acid rain or air pollution</li> </ul>	
<b>Baluster Stone</b>	Carboniferous English sandstone (Derbyshire) from the Millstone Grit Series	<ul style="list-style-type: none"> <li>• Buff/ grey in colour,</li> <li>• Fine- medium grained</li> </ul>	Unknown building, Stirling	NA	

From Curran et al. (2010), Ashurst and Dimes (1977); Clark (1995); BRE (2000b; 2000a); Blockstone (2008); Stancliffe Stone (2013)

### *Dungannon*

Dungannon stone has historically been quarried at Gortnauglush, Dungannon. The quarries are now inactive but the stone has been used for a large number of buildings in the Dungannon area (Fowler, 1961; Curran et al., 2010), see Figure 5a. Of the four sandstones studied, Dungannon has the highest open porosity and pore volume and the lowest density, making it the most porous of the group (see Table 4 and Figure 6). It has a relatively homogenous texture and is composed of fine, well-cemented grains (see Figure 7b). In appearance, it is the palest of the sandstones studied and has distinctive laminations (see Figure 8). Microscope observations indicate that these laminations are composed of very fine-grained material, more densely packed than surrounding material and are therefore likely to be clays/ silt in composition. After quartz, XRD analysis of sample chips identified kaolinite as the main constituent clay mineral (see Table 4).

### *St Bees*






St Bees is still actively quarried in Cumbria and has been used both as the primary construction material of buildings across the north-west of the UK, including in restoration work at Liverpool Cathedral (see Table 3) as well as for cladding and dressings, such as at St Polycarp's Church, Belfast (see Figure 5c). The sandstone has fine laminations and is primarily quartz based (66%) with a high percentage of clay content at 26% (Clark, 1995). This accounts for its distinctive red colour (see Figure 8). It is known to be a fairly durable building stone (Curran et al., 2010) and of the group of sandstones studied, it scores moderately in the standard tests falling in-between Dungannon (most porous) and Peak Moor/ baluster stone (least porous). It is fine-medium grained and appears to have a rougher surface than the other stones tested (see Figure 7).

**Figure 5: Examples of in-situ use of the three primary test stones**



*In situ green algal colonisation of Dungannon, St Bees and Peak Moor sandstones (scale= 1m):*  
**a.** Carved detailing (Dungannon stone), Allied Irish Bank, 15-17 High Street, Omagh (image source: Natural Stone Database (2008)). **b.** Peak Moor cladding, Scarva Street Presbyterian Church, County Down, Northern Ireland (image source: Cunningham Stone (2015)). **c.** Window dressings (St Bees stone), St Polycarp's Church, 104 Upper Lisburn Road, Finaghy, Belfast (image source St Polycarp's Church (2014)).

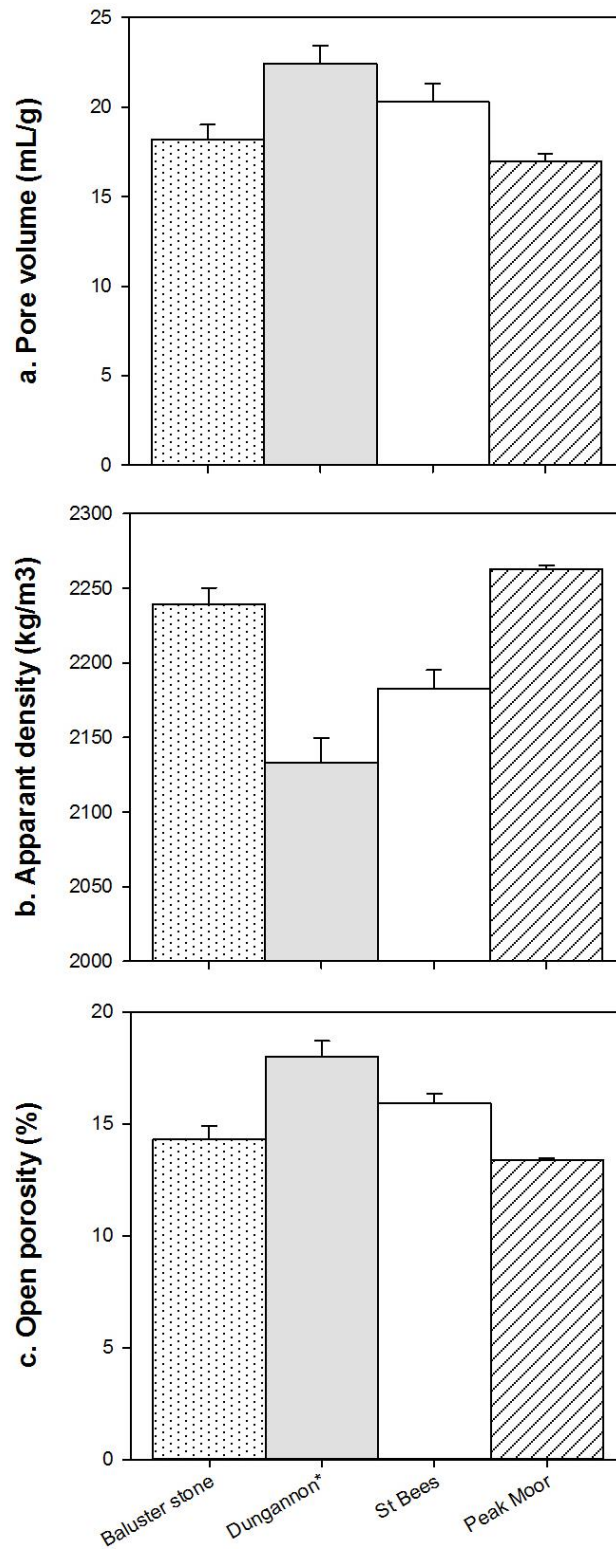
Table 4: Detailed comparison of sandstone properties

Stone Type	General Description	Source	SCE Colour (L*, a*, b*, C*, h)	Computed pseudo colour	Pore volume (ml)	Apparent density (kg/m <sup>3</sup> )	Open porosity (%)	Predominant mineral components (XRD results)	Notes
Dungannon	Buff grey. Fine- medium grained, carboniferous sandstone from the Millstone Grit Series	C. Fermanagh, Northern Ireland	L*= 73.27 a*= 2.72 b*= 14.00 C*= 14.27 h= 78.99		22.4*	2133.2*	18.0*	Quartz Kaolinite Muscovite Anorthite Albite Calcite	Two tone grey-yellow colour. Distinct clay laminations
St Bees	Dull red. Fine- medium grained, permio Triassic sandstone from Whitehaven, Cumbria	Whitehaven, Cumbria	L*= 48.17 a*= 11.77 b*= 14.05 C*= 18.33 h= 50.06		20.3	2182.8	15.9	Quartz Muscovite Hematite Albite Clinochlore (Chlorite)	Close bedded, fine grained. Fine clay laminations
Peak Moor	Buff golden. Fine- medium grained, carboniferous sandstone from the Millstone Grit Series	Matlock, Derbyshire	L*= 67.97 a*= 3.52 b*= 17.21 C*= 17.56 h= 78.45		17.0	2262.8	13.4	Quartz Kaolinite Clinochlore (Chlorite) Albite Anorthite Muscovite	Ranges from light pink- to cream in colour, fairly homogenous. Fine clay laminations
'Baluster stone'	Unweathered	Presumed Matlock, Derbyshire	L*= 66.19 a*= 5.71 b*= 18.68 C*= 19.53 h= 73.01		18.2	2239.2	14.3	Quartz Kaolinite Muscovite Calcite	Inhomogeneous appearance due to weathering and existing soiling/ crust formation
	Weathered		Presumed carboniferous, Millstone Grit	L*= 56.79 a*= 5.07 b*= 18.86 C*= 19.53 h= 73.01		NA	NA		

Tests to determine pore volume, apparent density and open porosity were conducted to BSi standard test guidelines (British Standards Institution, 2006) at the Oxford Rock Breakdown Laboratory, Oxford. A standard of six replicate samples were analysed and results averaged (mean) for each stone type. 'Unweathered' baluster stone refers to stone cut from the internal of a control baluster. 'Weathered' refers to stone cut from the exposed outer faces of this baluster.

\*Due to limited fresh stone supply, only three replicates were tested for Dungannon stone. Mineralogy was determined through XRD analysis of random sample chips.

**Figure 6: Standard test results for studied sandstones**



Tests to determine pore volume, apparent density and open porosity were conducted to BRE standard test guidelines (British Standards Institution, 2006) at the Oxford Rock Breakdown Laboratory, Oxford. A standard of six replicate samples were analysed and results averaged (mean) for each stone type. Only unweathered baluster stone (refers to stone cut from the internal area of a control baluster) is presented here. \*Due to limited fresh stone supply, only three replicates were tested for Dungannon stone. Error bars represent standard deviation from the mean

Figure 7: Thin sections of test sandstones

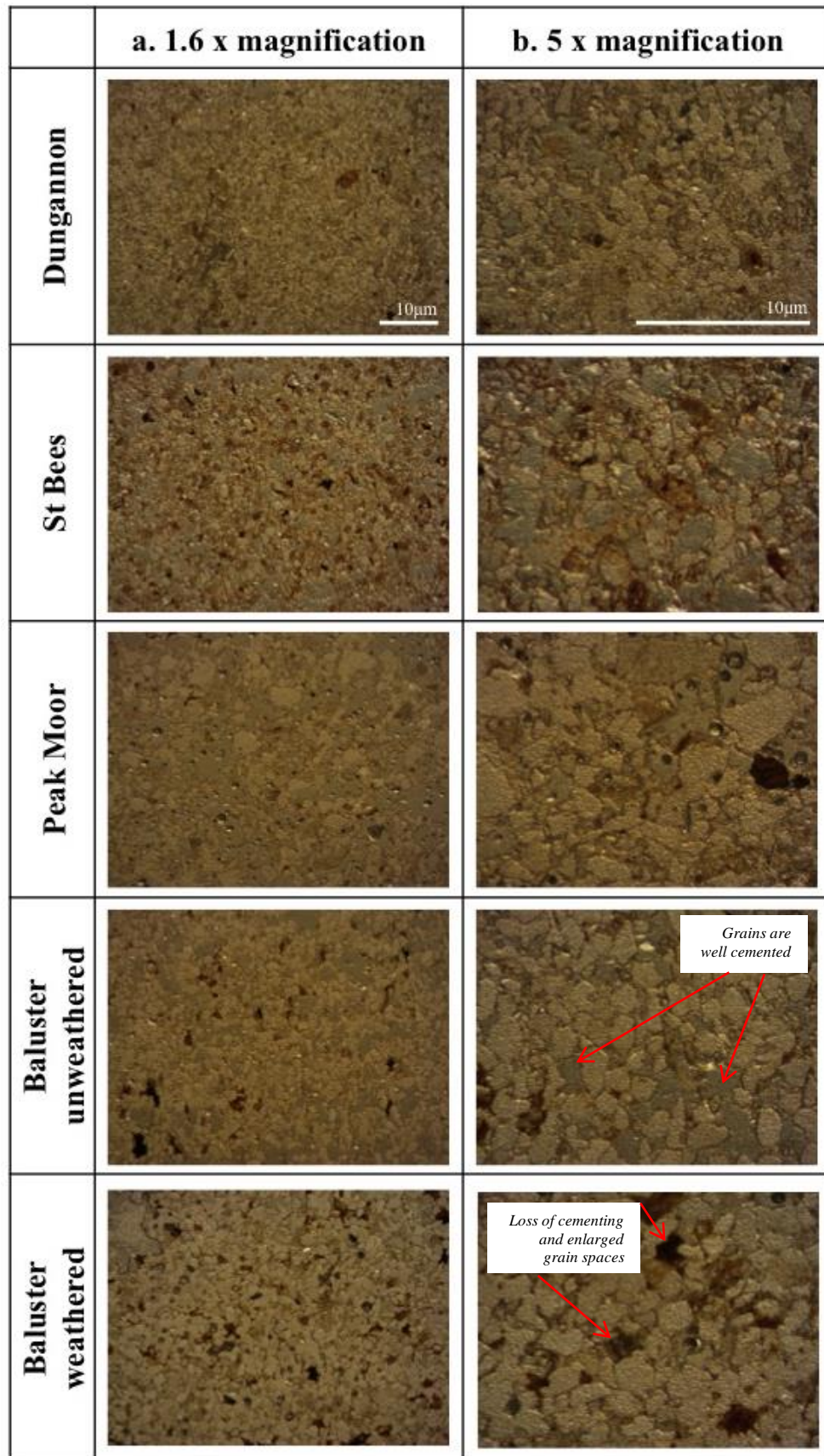
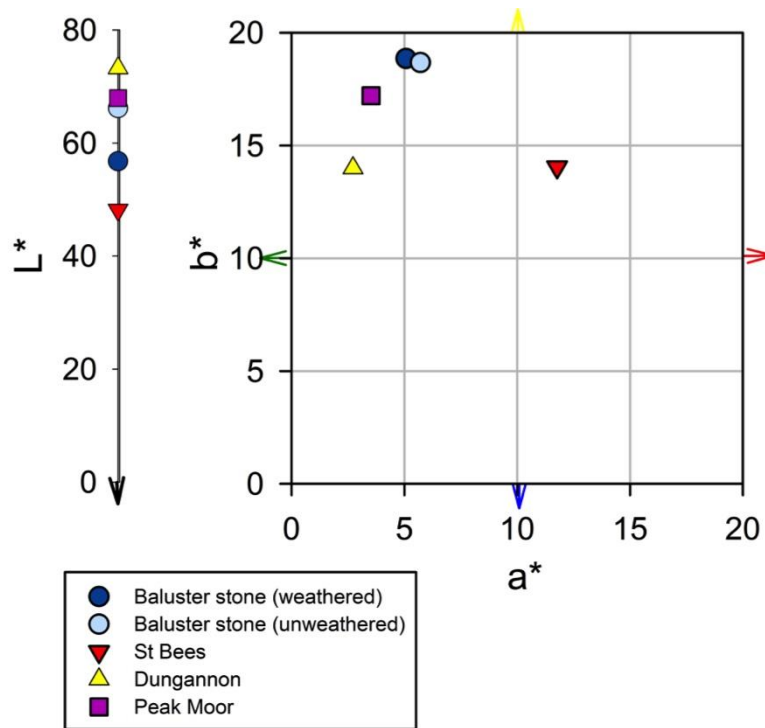


Figure 8: Comparison of sandstone base colours as plotted in CIE L\*a\*b colour space



Ten random point measurements were taken of a 50 x 50 x 50 mm block of each of the sandstones using a CM-700d Spectrophotometer (Konica Minolta, Japan) with 7 mm Medium Area View (MAV) aperture under standard illumination (D65: average daylight) conditions. Only 'Specular Component Excluded' (SCE) data are presented here

### Peak Moor

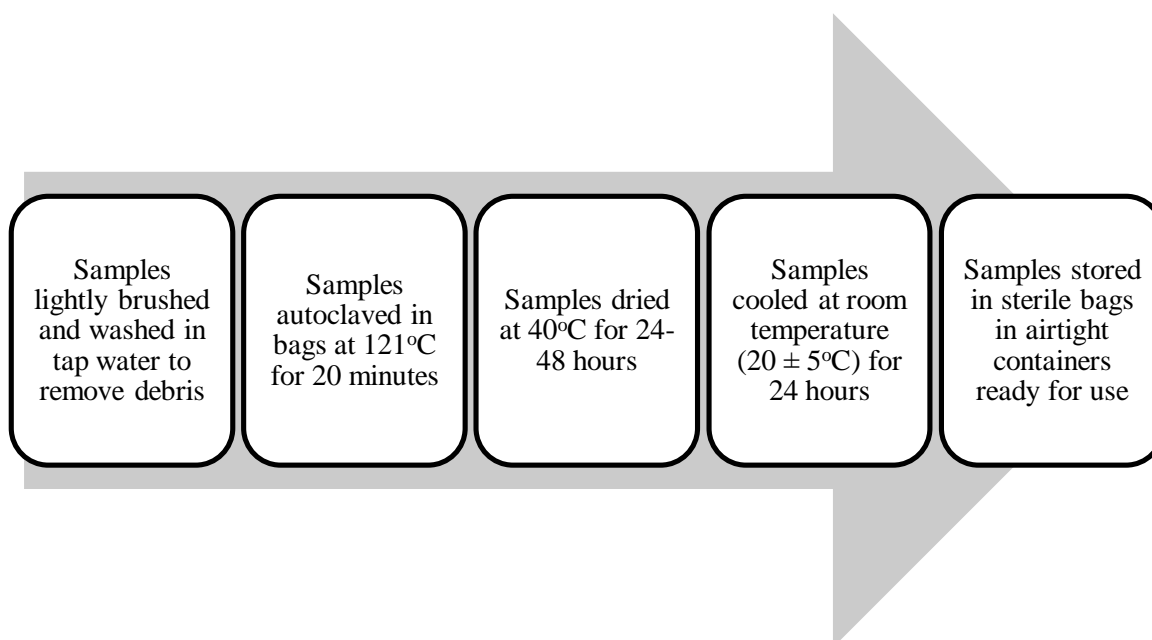
Peak Moor is still actively quarried and is a popular replacement stone. Not many historic examples of its use are known but it is commonly used as a replacement stone for weathered Stanton Moor (such as at Sheffield Cathedral) and has been used for recent construction projects including work to Scarva Street Presbyterian Church, County Down (see Figure 5c). Of the group of sandstones studied here, along with 'baluster stone' it has the lowest open porosity and pore volume and the highest apparent density (see Figure 6) making it one of the least porous of the sandstones. It is fine- medium grained and fairly homogenous in appearance (see Figure 7).

### *Baluster stone*

Six reclaimed sandstone balusters were sourced from a building in Stirling thought to date from the early 19<sup>th</sup> Century. One baluster was kept under controlled conditions in the laboratory whilst the other five were exposed in the field for a period of 12 months (see Chapter 7). After experimentation, the control baluster was cut up to enable standard tests to be conducted. Where possible, ‘unweathered’ (stone from the baluster interior) and ‘weathered’ (stone from the exposed faces of the baluster) were compared. Figure 7 highlights the effect of weathering on the texture of the stone, whereby cementing is reduced and grain spaces are enlarged. Colour measurements (see Figure 8) and microscopical observations of ‘unweathered’ baluster stone indicate that the texture and appearance are broadly similar to Peak Moor sandstone (see Figure 7). Standard tests also reveal a similarity between the two stones in terms of pore volume, apparent density and open porosity (see Table 4 and Figure 6). Of the four sandstones studied here, baluster stone is the coarsest with the largest grain sizes (see Figure 7).

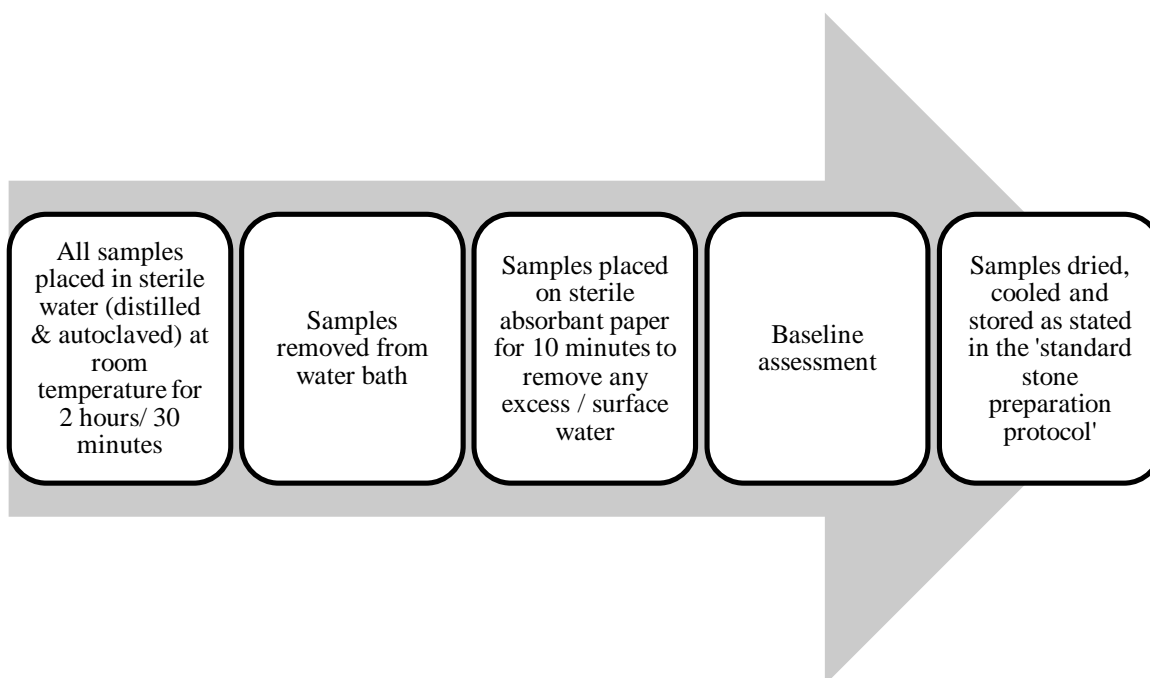
### *Stone preparation and storage*

For the majority of experimentation, uncolonised, freshly-cut stone has been used. The use of freshly-cut stone minimises the potential of interactive effects between pre-existing biological agents and those introduced to the stone as part of the experiment. To further ensure sterility, all fresh stone samples were sterilised according to the standard protocol outlined in Figure 9 and stored until required for use. Where weathered stones were used, every effort was made to obtain samples with a documented exposure history.

**Figure 9: Standard stone preparation protocol**

Prior to inoculation treatment or incubation in a growth cabinet, all stone samples (including controls) were subject to baseline assessment. This would act as the reference point for subsequent stone assessment, including weight, colour, moisture status and appearance. The standard protocol for baseline assessment is outlined in Figure 10. The length of time for which samples were soaked in sterile water was dependent upon the size of samples. All ‘block’ samples  $\geq 30 \text{ mm}^3$  in dimension were soaked for two hours, all samples smaller than this were soaked for 30 minutes. Pilot tests showed that these were the optimum amounts of time required for stone samples to become saturated.

**Figure 10: Standard baseline assessment protocol**



### 2.3 Selecting microbial cultures

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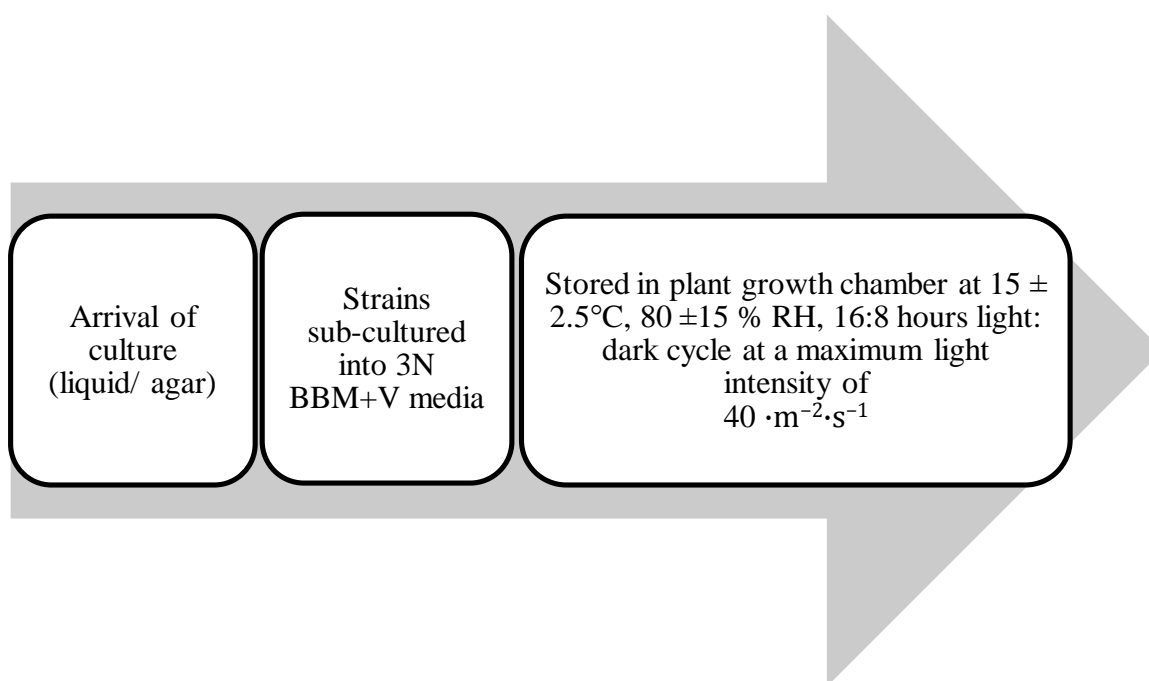
Field studies of naturally occurring lithobiontic biofilms have shown that they contain such great species diversity so as to be difficult to realistically replicate in the laboratory environment alone (Corenblit et al., 2011). Furthermore, studies of stone biofilms in Europe and elsewhere have shown the ubiquitous existence of a small number of dominating genera; in particular *Chlorella* and *Stichococcus* (Rindi et al., 2011). These genera are well known for their ubiquitous presence in both aquatic and terrestrial environments (George, 1957).

#### *Culture preparation and storage*

Cultures and medium were both obtained from maintained cultures at the Culture Collection of Algae and Protozoa (CCAP; Argyll, UK). All cultures were prepared

following the standard protocol outlined in Figure 11 and stored in a MLR-352H Plant Growth Cabinet (Panasonic, UK) until required for use.

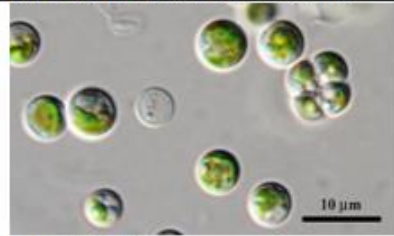

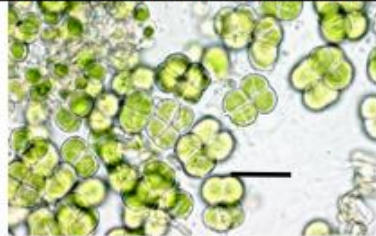
**Figure 11: Standard culture preparation protocol**



### *Comparison of microbial cultures*

*Chlorella vulgaris*, *Stichococcus bacillaris* and *Desmococcus olivaceus* algae were chosen to represent realistic lithobiontic colonisers due to their well-documented colonisation of stone structures in Europe and elsewhere (Tomaselli et al., 2000). Whilst these three species share ubiquity, they were chosen for comparison due to clear differences in the morphology of their cells (unicellular, uniseriate and sarcinoid respectively). The origin and basic characteristics of these algae are outlined in Table 5.

Table 5: Comparative characteristics of photobionts selected for experimentation

Organism	<i>Chlorella vulgaris</i>	<i>Stichococcus bacillaris</i>	<i>Desmococcus olivaceus</i>
Strain name	Beijerinck 1890	Nägeli 1849	Laundnon 1985
CCAP strain number	211/11B	379/1A	417/1
Division	Chlorophyta	Chlorophyta	Chlorophyta
Class	Trebouxiophyceae	Trebouxiophyceae	Trebouxiophyceae
General environment	Freshwater	Ubiquitous	Terrestrial
Cell Morphology	Unicellular	Uniseriate, unbranched filaments	Sarcinoid
Origin	Freshwater; Delft, Holland	Unknown	Rock; granitic rocks, Gerlache Inlet, Victoria Land, Antarctica
Case Studies <i>Type and location of stone structures with documented colonisation</i>	<ul style="list-style-type: none"> <li>• Wall paintings in Italy (Nugari et al., 2009)</li> <li>• Dolomitic outcrops, Piora Valley, Switzerland (Sigler et al., 2003)</li> <li>• Heritage monuments in Latin America (Jones et al., 1999).</li> <li>• Ecclesiastic monuments in Spain (Gómez-Alarcón et al., 1995)</li> </ul>	<ul style="list-style-type: none"> <li>• Wall paintings in Vallerano, Italy (Zucconi et al., 2012)</li> <li>• Tombstones in Bratislava, Slovakia (Bohuslav, 2008)</li> <li>• Stone fountain, Granada, Spain (Peraza Zurita et al., 2005)</li> </ul>	<ul style="list-style-type: none"> <li>• Tombstones in Bratislava, Slovakia (Bohuslav, 2008)</li> <li>• Canal lock in Bečej, Serbia (Grbić et al., 2009)</li> </ul>
General and metabolic effects on stone	Staining and dissolution leading to friability of colonised stone (Rifón-Lastra and Noguerol-Seoane, 2001)	Etching, change in pH (Welton et al., 2003)	Forms dense mucoid biofilms which may modify capillary water uptake (Ljaljević-Grbić Milica et al., 2010)
Image Scale: 10µm  Image source:	 <p>CCALA (2013a)</p>	 <p>CCALA (2013b)</p>	 <p>Guiry and Guiry (2013)</p>

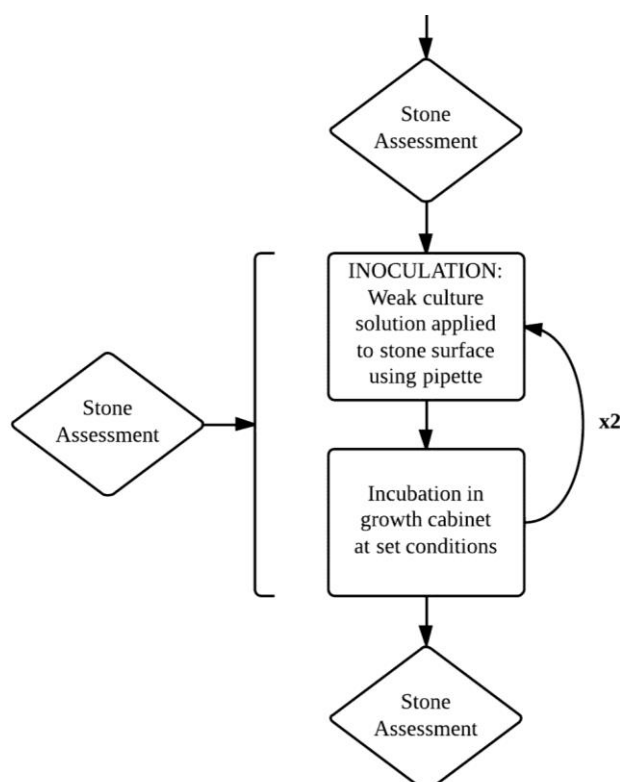
The metabolic effects of each species on stone are discussed in greater detail in subsequent chapters.

## 2.4 Laboratory experiments: characterising algal growth

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In order to better characterise stone-dwelling green algal biofilms, algal ‘simulations’ were carried out under laboratory conditions. ‘Simulations’ refers to an attempt to propagate growth of green algae on sandstone samples within a controlled laboratory environment. Within the context of this research, simulations involved inoculating small stone cubes or tablets with diluted single and mixed strain suspended solutions of lithobiontic green algae. The protocol for inoculation varied for each phase of experimentation and further details are given in the appropriate methodology sections. However, the basic protocol, is outlined in Figure 12.

**Figure 12: Basic protocol for stone inoculation**



It is widely understood that different microorganisms not only display very distinct growth patterns, but also have widely varying impacts on the underlying stone surface. Species grow very differently in terms of rate, metabolism and competition on the stone surface. This is due to the vast difference in environmental conditions between pure culture growth and growth at the often harsh, lithosphere-atmosphere boundary (Gorbushina and Broughton, 2009).

Within this body of research, the preference was to inoculate samples with single- strain cultures. The primary reason for choosing a single alga was to help ensure that interactive effects between two or more strains were eliminated. This decision was taken with the aim of more clearly being able to identify possible cause and effect relationships between biofilm development and biodeterioration/ bioprotection of the stone substrate. Where mixed cultures were used (as in Chapter 6), these were carefully measured and mixed and were composed of strains that had been tested individually as well.

As Figure 12 demonstrates, inoculation was carried out by pipetting between 1-5 ml (exact volume varied between experimental phases, this is discussed in more detail in following experimental chapters) of diluted suspended culture solution onto the stone surface. All cultures were diluted to the same concentration (see Table 6 below), mechanically stirred and mixed (at 80 rpm for 30 minutes) just prior to use. Control samples were treated with the same volume of autoclaved stock medium or autoclaved distilled water as required for each experiment.

**Table 6: Concentrations of algal solutions for inoculation**

Algal culture	Diluted concentration in ml (concentrated CCAP culture: 3N-BBM+V)
<i>C. vulgaris</i>	1:25
<i>S. bacillaris</i>	1:25
<i>D. olivaceus</i>	1:25

After initial treatment, samples were subject to an incubated propagation stage to allow development of a fledgling community. That is, samples were inoculated, incubated for two hours, inoculated again and then incubated for the remaining duration of the experiment. Two inoculation treatments were found to be sufficient to initiate and sustain a healthy colony for the experimental period.

Incubation of stone samples and suspended algal solutions was carried out in a MLR-352H Plant Growth Cabinet with humidity control (Panasonic, UK). The cabinet was calibrated to measure its general performance and fluctuations of humidity ( $\pm 5$  % error) and temperature ( $\pm 1$  °C error). An environmental regime to replicate temperate conditions and to encourage maximum algal growth was optimised in early laboratory experiments (see Chapter 3). This included: constant relative humidity of  $85 \pm 5$  %, temperature of  $20 \pm 1$  °C and cycling between 8 hours of dark and 16 hours of illumination. Lighting was set to a maximum light flux of 14 000 lux during ‘daytime’ hours.

For each experimental phase, stone assessment was carried out prior to, during and following the inoculation and propagation stages. These assessment techniques are discussed in greater detail in section 2.6.

## 2.5 Field experiments

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Field experiments were conducted in two locations. Chapter 4, part II includes results from 30-month exposure trials on a test structure in Derrygonnelly, Northern Ireland and Chapter 7 presents data from a 12 month exposure study of weathered balusters in a private garden in central Oxford. A survey of the literature demonstrates great variability in the ideal length proposed for field exposure trials (see Chapter 1, section 1.7). Therefore we chose to conduct two studies of varying lengths to better assess the time required to gauge information on algal greening in the natural environment.

### *Exposure trials: Derrygonnelly, Northern Ireland*

Extended exposure trials of 30 months were conducted at the Field Studies Centre, Derrygonnelly, Northern Ireland. These exposure trials are discussed in greater detail in Chapter 4, part II. This site is the location of purpose- built test-walls commissioned for a multi-disciplinary EPSRC project on green algal growths and climate change (Grant No. EP/G011338/1). The site is based around 2 hours away from central Belfast in a semi-rural location (see Figure 13).

**Figure 13: Location of project test structure, Derrygonnelly, Northern Ireland**



It has been identified as a ‘field laboratory’ (McAllister et al., 2012) for the study of algal colonisation due to its wet climate and moderated location, away from any direct pollution influence. The area is also home to an abundance of vulnerable sandstone heritage, making it a particularly appropriate study location. Temperature, humidity and rainfall data from the nearest maintained meteorological station (Lough Navar Field Station) was used to contextualise our findings.

#### *Worcester College Fellow’s garden, Central Oxford*

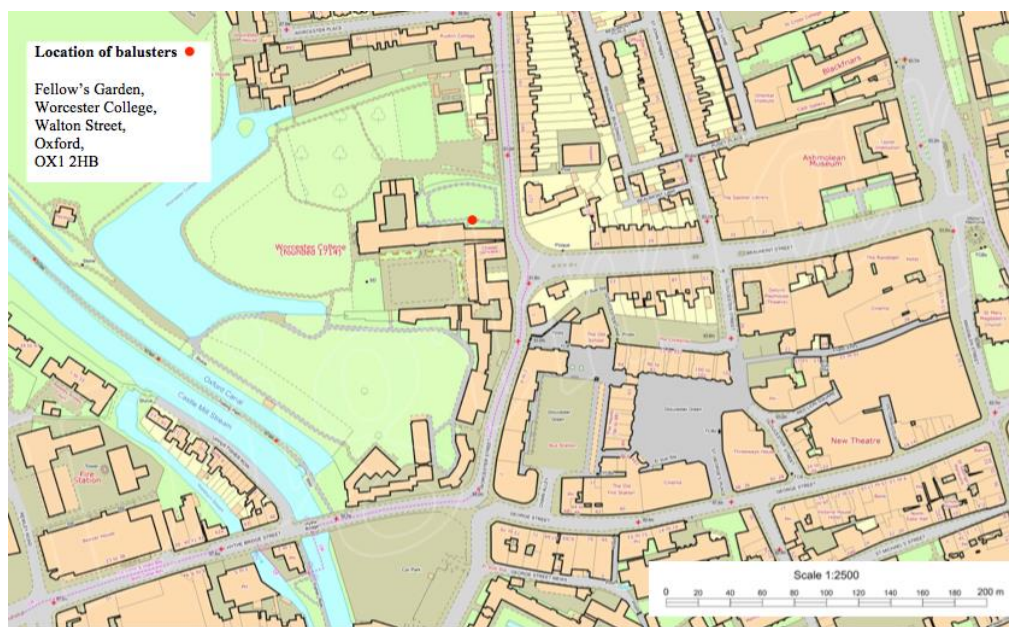
Architectural building features such as string courses, cornices, buttresses, carvings and columns have been observed to both encourage and inhibit colonisation by green algae. The aim of this experiment was to better understand the interactions between microclimate (temperature, relative humidity, rainfall and the influence these have on wetting/ drying patterns) and complex architectural features. Balusters were chosen to replicate the kind of architectural complexities observed on full-scale heritage buildings.

This experiment consisted of exposing weathered sandstone balusters in a private garden at Worcester College in central Oxford (see Figure 14). Within the garden balusters were positioned at one of two sites (exposed and shaded) for 12 months. Balusters were measured at bi-monthly and six-monthly intervals to monitor surface moisture movements, colour and general appearance.

Data from the Radcliffe Meteorological Station (RMS) was used to contextualise observations of the balusters. In addition to this, sensors were used to gather fine-resolution humidity and temperature measurements on two faces of each baluster. A Time Lapse Camera (TLC) was used intermittently to monitor rainfall status at the site and in

particular, to record wetting and drying patterns (these assessment techniques are discussed in greater detail in section 2.6 below).

**Figure 14: Worcester College Fellows Garden, central Oxford**



*Map source: Edina Digimap (2014)*

## 2.6 Assessment techniques

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The impact of microbial growth has been measured on stone at field sites as well as on stone blocks used in growth-chamber experimentation. In all instances, the goal has been to use relatively portable, non-destructive and non-specialist equipment. The reasoning behind this was to make methods reproducible and the findings accessible for a range of specialist and non-specialist stakeholders in the building conservation field. A number of parameters have been measured to monitor colonisation, moisture movements within the stone and to monitor microclimatic conditions at the field sites (see Table 7).

Table 7: General information about assessment techniques

	Parameter	Equipment and error values	Units of measure	Non/destructive	Non/ portable	Non/ specialist
Monitoring colonisation	General appearance	10 megapixel digital camera, visual observations	Photographs	Non- destructive	Portable	Non- specialist
	Biomass estimations	10 megapixel digital camera, Photoshop software	% Algal cover and density rating (1-5)	Non- destructive	Portable	Non- specialist
	Surface characteristics	Leica MZ10 F Stereomicroscope	Micrographs	Non- destructive	Non- portable	Non- specialist
	Colour	CM-700d Spectrophotometer and SpectraMagic software (Konica Minolta, Japan)	CIE L*a*b colour space units	Non- destructive	Portable	Basic training required
Moisture movements	Weight as a proxy for moisture absorption	Bench- top balance (error $\pm 0.001$ g).	Grams	Non- destructive	Portable	Non- specialist
	Water penetration	Vertical karsten tubes (TQC, Capelle aan den IJssel, The Netherlands)	ml/minute	Non- destructive	Portable	Non- specialist
	Surface moisture distribution	Protimeter Surveymaster moisture meter (GE; London)	Resistance (%WME)	Non- destructive	Portable	Non- specialist
	Near- surface moisture distribution	CEM DT-128 non-contact moisture meter (CEM, Shenzhen, China)	Capacitance (%)	Non- destructive	Portable	Non- specialist
Microclimate	Humidity and temperature	iButton® Hygrochron (Maxim; California, USA).	Temperature and relative humidity	Non- destructive	Portable	Non- specialist
	Wetting and drying	TLC 100 Time Lapse Camera (Brinno, Florida, USA)	Photographs/ videos	Non- destructive	Portable	Non- specialist

General information about these parameters is provided in Table 7, this includes device details and whether equipment was non-destructive, portable or required specialist training to use. The primary methods used are emboldened and the reasons for their choice as well as strengths and limitations are discussed in further detail below.

Due to limited incubation space in the growth cabinet and a need to maximise replicate numbers, the size of test blocks was often small (50 x 50 x 50 mm or 25 x 25 x 5 mm). Therefore, all methods used to monitor colonisation and measure moisture movements were selected to account for this (i.e. contact points had to be able to fit within the perimeters of individual stone blocks).

### *Monitoring colonisation*

A number of methods exist to quantify microbial biomass on stone surfaces. These are discussed and critiqued in greater detail in Chapter 4. However, limited methods exist to quantify and characterise the spatial variation in green algal colonisation over time.

We have used two primary methods to monitor surface colonisation, chosen for their accessibility and ease of use: surface colour change and biomass estimations. Surface colour measurements take advantage of the epilithic (surface-dwelling) nature of green algae and their distinct colour pigments ranging in hue from brown- green. Surface colour of samples was measured using a CM-700d Spectrophotometer and data analysed with SpectraMagic software (Konica Minolta, Japan). Spectrophotometry and methods used in the laboratory and field are discussed in greater depth in Chapter 4.

To our knowledge, no single low- tech method exists to measure both the spatial variation and density of green algal colonisation. Therefore, we have developed a simple semi-

quantitative method to map these parameters simultaneously. The method can be applied to a range of block sizes in the laboratory. The method maps colonisation based on photographs of the top inoculated surface of blocks. Photographs were taken under precise and controlled lighting conditions using a lightbox. The method involved overlaying a 10 square grid on photographs and calculating the percentage area covered by green growth. Each block was also assigned a weighting in terms of the relative density of the colonisation observed (see Figure 15).

**Figure 15: Colonisation density weightings**

- 1 Very light,
- 2 Light in some areas, some patchy heavier growth
- 3 Medium density
- 4 Fairly heavy growth- stone visible underneath
- 5 Heavy growth- no stone visible underneath

This method relies heavily on ensuring that all photographs are taken under the same consistent lighting conditions, this can be overcome by using a relatively inexpensive lightbox or tent and a fixed tripod set-up. As with all visual observation methods, the results have some degree of subjectivity depending on the observer. Therefore, it is advisable that where results are to be compared, the same observer conducts the analyses. Despite lighting constraints and observer subjectivity, the method is an inexpensive and low- tech way to chart the development of biofilms over time.

### *Moisture movements*

One of the objectives of this thesis (see section 1.9) has been to investigate the role of moisture in the development of algal greening. Therefore, the methods used here, relate to the moisture status of stone blocks in the laboratory and samples exposed in field experiments. In particular, we have used resistivity- and capacitance-based methods as proxy measures of surface (SM) and near-surface (NSM) moisture.

Surface moisture is the measure we have used most widely. For this we chose a portable, non- specialist, relatively inexpensive Protimeter Surveymaster moisture meter (GE; London). This device is designed for measuring the moisture content of timber, therefore when measuring other porous materials it measures moisture relative to known limits of wood or ‘Wood Moisture Equivalent- %WME’ (GE Sensing, 2007). In resistance mode, it has a measurement depth of approximately 12.7mm (Yardımcı and Sarp Tunçoku, 2008). The Protimeter has been used extensively in recent times to characterise moisture conditions in construction materials such as brick (Martinez- Garrido et al., 2014), limestone (Sass and Viles, 2010), sandstone (Mol and Viles, 2012) and mixed materials including plaster (Yardımcı and Sarp Tunçoku, 2008).

The main limitation of resistance- based measuring devices, such as the Protimeter is that readings can be influenced by material characteristics such as mineral content and soluble salts in stone (Akiner et al., 1992; Camuffo and Bertolin, 2012; Aly et al., 2015). However, we have found the Protimeter to provide a good *relative* indication of surface moisture content and its use is favoured due to its ease of operation and applicability in both the field and laboratory environments. Indeed, rigorous testing of the use of the Protimeter (and similar, hand held moisture meters) for estimating the relative moisture

content of stone substrates under controlled conditions has been conducted by the Oxford Rock Breakdown Laboratory (see Eklund et al., 2011).

We also used a CEM DT-128 non-contact moisture meter (CEM, Shenzhen, China) to measure near- surface moisture in samples. In contrast to the Protimeter, this device works on the capacitance principle to estimate moisture in porous materials at a depth of 20- 40 mm, tracing moisture on a range from 0 (dry) to 100 (wet) (CEM, 2015). Unlike the Protimeter, it is specifically designed for hardened construction materials and is gaining popularity in studies of moisture and weathering of building stone (Eklund et al., 2011; Hanssen and Viles, 2014). Whilst capacitance-based measurements are not as vulnerable to influence of salinity as resistance-based methods, they have been shown to be slightly affected (Eklund et al., 2011). The device is also vulnerable to interference from external sources of high frequency and human interaction; however these can be minimised by holding the device near the base (CEM, 2015).

Both devices are subject to influence by environmental conditions. Therefore, we ensured that they were zeroed and calibrated according to manufacturer guidelines prior to each use.

### *Microclimate*

As discussed previously, ambient conditions at both field sites were monitored using data from maintained meteorological stations. Due to the nature of the study, high-resolution microclimatic data (temperature and relative humidity) was also required for the field study at Worcester College, Oxford to measure inter-site variability. Therefore, iButton®

Hygrochron devices (Maxim; California, USA) were attached to the top front and back of each of the five balusters exposed in the garden and data logged hourly. These data loggers are easy to use and light-weight allowing them to be attached directly to the stone surface with non-toxic and removable adhesive tack.

I- buttons have been used widely to monitor microclimatic conditions in field and laboratory-based stone weathering studies (Sternberg et al., 2011; Briones, 2013; Gowell et al., 2015). They have particular potential for use in the study of heritage buildings due to the wide range of intra-site microclimates created around a single building due to its cardinal aspects and architectural features such as buttresses, porches, arches and pillars.

In our experience, the loggers are not able to tolerate consistently high relative humidities of above 80%, which can occur frequently in British mid-winter or during particularly wet or rainy periods throughout the year. In these instances, the humidity values drift over time due to saturation of the logger sensor. However, by applying a saturation drift compensation equation in line with manufacturer guidelines (Maxim Integrated Products Inc, 2015) drifted values can be corrected (see Appendix 4). Despite this, we have found iButton® Hygrochrons to be effective in monitoring microclimatic differences between sites.

In order to record wetting and drying patterns on exposed balusters, we set up a TLC 100 Time Lapse Camera (Brinno, Florida, USA) to log photographs every five minutes during forecasted rain events. These cameras are traditionally used for monitoring growth of crops or movement of small mammals in remote areas. Whilst time lapse photography has been used in more traditional geomorphological studies to monitor weathering rates (Eckerstorfer et al., 2013), its use to monitor run-off and drying patterns of geometrically-

complex building stone is still comparatively limited.

The following chapters (Chapters 3- 7) present the laboratory and field experiments conducted using the methods detailed in this chapter. In particular, Chapter 3 focuses on refining laboratory methods to reproduce algal colonisation under laboratory conditions whereas Chapter 4 explores spectrophotometry as a method to monitor green algal biofilm development in the laboratory and field settings. The subsequent chapters (Chapters 5, 6 and 7) build on the methods reviewed here and tested in Chapters 3 and 4.

# Chapter 3:

## Characterising green algal colonisation under ideal conditions in the laboratory

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### Chapter Summary

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Limited work has been done to simulate and characterise algal greening on British sandstones under laboratory conditions. This chapter focuses on developing and trialling a suitable method to inoculate, grow and monitor green algal biofilms on sandstone blocks in a growth cabinet. This chapter partially fulfils Objective 1 of the overall thesis (see section 1.9). 50 x 50 x 50 mm cubes of Peak Moor sandstone were inoculated with an axenic culture of *C. vulgaris* and incubated under optimum conditions. A large number (19) of replicates were used to evaluate inter-block variability. A range of analyses was conducted to monitor the morphological change of biofilms over time, as well as looking at the receptivity of Peak Moor to algal growth. The experiment demonstrated that even under optimal environmental growth conditions, inhomogeneous concentric patterns of colonisation were observed. These results go some way in explaining patterns of patchy colonisation observed on heritage monuments in the UK. Sandstone blocks colonised with green algae retained more moisture than uncolonised samples. In addition, interesting relationships were observed between surface moisture movements and the spatial distribution of algal biomass.

### 3.1 Introduction

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The growth of green algal biofilms on important components of our sandstone heritage has become a growing heritage conservation concern in recent times. An increasingly warm and wet climate is likely to produce favourable conditions for algal biofilm development (Smith et al., 2004). This is of particular concern in Northern Ireland where the rate and extent of greening has increased over the past 30 years (McCabe et al., 2011). Whilst these growths often have a profound aesthetic impact, their morphology, ecology and physical impact upon the underlying sandstone is still relatively uncertain (Cutler and Viles, 2010). There is a need for geomorphological studies to understand whether these growths are benign, or even bioprotective or conversely if they are detrimental and biodeteriorative.

Some studies have hypothesised that green algae have a direct biodeteriorative impact through penetration of algal filaments (Warke et al., 1999) expansion and contraction of cells due to wetting and drying cycles (Lopez et al., 1999), excretion of harmful organic acids (Bland and Rolls, 1998) and production of microbial metabolites (Urzi and Krumbein, 1992) which contribute to stone dissolution. Welton et al. (2003) for example, analysed the impact of a mixed culture of a number of photoautotrophs including *Stichococcus bacillaris* on pure mineral chips immersed in a liquid medium. They found evidence of mineral etching mediated by the biological agents present and an impact on the pH of the surrounding medium.

However, the majority of field studies of *epilithic* (surface-dwelling) green algal biofilms have shown little evidence of a direct relationship between growth and stone decay. A positive feedback loop is created whereby colonisation encourages entrapment of dust

particles (Warke et al., 1999) and retention of moisture, thereby catalysing further algal growth (McCabe et al., 2007). In so doing, green algal biofilms act as a precursor to subsequent ecological colonisers such as fungi, mosses and vascular plants; these organisms have been shown to have a significant role in stone decay (Gómez-Alarcón et al., 1995). Through this precursory role, green algal biofilms appear to have an indirect role in stone deterioration.

Carter and Viles (2005) summarise the study of the *bioprotective* role of a range of microbial organisms growing on natural stone outcrops as well as on built heritage. In particular, they highlight the relatively well-studied role of lichens. For example, Ariño et al. (1995) whilst noting the micro scale lichen-induced biodeterioration of sandstone flagstones in Spain, found the rate of this to be much slower than traditional processes of abiotic weathering. They propose that, in this case, lichens provide a preferential, protective cover. A more recent study by Cutler et al. (2013b) demonstrates that through regulation of moisture regimes, epilithic green algae may also provide a protective covering. Generally however, comparatively less work has been done to explore the bioprotective role of green algae than of lichens and higher organisms. Nevertheless, observations of the growth and behaviour of these organisms may give an indication of the bioprotective potential of green algae.

Miller et al. (2009) highlight the need for laboratory-based stone colonisation experiments to provide better data on the impacts of green algal colonisation on stone substrates. An integrated approach to the evaluation of the impact of a biofilm (whether neutral, positive or negative) is required as the processes involved are often complex and interrelated (Carter and Viles, 2005). Prior to this evaluation however, we must first understand the

fundamental *reasons* for biofilm development. The factors encouraging biofilm growth can be divided into *external* (e.g. environmental and climatic conditions) and *internal* (e.g. characteristics of the substrate/ materials on which the organisms are growing). The aim of this chapter was to contribute to developing improved laboratory experimental methods to control and monitor algal growth (see Objective 1, section 1.9) by studying the growth and impacts of a green alga under optimal environmental conditions.

An experiment was set up to reproduce a lithobiotic green algal biofilm under laboratory conditions. We aimed to develop a robust methodology to inoculate stone, propagate growth and to grow and sustain an epilithic green algal biofilm on sandstone blocks under laboratory conditions. This included trialling an environmental regime to stimulate growth as well as testing the methodological set-up (for instance, the appropriate number of replicate and control blocks to use). We then monitored morphological changes in the biofilm over time and evaluate any impacts on the host substrate. Establishing robust laboratory experimental methods for growing and monitoring green algal biofilms on sandstone under controlled environmental conditions is an essential precursor for the experiments reported in the latter chapters of this thesis.

A pared- down, controlled, experiment was set up to promote development of a fledgling algal community. This involved inoculation of sandstone blocks with a single-strain axenic culture of *Chlorella vulgaris*. Blocks were subject to incubation in a growth cabinet under optimum conditions with the objective of maximising algal yield. Optimising growth conditions allowed observation of changes in an ‘ideal’ or healthy biofilm over time. This also allowed for exploration of the impacts of growth on the host stone in a controlled situation where algal growth was allowed to propagate without

inhibition or human interference. Changes in surface appearance, moisture movements and weight were assessed prior to, during and following colonisation.

### 3.2 Research questions

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1. What kind of methodological set-up is required to produce a green algal biofilm under laboratory conditions?
  - i. How many replicates and controls are needed to gain a representative picture of algal growth on sandstone blocks?
  - ii. How should samples be inoculated with green algae?
  - iii. What environmental conditions are required to promote growth?
  - iv. How long should the experiment run for?
2. What are the spatial and temporal characteristics of algal colonisation on sandstone blocks under ideal conditions?
3. Under ideal environmental conditions, does algal colonisation affect moisture regimes within sandstone blocks?

### 3.3 Materials and methods

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#### *Sample preparation*

From a survey of the literature it appears that a range of sample shapes and sizes have been used in laboratory experiments to simulate biofilm growth. These include cubes (Guillitte and Dreesen, 1995), square tablets (Tiano et al., 1995; Rogerio-Candelera et al.,

2011; Manso et al., 2014) and cylindrical probes or discs (Papida et al., 2000; Miller et al., 2006). Similarly to Guillitte and Dreesen (1995), we have chosen to use small cubes of 50 x 50 x 50 mm. These better represent the depth of load-bearing masonry than shallow tablets or discs. In addition, samples of greater depth will retain more moisture, which is known to be conducive to biological colonisation (Gorbushina, 2007).

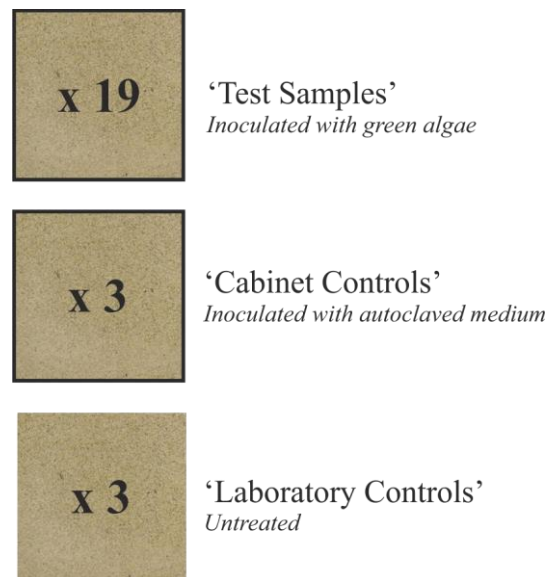
Twenty five 50 mm x 50 mm x 50 mm cubes were cut from a single piece of fresh buff-coloured Peak Moor sandstone, quarried at Stanton Moor near Matlock in Derbyshire (Blockstone, 2008). This was the maximum number of cubes that could be cut from the single block available. A detailed description of the stone type is provided in Chapter 2, section 2.2.

Of the 25 cubes cut, six blocks were kept as controls (see Figure 16). Of the controls, three were treated with autoclaved medium only (herein referred to as cabinet controls). The three remaining blocks were not treated at all and were kept uncovered in the laboratory (laboratory controls). As we wanted to maximise the number of replicates to test the significance of any findings, three controls of each type were thought sufficient as Peak Moor is a homogenous and relatively- well documented stone type. All nineteen remaining cubes (see Figure 16) were taken as replicates. These were inoculated with green algae (herein referred to as test samples).

After cutting and washing with de-ionised water, cubes were placed into autoclave bags and autoclaved, dried and cooled as described in the Methodology section (see Figure 9: Standard stone preparation protocol). All blocks, except for the three laboratory controls, were wrapped in two layers of impermeable black duct tape on four side faces leaving the

top and bottom face of each block exposed. Blocks were wrapped in this way to isolate the top surface for algal inoculation and to prevent evaporation from side faces whilst allowing moisture uptake through the bottom face via capillary rise.

**Figure 16: Sample coding**



*Emboldened samples ('test samples' and 'cabinet controls') were all wrapped with impermeable tape and incubated in a growth cabinet. 'Laboratory controls' were kept uncovered in the laboratory*

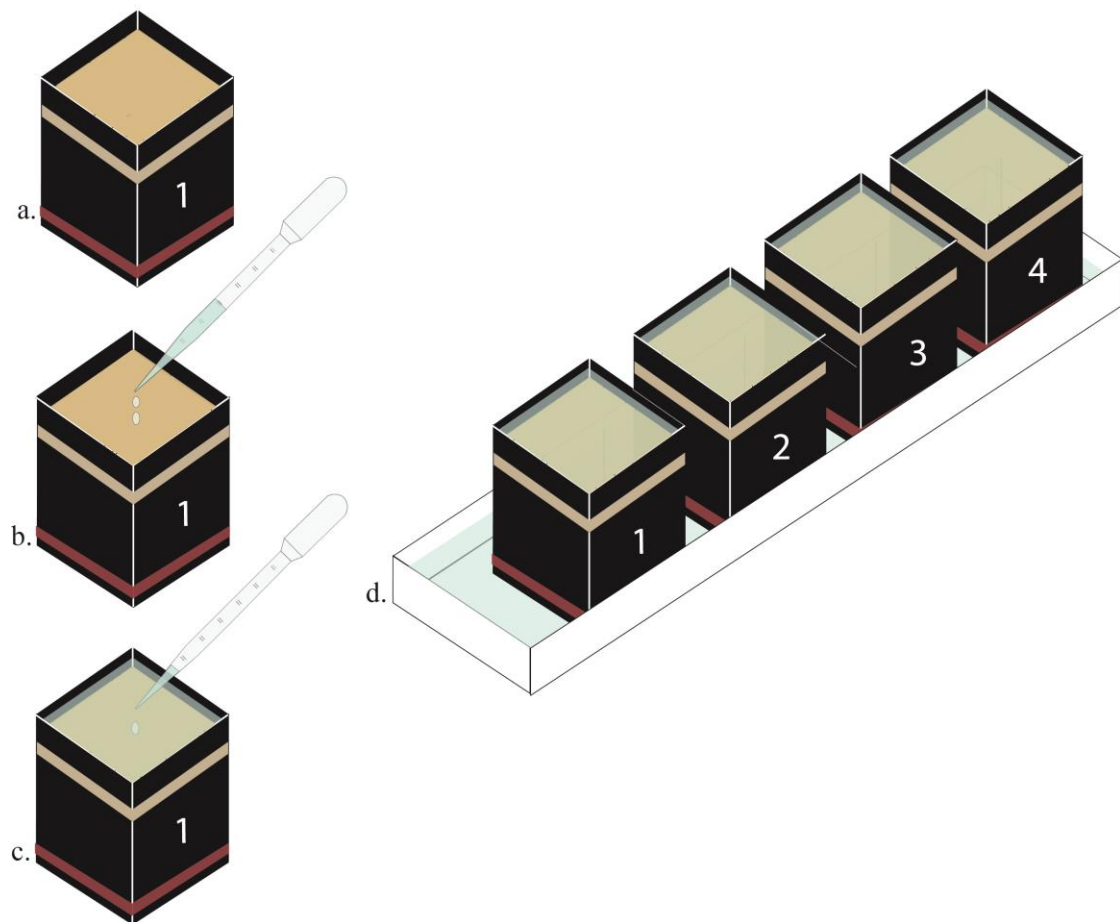
A 3 mm protruding 'lip' of duct tape was left around the uppermost exposed surface. The purpose of this 'lip' was to prevent the pipetted algal solutions from running off the treatment surface too quickly (see Figure 17c). Rubber bands were tightly wrapped around the top and bottom of each stone block to facilitate tape adhesion and to prevent moisture loss during the experiment.

### *Optimising growth conditions*

Testing and defining the optimum environmental conditions required to promote green algal growth is of key relevance in understanding how future climate change might affect

sandstone greening. For instance, projections for the north west of the UK over the next 100 years includes an expectation of increasingly dry summers and moderately wetter winters (McAuley, 2004; Arkell et al., 2007).

**Figure 17: Inoculation procedure**



*All test samples and cabinet controls were a. wrapped with duct tape and sealed with rubber bands and b. treated with either a weak algal solution or autoclaved stock medium. c. 2.5ml liquid was enough to cover the top, exposed surface evenly. The tape 'lip' prevented surface run-off. d. Samples were placed in sterile trays filled with distilled water.*

Green algae are hypertolerant organisms and are capable of growing in a diversity of environmental conditions and locations, requiring only a few basic elements to propagate growth; moisture, light and trace minerals (Warscheid and Braams, 2000). However, it is

likely that certain conditions will favour algal greening; it is these conditions which this study hopes to explore.

The purpose of this study was to develop methods to grow, record and observe the behaviour of a healthy biofilm. The aim was to monitor algal growth without the influence of environmental stressors, which could otherwise stunt cell development and biofilm morphology. Therefore, the environmental regime was optimised to encourage maximum algal yield.

It has been argued by many that the most influential factor controlling the ecology of lithobiontic algae and the degree of biological soiling is *moisture availability* (Hoffmann, 1989; Tomaselli et al., 1999; Crispim et al., 2003; Gorbushina, 2007). Water may accumulate on the substrate directly from precipitation or indirectly through condensation and rising damp (Warscheid, 2005). According to the literature, there appears to be a close relationship between moisture availability and microbial growth rates. Where there is greater moisture accumulation therefore, one would expect a greater degree of microbial growth. In particular, it is the increased time-of-wetness of stone surfaces predicted under future climatic conditions which are likely to be conducive to biological colonisation (Smith et al., 2004). In the natural environment, a simple combination of mild and moist or temperate conditions are known to encourage biofilm development (Schlichting, 1975). Indeed, relative humidities of around 90% are required for prolific growth of green algae (Jones et al., 1999).

A number of studies have explored the optimum temperature conditions for growth of green algae on stone surfaces. The temperature range for cell survival is very large, between 0 to 110°C for some species (Urzi and Krumbein, 1992). In most instances,

strains of chlorophyta require a range of 16-35°C (Warscheid, 2005) for active growth with optimum growth rates occurring between 20-23°C (Guillitte and Dreesen, 1995; Häubner et al., 2006). The ability to photosynthesise is a fundamental requirement for a healthy colony. Whilst phototrophic species have been shown to colonise all aspects on buildings, including often dark and damp areas (Nugari et al., 2009), some light is fundamental for healthy cell growth and division. Andersen (2005) recommends intensities of 10-100  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for healthy green algal biofilm development. The photoperiod is also of key importance, with long day conditions of 16 hours light to eight hours of dark providing a suitable balance (Guillitte and Dreesen, 1995; Rindi and Guiry, 2002).

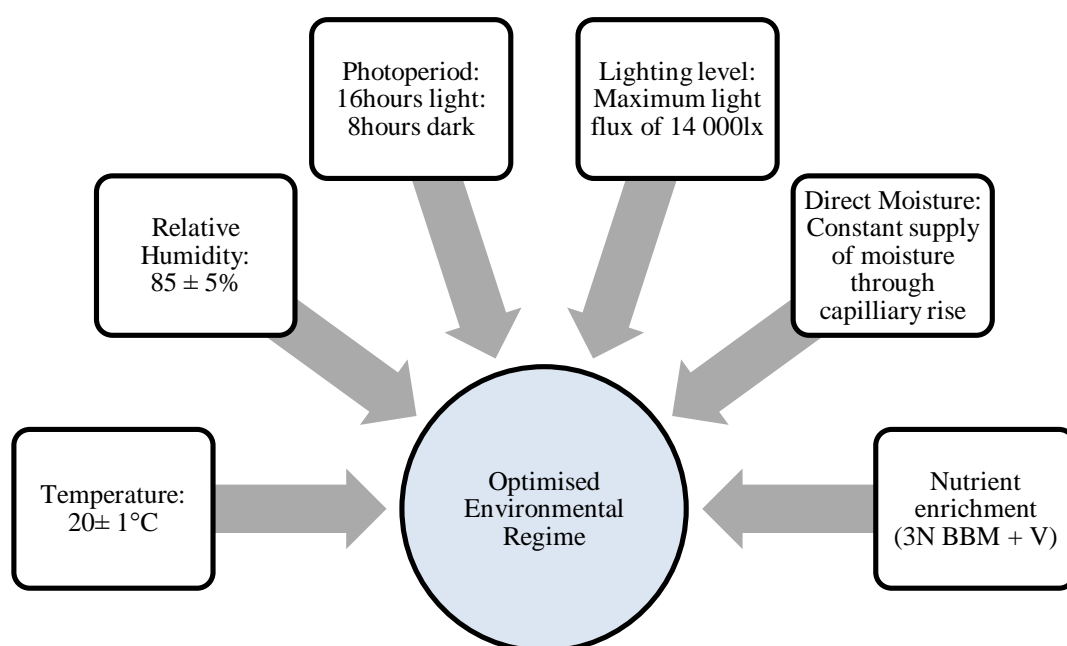
Typically, building surfaces are oligotrophic and in particular, lack available nitrogen (Cutler and Viles, 2010). Being hypotolerant, green algae are able to survive under these harsh conditions, but often need some form of nutrient enrichment to proliferate. For instance, it has been hypothesised that the increase in sandstone greening observed over the past 30 years has been aided by declining levels of atmospheric sulphur dioxide and an increase in local levels of NO<sub>x</sub> (McCabe et al., 2011). Anthropogenic pollutants such as NO<sub>x</sub> (industrial and agricultural sources) have been argued to be solely sufficient for serving the nutrient-demand of lithobiontic organisms (Warscheid and Braams, 2000).

In reality, it is a combination of environmental conditions that work together to encourage biofilm growth. Many parameters are interdependent and cannot be seen in isolation; therefore, an optimised *environmental regime* needs to be established to facilitate growth. Given this requirement and the existing literature on environmental controls on green algal growth, an optimised regime for incubation was devised and is outlined in Figure

18. All test samples and cabinet controls were incubated under the conditions described, in a MLR-352H Plant Growth Cabinet (Panasonic, UK) as depicted in Figure 19b. Laboratory control samples were kept uncovered in a drying cabinet set at 15°C for the duration of the experiment.

Green algal cultures were grown in nutrient-rich growth medium containing a combination of trace metals and vitamins required to promote growth (see Appendix 1: Recipe for 3N-BBM+V-growth medium).

**Figure 18: Establishing an optimised regime for growth of *C. vulgaris***



**Figure 19: Experimental set-up**



- a. Samples were placed in sterile plastic trays and treated under controlled laboratory conditions.*  
*b. Trays were placed on separate shelves in a growth cabinet under 'optimum' environmental conditions. Shelves and trays were all rotated randomly every 3 days.*

As test samples were inoculated with this solution, dissolved nutrients were directly introduced onto the stone surface. Cabinet controls were also treated with autoclaved growth medium to enable direct comparison.

### *Culture selection and preparation*

*Chlorella vulgaris* Beijerinck (CCAP 211/11b, herein referred to as *C. vulgaris*) was chosen to represent the kind of green algae known to colonise sandstone buildings. *C. vulgaris* is a stone-dwelling green alga commonly found on stone surfaces in Europe (Tomaselli et al., 2000; Macedo et al., 2009) and in particular, moist areas of walls and monuments (Nugari et al., 2009). It is, in general, one of the most well-documented and ubiquitous lithobionts (George, 1957).

*Chlorella* cells are composed of a single large chloroplast (Wakasugi et al., 1997) surrounded by a thick cell wall (Kasai and Hatakeyama, 1993) containing sporopollenin; an extremely tough organic component common in pollen of higher plants. Studies have shown that the presence of sporopollenin within *Chlorella* cells helps them to tolerate extreme environmental change (Atkinson et al., 1972; Kasai and Hatakeyama, 1993). These extremes may include desiccation, and particularly low or high temperatures (Gaylarde and Gaylarde, 2005). Indeed, in unpublished tests carried out by the author, standard DNA extraction protocols involving the vigorous lysis techniques of bead beating (Müller et al., 2005), freeze thaw (Palla et al., 2002) and use of lysing enzymes (Sivan and Chet, 1989) were largely unsuccessful in breaking open the cell walls of *C. vulgaris* in pure culture. The resilience afforded by the morphology of *C. vulgaris*' cell structure, may therefore explain the alga's ability to colonise a huge diversity of substrates in the natural environment.

A weak (4ml concentrated CCAP culture subcultured in 100ml stock medium), axenic sub culture of *C. vulgaris* suspended in 3N-BBM+V nutrient- enriched liquid medium (see Appendix 1) was used to treat test samples. Autoclaved stock medium was used to treat control samples (see Methodology section for details). Cultures and medium were both obtained from the Culture Collection of Algae and Protozoa (CCAP, Argyll).

### *Stone inoculation*

Inoculation was carried out by pipetting 2.5 ml of weak suspended culture solution onto the stone surface of test samples. This was enough liquid to completely cover the exposed top surface of each sample block (see Figure 17c). Cabinet control samples were treated

with the same volume of autoclaved stock medium. All blocks (apart from three laboratory controls) were then placed in sterilised white plastic trays. Trays were filled with enough distilled water to submerge the bottom 3 mm of each block (see Figure 17d). The level of water was checked and maintained daily for the duration of the experiment. The idea was to allow the blocks a moderated, but constant supply of direct moisture via capillary rise. Test and cabinet control samples were kept in separate trays and on separate shelves in the growth cabinet to minimise cross-contamination (see Figure 19).

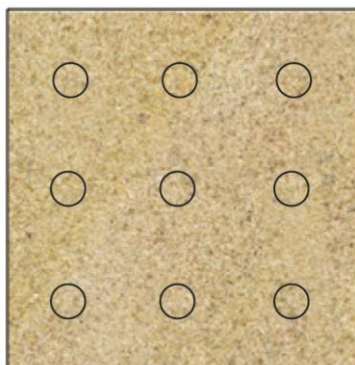
After the first treatment, samples were incubated for two hours to allow full absorption of the inoculant. Following this, samples were re-treated with a further 2.5 ml of either liquid culture or autoclaved medium. Two inoculation treatments were found to be sufficient to initiate and sustain a healthy colony for the experimental period. Samples were incubated for a total duration of 30 days.

#### *Analysis of stone samples – monitoring biofilm morphology and surface moisture*

Visual appearance of the blocks was documented every seven days through digital photography, under controlled lighting conditions. In combination with observational notes, photographs were used to record change in biofilm morphology. The spatial area colonised by *C. vulgaris* (herein referred to as ‘percentage cover’) was mapped by overlaying a 5 mm grid over magnified, scaled photographs. In addition, each sample surface was assigned a semi-quantitative rank of the relative ‘density’ of colonisation (judged by the visual saturation or depth of green colour), as specified in Figure 15. Percentage cover and density were used as proxy measures for surface biomass, further details on calculation can be found in the Methodology chapter.

Moisture movements were measured indirectly using two methods, weight change and a hand-held moisture meter. Weight change was used as a proxy measure of moisture ingress and egress from samples. Weight was measured prior to initial treatment and subsequently every 7 days at the same time and under the same environmental conditions. Moisture content at the stone surface was measured every 7 days using a Protimeter Surveymaster moisture meter (GE; London). The Protimeter device displays results in the units of Wood Moisture Equivalent (WME%); please see Chapter 2, section 2.6 for further details. Nine measurement points were assigned across the top exposed surface to ensure fine data resolution (see Figure 20).

**Figure 20: Surface moisture measurement point schematic**



### 3.4 Experimental results

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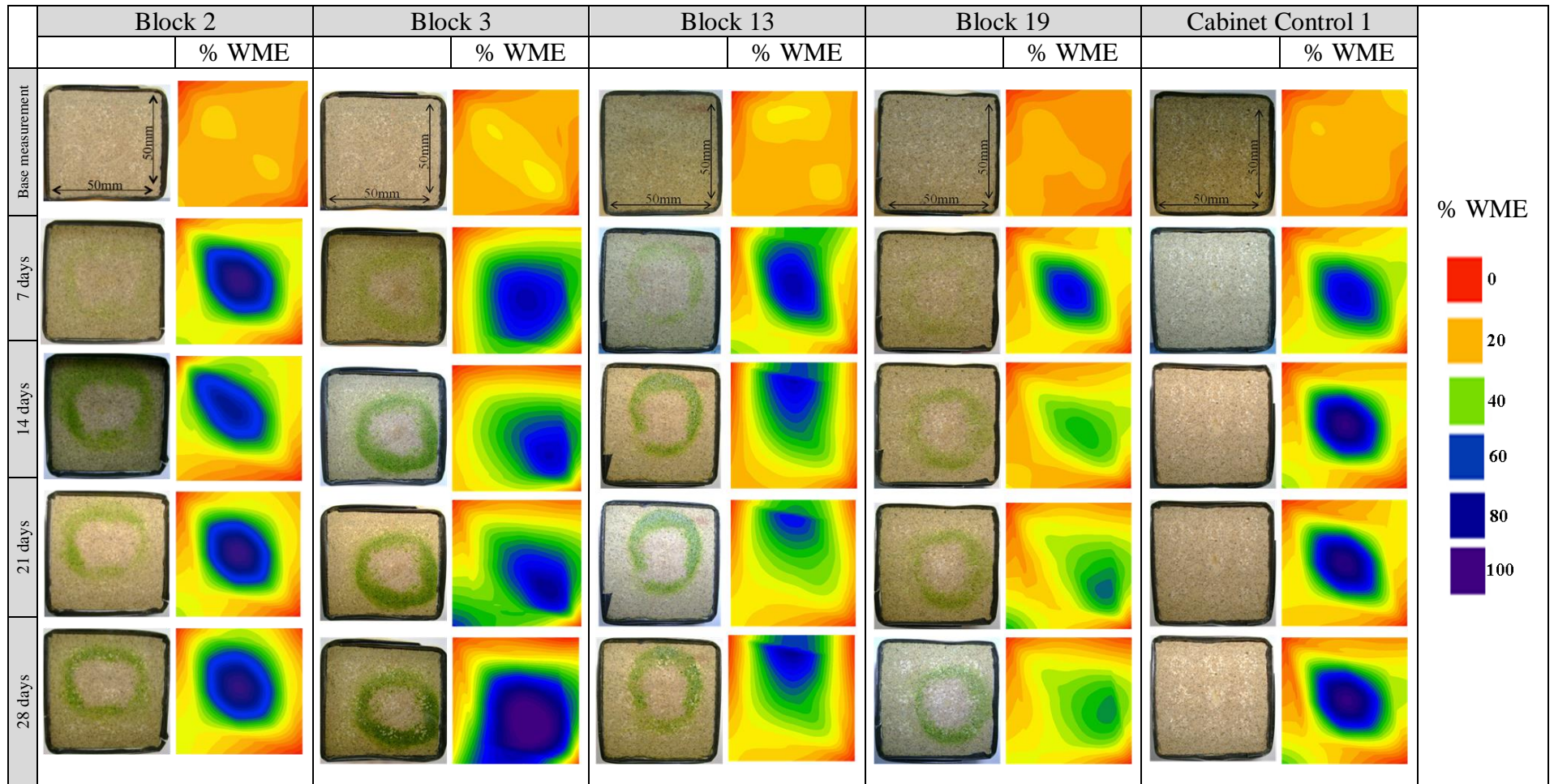
#### *Trends in algal biofilm growth*

Test samples began to exhibit visual signs of greening at five to seven days after initial inoculation (see Figure 21). After seven days a biofilm started to form on all test samples. The morphology or ‘shape’ of these biofilms was not clear at this stage. Indeed, it appeared that whilst low in biomass, colonisation was evenly spread across the surface. By  $10 \pm 2$  days, the outline shape of each biofilm had been established on all blocks.

As depicted in Figure 22, after 28 days of incubation a clear ‘ring’ of greening was visible on the surface of all test samples. Whilst the morphology of these ‘rings’ was variable between blocks, a clear visual trend was apparent across all surfaces. *C. vulgaris* favoured an intermediate zone avoiding colonisation of the central zones entirely. By the 28<sup>th</sup> day, colonisation was beginning to spread out from the intermediate ‘ring’ towards the outer perimeter. Small clusters of cells were visible along the tape edges on most test samples by the end of the experimental period.

After 30 days of incubation, cabinet control samples began to exhibit signs of initial colonisation. Therefore at this stage it was decided that the experiment should cease, as the control samples were no longer true controls. Twenty-eight days was taken as the cut-off point for the experiment. After the end of the experiment, all samples were removed from the growth cabinet and placed in a north-facing windowsill in the open laboratory. After two weeks of exposure in this setting, samples had become subsequently colonised with fungi and mould spores (see Figure 23).

Figure 21: Comparison of biofilm morphology and changes in surface moisture distribution over time



Changes in biofilm morphology showed a good degree of correspondence with changes in surface resistivity over time.

Four random test samples are presented here and one representative cabinet control.

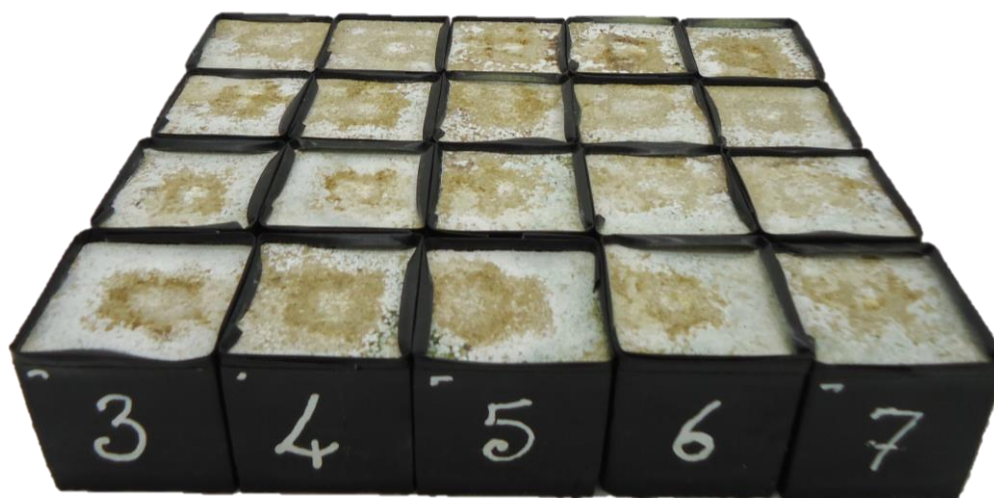
The distribution of surface resistivity readings for control blocks did not alter much past 14 days of incubation.

**Figure 22: Biofilm development after 28 days**



*Colonisation was concentrated in intermediate 'rings' on exposed surfaces after 28 days of incubation*

**Figure 23: Secondary colonisation of samples post experimentation**



*After the end of incubation, test samples and cabinet controls were placed on a North-facing windowsill. Within two weeks the top surface of blocks had become colonised by mould and fungal spores*

Miller et al. (2008) found similar white growth of fungi on limestone samples after 3 months of incubation. Test samples exhibited both faster and more vigorous colonisation by these organisms in comparison to the cabinet controls. This would indicate that inoculated samples had a higher secondary bioreceptivity than untreated cabinet controls.

### *Trends in moisture regimes*

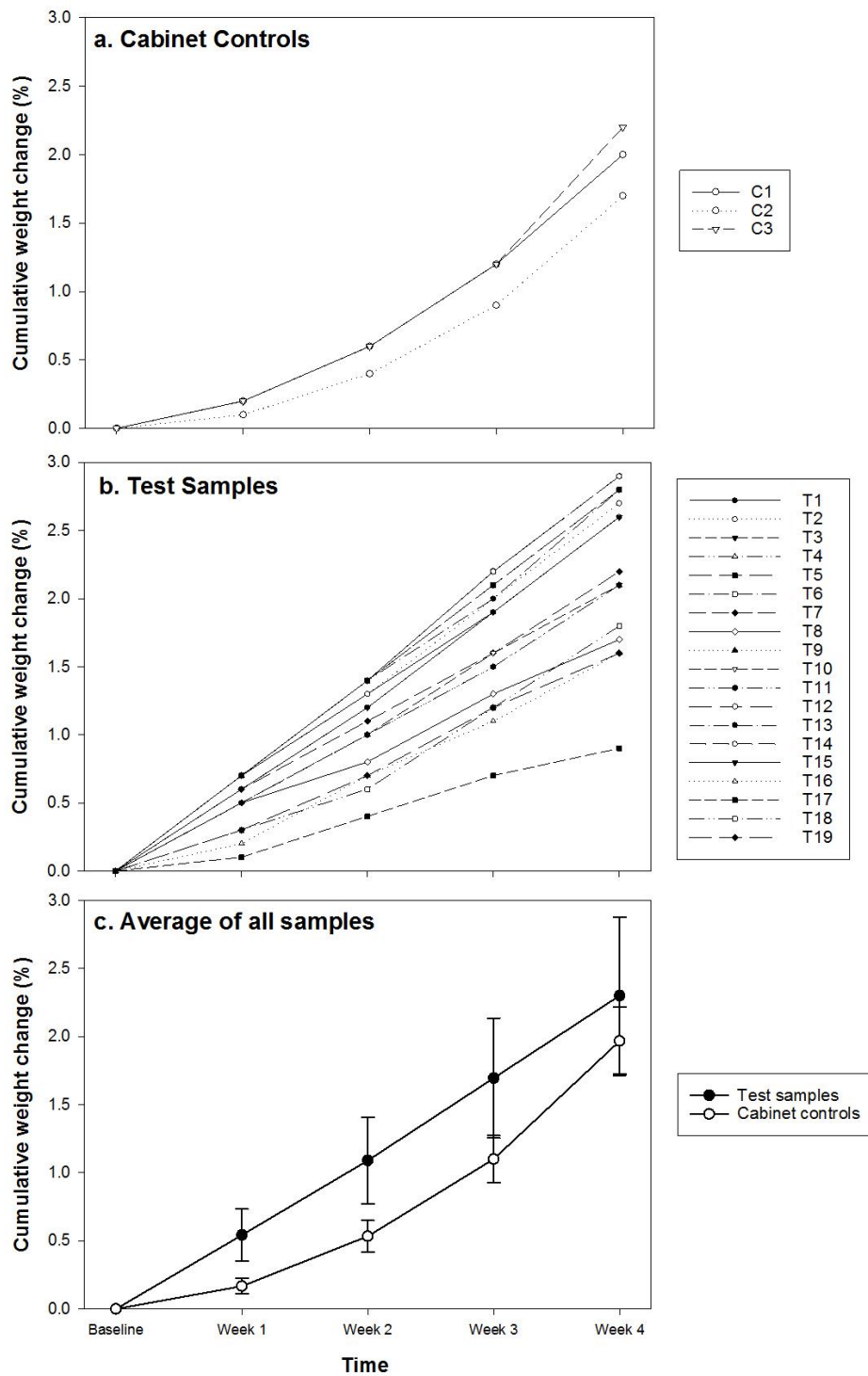
The weight of laboratory control samples remained constant over time within the range of balance error ( $\pm 0.001$ g) and %WME readings consistently measured below the lower threshold reading ability of the Protimeter device. Therefore, the moisture results from these blocks are not presented here as we can assume that their moisture status remained unaltered for the duration of the experiment.

All incubated samples gained weight over time (see Figure 24). Cabinet controls gained weight over time at an average rate of around 0.4% per 7 days (see Figure 24c). Conversely, test samples (which all became visibly colonised by green algae) gained weight at a faster rate of 0.56% per seven days, than cabinet controls (see Figure 24c). Test samples gained more weight overall than cabinet controls (see Figure 24c). By the 28<sup>th</sup> day of incubation, test samples had gained an average of 0.09% more weight than cabinet controls, a statistically significant difference (T-Test: P-0.05, CI- 95%). Results indicate that algal colonisation may lead to more rapid weight gain over time (straight line) than observed in cabinet controls (curved line). As the weight of algal biomass itself is unlikely to contribute significantly to overall weight change, results demonstrate that biofilm development can contribute to moisture retention within sandstone under conditions of constant moisture delivery and evaporation.

### *Monitoring moisture movements*

In all 19 test samples, a clear visual pattern in near-surface moisture was observed; maximum algal colonisation at 28 days corresponded with resistance readings of 35-40%WME (see Figure 25b and c). Despite optimum growth conditions for *C. vulgaris* being maintained, non-homogenous patterns of growth occurred.

Figure 24: Cumulative percentage weight change over time



Weight change was calculated from the 'baseline' point and graphed cumulatively. The weight of laboratory control samples remained constant over time within the range of balance error ( $\pm 0.001$ g), therefore they are not presented here.

Graph c. plots mean weekly weight change data for each sample group. Error bars represent standard deviation from the mean.

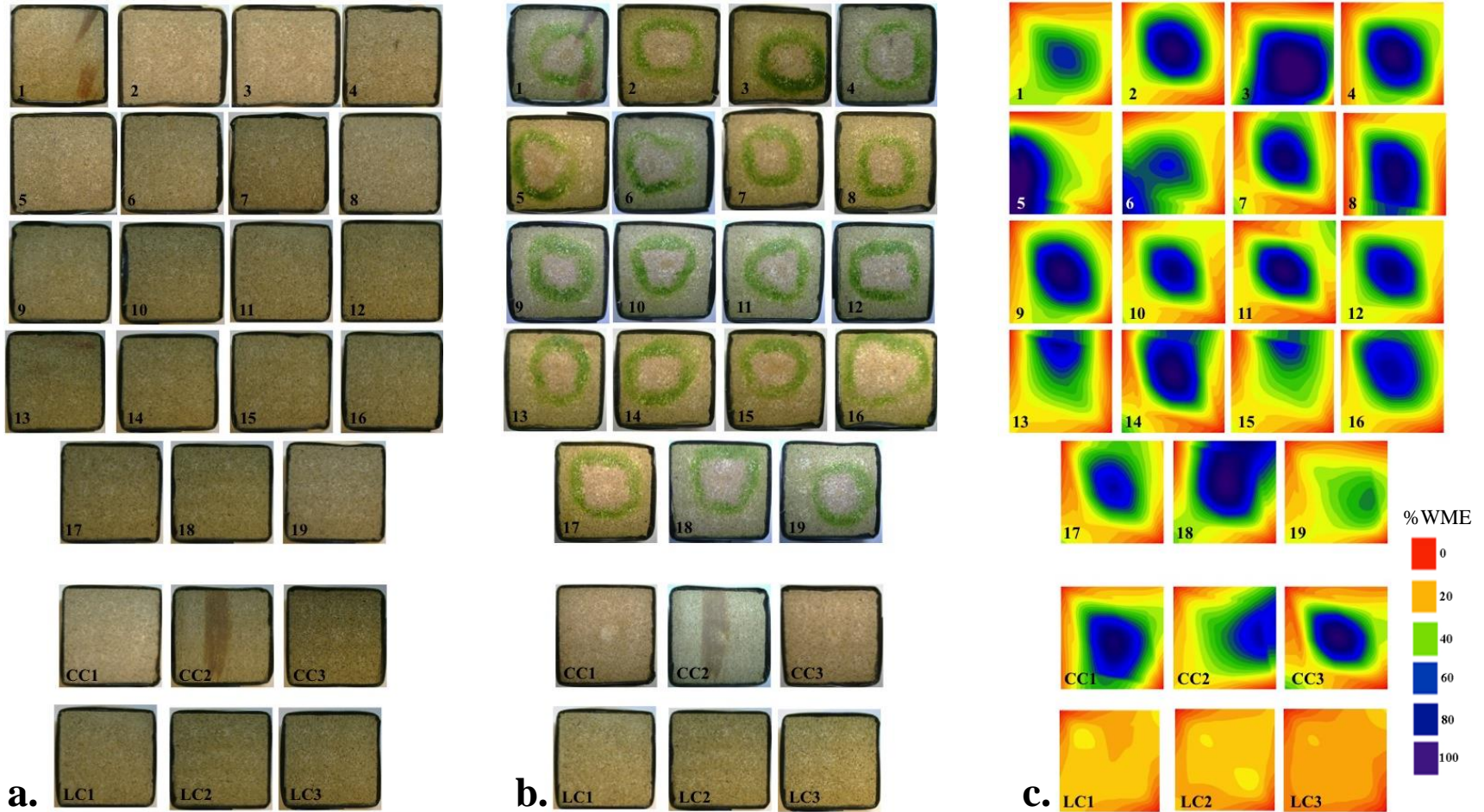
Observations indicated that on all blocks the central zone of the block remained entirely uncolonised, even after one month of incubation (see Figure 25b).

By the end of the incubation period, surface salt accumulation was visible at the centre of all cabinet controls and some colonised samples, indicating the inhibiting effect salts may have on growth. This accumulation of salts may also explain the high WME readings for the central zone of all incubated blocks. It is well known that resistivity measures are affected by fluctuations in salt content (Cutler et al., 2013b). Therefore, maximum conductivity readings (see Figure 21 and Figure 25c) may have been due to either high moisture content or salt accumulation on the near- surface centre of blocks, or a combination thereof.

Contour plots depicting inferred surface moisture at the beginning of incubation (up to day 7) were very similar in morphology for both test samples and cabinet controls (see Figure 21). This period overlaps with the initial phase of growth (between day 7-14), where most biofilm growth was observed on test samples. This indicates that formation of the distinct 'rings' of growth observed on test samples was due to the kind of initial moisture movements documented for *all* incubated samples. After 14 days, the distribution of resistivity readings remained relatively consistent for all control samples (see Figure 21).

However, beyond 14 days of incubation the surface moisture distribution of colonised samples began to exhibit a far greater degree of variability than cabinet controls. This might indicate that as a biofilm enters maturity, *it* may begin to exert an influence on the distribution of surface moisture.

Figure 25: Change in visual appearance of samples after 28 days in relation to surface moisture distribution



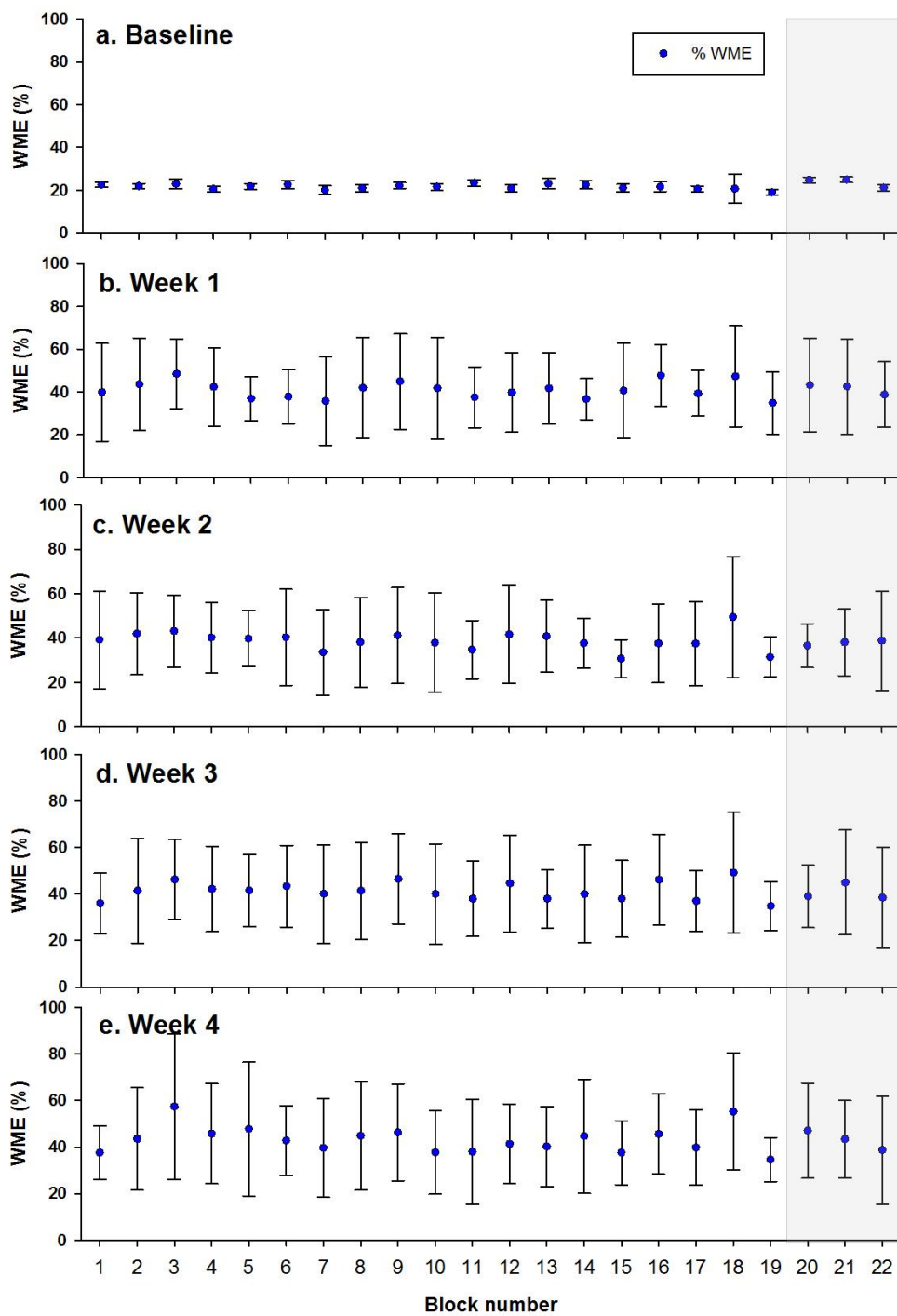
a. Photographs taken prior to initial inoculation treatment, b. Photographs taken after 28 days of incubation, c. Moisture vectors depicting surface moisture (in %WME) distribution after 35 days of incubation.

Contour plots (see Figure 21 and Figure 25c) are a useful indicator of resistivity and therefore inferred surface moisture distribution. However, average resistivity measurements (this is the mean of the nine surface measurements for each block) can give an overall picture of differences between samples (see Figure 26). As Figure 26 demonstrates, there was a lot of intra- block variability for all samples, as demonstrated by high standard deviation values (see Figure 26 error bars). This is owing to the spatially variable distribution of resistivity readings on each block (i.e. peak readings in surface centre and lower readings in the peripheries, as discussed earlier).

There appeared however to be much less inter- block variability. That is, when looking at mean resistivity values, test samples and cabinet controls behaved very similarly over time. This is exemplified in Figure 27 where it is evident that mean resistivity readings for all samples increased over time (indicating an increase in surface moisture) but there was not a noticeable difference between test samples and cabinet controls. However, the difference in average resistivity readings between the two sample sets was not significant (T-Test: P-0.05, CI- 95%).

Our results indicate that surface colonisation correlates with resistivity readings of between 35-40% WME. This appears to be a moderated region between peak readings in the centre and low readings in the outer perimeter of blocks. Whilst the distribution of resistivity readings (and therefore inferred moisture) was more variable on colonised test samples than cabinet controls, a significant difference in average readings did not occur between the two sample groups.

Figure 26: Change in average surface moisture over time

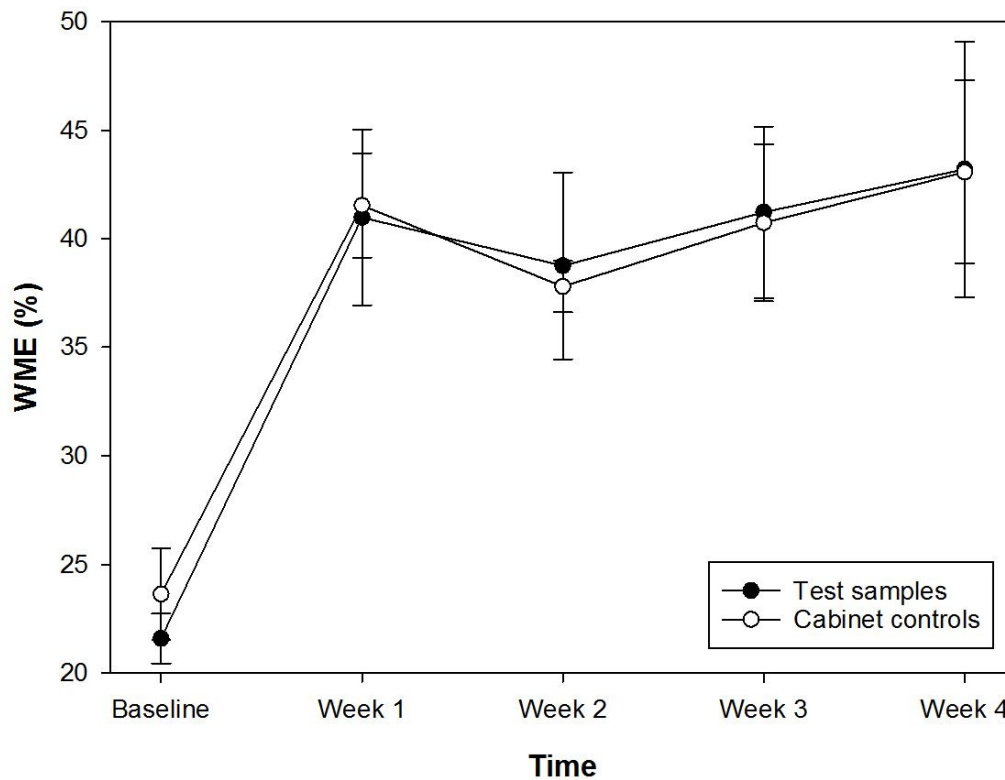


Average surface moisture is calculated by averaging (mean) the nine surface measurement points on the top faces of samples. Error bars representation standard deviation from the mean.

Shaded blocks 20-22 refer to the cabinet controls.

Laboratory controls measured below the bottom threshold of the Protimeter device, therefore they are not presented here

**Figure 27: Mean resistivity readings for test samples and cabinet controls**

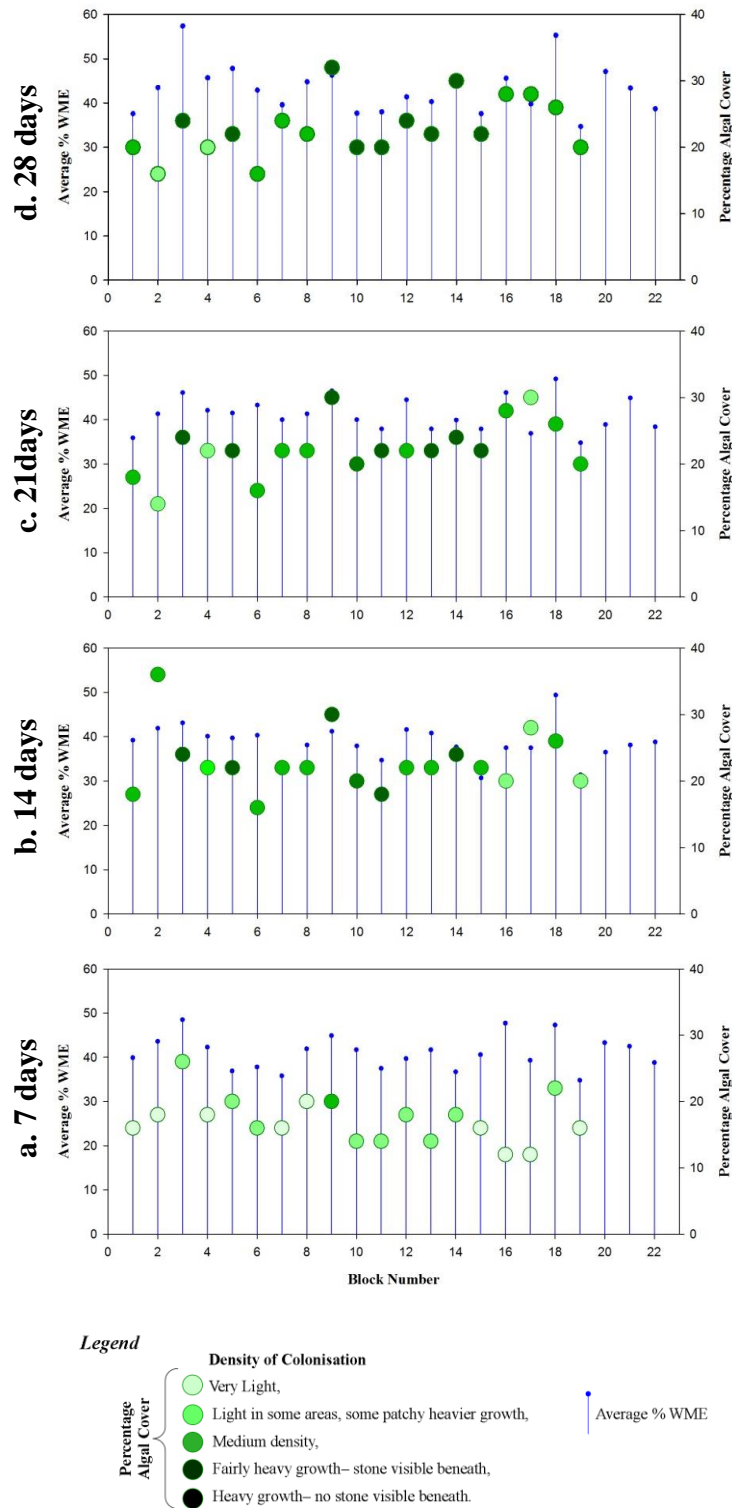


*Symbols represent the mean surface resistivity readings for all blocks in either the 'test sample' (blocks 1-19) or 'cabinet control' (blocks 20-22) groups. Error bars represent the standard deviation from the mean*

### **Monitoring changes in biomass (using algal cover and density as proxies)**

In combination, percentage algal cover and density of colonisation were used as a proxy for biomass using the methods described previously (see Chapter 2). Figure 28 illustrates the relationship between changes in biomass and surface resistivity readings over time. The height of blue lines represent average resistivity (% WME- this is the mean of all 9 point measurements, as discussed previously) and the position of green circular symbols represent percentage algal cover. The colour of circular symbols refers to the ranked density of colonisation.

**Figure 28: Surface greening- the relationship between percentage algal cover, density of colonisation and inferred surface moisture over time**



*Blocks 1-19= test samples, 20-22= cabinet controls.  
 The height of blue lines represent %WME (left axis) and the height of green circular symbols represent percentage algal cover (right axis). The colour of the green circular symbols refers to the density of colonisation as presented in the legend*

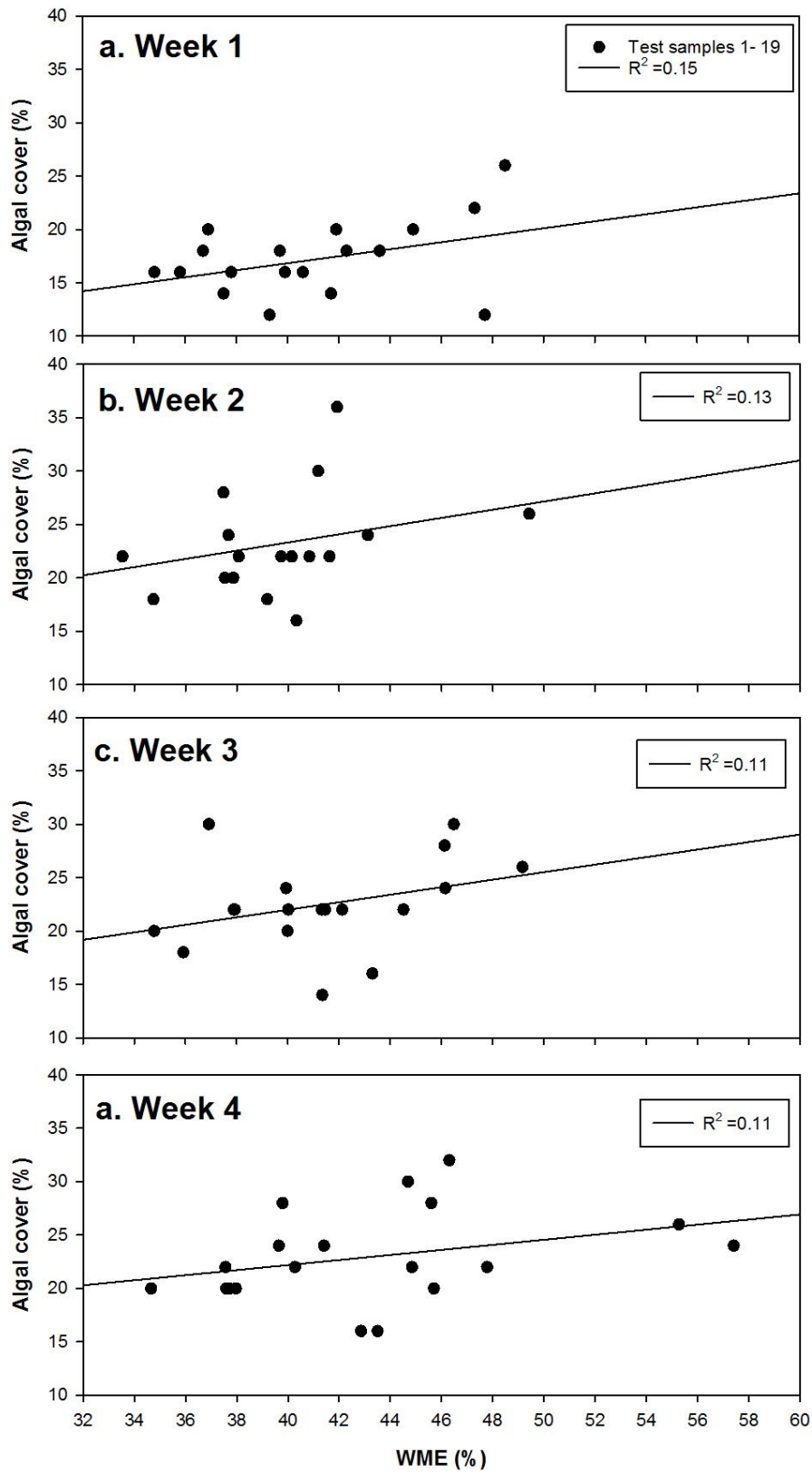
Whilst average surface resistivity readings did not change greatly over time, both algal cover and density did seem to increase. In particular, the density of colonisation reached ‘fairly- heavy growth’ on 47% of the colonised blocks by the fourth week of incubation (see Figure 28d). Overall on test samples, algal cover increased over time as did surface resistivity readings and therefore, inferred moisture (see Figure 29).

Whilst a relationship does appear to exist between the two variables,  $r^2$  values are very low (see Figure 29), demonstrating that this relationship is not very strong. Percentage algal cover did not increase as incrementally over time as density of colonisation, but seemed to fluctuate in a number of cases. For example on Block 2 (see Figure 21 and Figure 28b), biofilm cover and density of colonisation peaked at around 14 days of incubation. Beyond this point greening began to decline. This demonstrates a degree of dynamism in the proliferation of algal colonies and consequently in the morphology of the biofilms produced.

By 14 days of exposure, the surface of all test samples appeared moist and shiny in comparison to exposed surfaces of cabinet controls (see Figure 21). Cabinet controls did appear darker, possibly due to moisture retention but resistivity readings did not indicate that they were excessively moist at the surface (see Figure 27).

The visibility of a ‘moist’ layer on the surface of colonised samples peaked around 7-14 days of incubation in line with the period of heaviest growth. Formation of this moist, EPS- rich layer indicated that algal colonies had become established on the surface and signified unification of individual colonies as an epilithic biofilm.

**Figure 29: Relationship between surface resistivity and algal cover over time**



*This scatter plot graphs algal cover (%) on all 19 inoculated test blocks (black circular symbols) against average surface resistivity (mean of 9 point measurements, %WME) at seven day intervals. Regression lines are 'best fit' and the R<sup>2</sup> value is presented in the corner of each plot.*

### 3.5 Discussion- addressing the research questions

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A simple protocol was devised to grow and characterise a green algal biofilm on sandstone, under laboratory conditions. By inoculating stone samples with a single algal species culture and incubating under ‘optimum’ environmental conditions it was possible to initiate and sustain biofilm growth over a 28 day period. This study shows that it is possible to reproduce the kind of epilithic biofilms seen on our built heritage, in the laboratory environment on sandstone blocks under realistic conditions. By recreating green biofilms in a controlled environment, we have the potential to assess the bioreceptivity of the stone substrate and the environmental conditions required to promote and sustain growth. We can also closely monitor the morphology of the biofilm itself and the impact it may have on the underlying stone substrate.

After 28 days of incubation, all samples (test samples and cabinet controls) were stored in the open laboratory environment and quickly became colonised by bacteria and fungi (see Figure 23). This demonstrates the likely speed of colonisation by higher organisms in an uncontrolled environment. This observation highlights the need to keep treated samples in a sterile, isolated location away from contaminants in the general environment. It also hints at the vulnerability of stones colonised by green algae, to subsequent colonisation by organisms capable of causing greater damage (Gómez-Alarcón et al., 1995).

#### *R1. What kind of methodological set-up is required to produce a green algal biofilm under laboratory conditions?*

Based on a survey of the literature, we chose to use 50 x 50 x 50 mm cubes of Peak Moor sandstone for analysis. Of the 19 test samples inoculated with algae, all became colonised

within the first seven days of incubation and whilst the exact morphology of biofilms was variable between blocks, all developed similar ring- like surface biofilms by 28 days of incubation. These rings occurred in the middle regions around the centre of blocks. Cabinet control samples remained entirely free of colonisation for 28 days of incubation.

As the general morphology of the biofilms was similar across all inoculated blocks, this suggests that a reduced number of replicates could be used in future experiments. This is likely to be a consideration where the total number of replicates (including controls) must be constrained due to limited availability of stone and or the limited storage capacity of the growth cabinet used for incubation.

We inoculated algae by trialling a simple two- phase inoculation protocol as described in the Methodology chapter. Briefly, this included pipetting a predetermined volume of inoculant (algae suspended in growth medium) on to the stone surface. Samples were then incubated for two hours to ensure this treatment had been fully absorbed. Samples were then re- treated with the same volume of inoculant and incubated for the remaining duration of the experiment. Samples had been wrapped in impermeable tape and a ‘lip’ left around the upper rim to reduce the likelihood of inoculant washing down the sides of blocks. This procedure was effective in introducing algal cells to the surface of stone samples leading to the development of a primary biofilm within 28 days.

Having surveyed the literature and identified the optimum environmental conditions required for chlorophyta to thrive, we incubated test samples and cabinet controls in a growth cabinet under the regime depicted in Figure 18. As discussed, we were able to obtain healthy colonisation in a relatively short period of 28 days, with initial colonisation

visible within seven days on all test samples. The establishment of healthy biofilms was indicated by a moist and waxy surface biomass and the deposition of visible EPS on all inoculated test samples. As the inoculation protocol and environmental regime were successful in producing healthy colonisation, we recommend their use in subsequent laboratory experimentation.

As discussed in the results section, the duration of the experiment was limited by the time for which cabinet controls remained sterile. That is, the length of time before cabinet control samples began to show signs of being colonised by algal propagules. In this instance, by the 30<sup>th</sup> day of incubation. These observations indicate that the viability of such experiments is limited to around  $28 \pm 7$  days in a growth cabinet setting, in which it is not possible to prevent algal propagation (in moist air) onto control blocks.

*R2. What are the spatial and temporal characteristics of algal colonisation on sandstone blocks under ideal conditions?*

Up to seven days of incubation, initial colonisation on all test samples was relatively evenly spread across treated surfaces. Beyond this point, clear ‘rings’ of growth formed; the shape of which became more pronounced over time. Whilst ‘ring-like’ biofilms were apparent on all test samples, the morphology of these biofilms was variable between individual samples. There was however, a good correspondence between the outlines of growth and the distribution of surface resistivity readings (and therefore, inferred surface moisture- see Figure 25b and c).

It is well established that green algae require moisture for growth. Indeed, the water requirement of algae and cyanobacteria is significantly higher than that of both fungi and

lichen (Urzi and Krumbein, 1992) which also dominate epilithic biofilms in the natural environment. Despite the well-documented need for moisture, no algal colonisation occurred on the surface centre of blocks. Moreover, resistivity readings were the highest at this location, from which we might infer that the centres of blocks were saturated (see Figure 21 and Figure 25c). However, as highlighted by Eklund et al. (2011) and Cutler et al. (2013b) resistivity readings are strongly influenced by the presence of soluble salts. Therefore, a lack of colonisation in the centre of blocks was likely due to saturation of the stone or the presence of inhibiting salts. Further evidence of high salinity was the precipitation of mineral efflorescence on the surface centre of all cabinet controls after 28 days of incubation.

The patchy nature of growth observed in this experiment is reminiscent of the kind of uneven growth observed by the authors on monuments in Belfast (see Figure 30).

**Figure 30: Patchy algal colonisation observed on sandstone buildings in central Belfast**



*a) Fitzroy Church, b) Elmwood Hall, c) All Souls Church (scale: 1m)*

It appears that even under optimal conditions of growth, certain factors affect the bioreceptivity of a stone surface. These factors may be inherent, such as the porosity and

surface texture of the substrate or due to external factors (such as limited access to moisture and nutrients required for healthy cell metabolism).

*C. vulgaris* growth was concentrated within a distinct zone on the surface of blocks. We observed an increase in the production of EPS between days 7 – 14 of incubation, concentrated in this zone. This was visible on the surface as a moist and translucent gelatinous layer. Studies have demonstrated the enhanced production of EPS in *Chlorella* species in the initial, enhanced phase of growth (Kaplan et al., 1987). The chelating properties of EPS in some species of *Chlorella* (Kaplan et al., 1987) may have a biodeteriorative impact on stone (Miller et al., 2010). Whilst we observed that cells were well- adhered to the surface of blocks, we did not observe any obvious evidence of biodeterioration.

The production of EPS has a buffering effect whereby algal cells which become embedded in the matrix, are protected from desiccation and isolation from minerals and nutrients required for healthy metabolic activity (Wakefield et al., 1996b). The production of EPS not only signals a healthy biofilm, but may initiate a positive feedback mechanism which encourages further greening. This is due to increased retention of moisture and production of nutrient-rich organic matter (Miller et al., 2009) which ‘feeds’ the growing biomass. Whilst initial growth was very pronounced, we noted that the percentage cover of colonisation did not increase much beyond the areas colonised by the 14<sup>th</sup> day of incubation (see Figure 21). However, the density of colonisation did increase more evenly over time (see Figure 28). This was demonstrated by a change in the visual appearance of the central ‘ring’ of growth, which became darker and the green colour more saturated over time.

*R3. Under ideal conditions, does algal colonisation affect moisture regimes within sandstone blocks?*

Primary bioreceptivity, that is, the initial potential of stone to become colonised by microorganisms (Miller et al., 2010) appeared to be controlled by movements of moisture within the stone surface and subsurface. On test samples however, once the biofilm was established, *it* began to exert greater control over movements and accumulation of moisture. Indeed, the distribution of surface moisture appeared very similar for both colonised test samples and uncolonised cabinet controls within the first 7 days of incubation (see Figure 21). However, a divergence in moisture regimes set in after 14 days of incubation, signalling the increasing influence of colonisation on surface moisture distribution.

By the 28<sup>th</sup> day of incubation, colonised test samples had gained significantly more weight than cabinet control samples (see Figure 24). Test samples gained weight at a faster rate of 0.6% every seven days compared to 0.4% for cabinet control samples. This indicates that algal colonisation led to greater moisture accumulation over time.

Changes in biofilm morphology in relation to changes in surface moisture would have to be monitored over a longer time period to establish a cause effect relationship. In particular, it is the increased time-of-wetness of stone surfaces predicted under future climatic conditions which are likely to be conducive to biological colonisation (Smith et al., 2004).

### 3.6 Conclusions

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This study provides a useful methodological base for future laboratory experiments. The inoculation protocol and environmental incubation regime tested were both successful in introducing and sustaining an epilithic biofilm for 28 days. Observations indicate that the viability of experiments such as this is limited to around  $28 \pm 7$  days in a growth cabinet setting.

All 19 inoculated samples developed ring- like surface biofilms and displayed similar patterns of weight gain and change in average resistance (and therefore, inferred surface moisture) over time. The similar behaviour of replicate blocks in this study suggests that it may be sufficient to use a smaller number of replicates in future experiments where test stone and incubation space may be limited.

Colonised test samples gained significantly more weight and at a faster rate over time than uncolonised, cabinet controls. This indicates that algal colonisation results in greater moisture retention which has important implications for our built heritage.

It has been possible to reproduce the kind of green algal biofilms observed on sandstone monuments in the laboratory environment. The patterns of patchy colonisation observed in the laboratory, relate to patterns observed on building facades in the natural environment. Whilst optimum growth conditions for *C. vulgaris* were maintained, non-homogeneous patterns of colonisation were observed. Maximum surface greening (percentage cover and density) was observed within a limited zone and no growth was observed in the centre of blocks, which also had the highest resistivity readings. Therefore, inhibited growth in the centre of blocks can be attributed either to stone

saturation or high levels of soluble salts, or a combination thereof. These observations highlight that the role of soluble salts in inhibiting growth on sandstone substrates requires further study.

# Chapter 4

## Assessing the applicability of spectrophotometry to monitor green algal biofilm development on sandstone structures

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### Chapter Summary

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This chapter addresses Objective 1 (see section 1.9) of the overall thesis by assessing the potential of spectrophotometry to monitor the level of biocolonisation on sandstone. Part 1 of the chapter tests a simple methodology for monitoring colour change of colonised sandstone tablets under controlled conditions in the laboratory. Results indicate that CIELAB provides a comprehensive system to chart change in surface colour and the parameters of total colour change (DE2000\*), Lightness (dL\*), and greening (da\*) are the most useful in making inferences about surface colonisation. The proposed methodology was successfully tested and is recommended for future work to monitor biological growth in more complex laboratory studies as well as field exposure trials. Part 2 applies the tested methodology to monitor biological colonisation in 30-month field exposure trials in Northern Ireland. Spectrophotometry was successfully used to differentiate changes in surface colour (and therefore, colonisation) due to exposure time and aspect. Overall, this chapter demonstrates the applicability of spectrophotometry in monitoring surface colour change caused by biological colonisation on sandstone, in both the laboratory and field environment.

## **Part 1: Testing the use of spectrophotometric methods to monitor algal biofilms in the laboratory, under controlled conditions**

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### 4.1.1 Summary

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A simple methodology to assess the applicability of spectrophotometry in measuring early colonisation and to monitor biofilm morphology was tested. ‘Test’ tablets of Dungannon sandstone were inoculated with a single strain algal culture and incubated under ideal conditions of growth for five weeks. Samples were weighed and their surface colour was measured using a portable spectrophotometer device (CM-700d; Konica Minolta, Japan), using the CIELAB colour system. Inoculated samples were compared to untreated ‘control’ samples. After five weeks of incubation, there was a significant difference in total colour change between the two sample groups. In particular,  $a^*$  (red-green colour scale) and  $L^*$  (lightness scale) values were affected by surface colonisation. By plotting the movement of samples through colour space, a strong colour bias was found towards the yellow end of the chromatic spectrum illustrating that the algal biofilms caused greening- yellowing of the surface over time. Peaks in greening and darkening were closely related demonstrating the influence of algal colonisation on these parameters.

### 4.1.2 Introduction

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Aesthetic biodeterioration refers to the existence of a surface layer of microorganisms in addition to the metabolites they produce (Allsopp et al., 2004). Change in surface colour or staining caused by colonising microorganisms is often the most displeasing aspect of an epilithic biofilm growing on cultural heritage materials (Warscheid, 1999). Biofilms

can vary in colour depending on their biological constituents (Urzi and Realini, 1998) and these colours can vary in the range between red-brown and blue-green. Chlorophyll (*a* and *b*), carotenoids and other accessory pigments are the primary compounds involved in surface staining by microorganisms (McStay et al., 1996). The perceived negative visual impact is often worsened by subsequent dark soiling from particulate air pollutants in urban environments and the formation of biogenic crusts. Research has shown a link between the formation of gypsum crusts and accumulation of organic matter (Turkington et al., 2003; Smith et al., 2011), with one enhancing the other and vice versa.

The aesthetic appearance of heavily pigmented biofilms is often undesirable on monuments of significant cultural heritage and or beauty (Tomaselli et al., 1999; Nugari et al., 2009). In 'green' biofilms, discoloration is caused by chlorophyll pigments in photosynthetic algae (Gaylarde et al., 2003) which often dominate subaerial biofilms in temperate locations.

Human cone cells are most sensitive to parts of the spectrum falling around 420, 530 and 560nm, which correspond to the hues 'blue', 'green' and yellow-green' respectively (Hill, 1997). Therefore, green staining is particularly noticeable by the human eye. Buildings exhibiting particularly dense 'green' colonisation (herein referred to as 'greening') are often perceived to be aesthetically unsightly. This may be due to the apparent discernibility of the colour green on building materials or because colonisation may imply a lack of maintenance, neglect and possible biodeterioration. Fitzroy Presbyterian Church (Belfast, Northern Ireland) for example, has experienced rapid and heavy greening after a history of unsuccessful cleaning attempts (Warke et al., 1999). Monuments such as this

often become the subject of debate and notoriety due to their ‘extreme’ appearance (see Figure 31).

**Figure 31: Fitzroy Presbyterian Church (Belfast, NI)**



*a. Heavy colonisation on windowsill associated with spalling surface crusts, b. General greening of front façade, c. dense biofilm development on ‘rusticated’ stone surfaces in a semi-sheltered location, northern façade. (Scale: 30cm)*

In addition to aesthetic biodeterioration, biofilms may be associated with physical deterioration of the stone surface either directly through excretion of organic acids (Gómez-Alarcón et al., 1994; Grbić and Vukojević, 2009) and chelation by organic metabolites (Kaplan et al., 1987) or indirectly by increasing the level of surface and subsurface moisture (Smith et al., 2011) and consequent stone dissolution. Surface deterioration can often clearly be associated with algal greening (see Figure 31a). Algal greening often leads to an increase in surface moisture and the initiation of a positive feedback loop, thereby facilitating further biofilm growth (Jim and Chen, 2011). In reality, the relationship between the stone surface and algal colonisation is dynamic.

### *Biomass mapping*

There is a distinct need for methods to map the change in epilithic biomass over time due

to the potentially deteriorative impact microorganisms may have. As photosynthetic microorganisms are primary colonisers of the stone surface (Wakefield et al., 1996b), their early detection acts as a useful means by which to predict subsequent colonisation by higher, often more detrimental organisms. They are also often the first species to recolonise freshly cleaned stone (McStay et al., 1996). Biomass mapping is also a prerequisite for evaluating the bioreceptivity of different lithotypes and the efficacy of removal strategies (McStay et al., 1996).

A number of methods exist to quantify microbial biomass on stone substrates. These include specialist DNA analyses to identify and measure abundance of bacteria (Sigler et al., 2003; Horath and Bachofen, 2009) lichen (De los Ríos et al., 2005) and more recently green algae (Cutler et al., 2013a). These methods require specialist knowledge and equipment to extract, amplify, identify and quantify abundance of species.

Studies have also measured the fluorescence of chlorophyll *a* pigments as a means to quantify microbial biomass on stone surfaces. However, these methods often require destructive sampling of the stone surface and the efficacy of the method may be impeded by lithotype characteristics (Prieto et al., 2004). Whilst, non-destructive *in vivo* methods have been tested more recently (Miller et al., 2010; Mandal and Rath, 2012 ), these involve the use of specialist and often expensive, spectrofluorometers. Other, more easily available methods can also provide good information. Prieto et al. (2004) compared three commonly used methods for the determination of biomass; amount of chlorophyll *a*, fluorescein diacetate hydrolysis and changes in colour. They found good correspondence between estimates of biomass derived from both Chlorophyll *a* determination and colour with actual biomass determined from more traditional cell counts.

Photographic methods have also been used to map biomass with varying success and applicability in both the field and laboratory setting. For example, novel data analysis techniques using Principal Component Analysis (PCA) have been used in both settings to estimate the spatial area covered by biofilm growth, from digital photographs (Rogerio-Candelera et al., 2011; Coutinho et al., 2012 ). Simple imaging techniques used to map inorganic soiling and crust formation on facades have the potential to be used to map colonisation patterns. The characteristic and diagnostic colour of epilithic biofilms (particularly those occurring on buff or light coloured substrates) enables this.

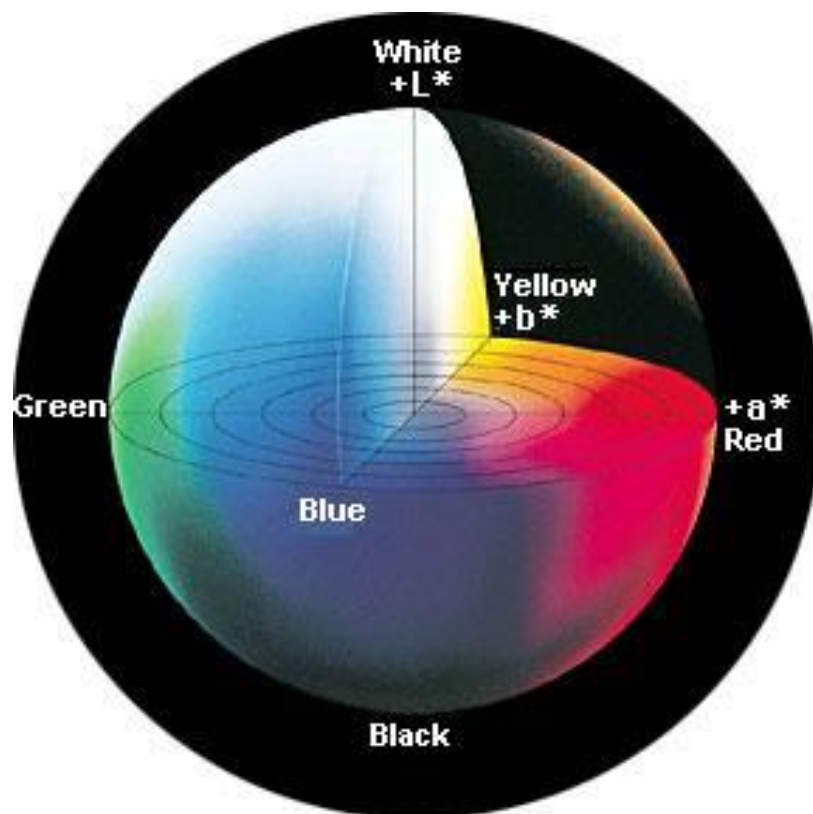
### *Colour measurements*

Whilst several papers have documented surface colour change in association with epilithic colonisation over the past 20 years (Wakefield et al., 1996b), far fewer have quantitatively monitored the influence of colonisation on the constituent components of colour change. Green algae are heavily pigmented organisms as are cyanobacteria and various species of fungi and lichen. Initial growth of an algal colony may not be visible within the range of the human eye particularly where darker stone substrates or black crusts are concerned. Fortunately, colorimeters are able to quantify very minute changes in colour (Prieto et al., 2004).

Most commonly, colour change is used as an intuitive measure where an increase in total colour change or the saturation of a specific colour-way denotes an increase in biomass over time. The most widely expressed colour standard is CIELAB  $L^*a^*b^*$  colour space (Prieto et al., 2004) which involves the three components of colour: lightness, chroma and hue. Colours are plotted in standard 'colour space' using the  $l^*$ -  $a^*$ -  $b^*$  colour coordinates as depicted in Figure 32. Lightness or  $L^*$  varies from 0 (absolute black) to 100

(absolute white),  $a^*$  represents colour on the red-green colour axis (where negative change indicates greening and positive change indicates reddening) and  $b^*$  represents colour on the blue-yellow scale (where negative change indicates an increase in the blue hue of a sample and positive change indicates yellowing) (Sanmartín et al., 2011). The standard is used widely and has been used to monitor biocolonisation using colorimeters as well as retrophotographic techniques (Thornbush, 2013).

**Figure 32: Representation of colour solid for  $L^*a^*b^*$  colour space**



*From Konica Minolta (2007b) p.19*

A number of studies have highlighted the efficacy of colour quantification as a proxy measurement for biomass. Young and Urquhart (1999) used colour in this way to monitor algal and non-algal soiling rates of exposed sandstone blocks over time. The rate of soiling was deduced by the percentage reduction in lightness ( $L^*$ ). Similarly, Prieto et al.

(2004) used total colour change ( $dE$ ) in their study of granites to monitor changes in biomass having noted a strong positive correlation between total colour change and number of cell aggregates. Whereas Marques et al. (2015) used total colour measurements to monitor cyanobacterial growth on schist under laboratory conditions. In their study of riverbed sediments Sanmartín et al. (2011) found a strong correlation between changes in CIELAB  $L^*a^*b^*$  co-ordinates and phytopigments, thereby proposing their colorimetric methodology as a useful means by which to determine phytopigment content in riverbed sediment cores. There is limited research on monitoring algal biofilm growth on British sandstones in both the laboratory and field setting using colorimetry.

Recently, field studies have used the  $a^*$  chromatic scale to map 'green' epilithic biofilms on test blocks and building facades. In exposure trials, Adamson et al. (2013a) found preferential greening (lower  $a^*$  values, denoting greening) of north-facing blocks in comparison to south-facing blocks, due to increased time-of-wetness and slower drying times on northern faces. Cutler et al. (2013b) were able to formulate links between surface greening and patterns of surface temperature distribution on monuments in Belfast, noting in particular that temperature accounted for 14% of the total variance in  $da^*$ . These findings are supported by laboratory studies in which Prieto et al. (2002) found a strong correlation ( $r^2 = 0.93$ ) between the number of organisms and greening. Their findings indicate that a decrease in the  $a^*$  scale (which denotes greening) corresponds well with an increase in biomass.

There are some key challenges associated with measuring the colour of epilithic biofilms as a proxy for biomass. The first is due to the patchy nature of colonisation on building facades, as demonstrated in Figure 31b. Naturally, microorganisms convene in certain

areas of a building façade; often areas with moderated temperature, light and moisture conditions. Therefore, large areas need to be measured to gain an overall view of biomass.

Biofilm growth and consequently surface colour and appearance can be dynamic over time and season (Urzi and Krumbein, 1992; May et al., 1999) as photosynthetic algae require water for healthy metabolic activity. Peak growth occurs in temperate locations in late winter to early spring (Young and Urquhart, 1999) where abundant rainfall is met with moderate temperature conditions and longer daylight conditions. Algal cells have been observed to become desiccated and inactive in dry conditions. Changes in the physiological state of microbial cells have been shown to significantly affect their colour (Urzi and Realini, 1998; Prieto et al., 2002). Surface roughness and the actual morphology or shape of the underlying substrate can also affect our ability to observe colour and changes in visual appearance (See Figure 31c). The use of colorimeters to measure colour objectively can go some way in overcoming these challenges.

Little work has been conducted on assessing the colour of epilithic biofilms on British sandstones and in particular on testing methods in the laboratory, for subsequent application in the field. A simple methodology is presented here to assess the applicability of spectrophotometry to monitor changes in biofilm development on sandstone samples in a laboratory setting. Sandstone tablets were inoculated with a single-strain algal solution and changes in weight and surface colour monitored over a period of five weeks. The primary aim of this investigation was to test the efficacy of colour measurements in the identification of early colonisation of a substrate and to make comparisons with traditional visual observation and digital photography. In particular, the impacts of

colonisation on the different components of colour (lightness, chroma and hue) were examined.

### 4.1.3 Hypothesised trajectory of greening

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Intuitively, one would expect the colour of a substrate to ‘green’ over time when colonised by photosynthetic green algae. In particular, one might expect a decrease in the  $da^*$  value and an increase in total colour change (expressed as  $dE^*$ ). Over time, one could expect the developing biofilm to retain moisture at the surface and possibly the sub-surface level. This would have a darkening effect on surface colour resulting in a reduction in ‘lightness’. In a typical scenario, we would expect the components of colour to change as summarised in Table 8.

### 4.1.4 Materials and methods


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A simple laboratory experiment was designed to assess the efficacy of colour as a proxy measure for biomass change and to monitor the trajectory of ‘greening’ in colour space over time. Eight 25 mm x 25 mm x 5 mm tablets of Dungannon sandstone (see Table 9) were sterilised and dried as described in Chapter 2, Figure 9. Further details about the stone type can be found in Chapter 2, section 2.2. All samples were placed in individual, sterile 5 cm diameter petri dishes.

Table 8: Expected influence of algal greening on the component parameters of colour

Parameter		Expected change in sample over time	Cause
Total colour change	dE*	Increases	An increase in biomass will result in an increase in overall colour change over time
Lightness	L*	Darkens	Due to increase in surface moisture as well as colour change (e.g. greening)
Colour space direction	a*	Moves from red to green	Greener and yellower due to chlorophyll and other ancillary cell pigments
Colour space direction	b*	Moves from blue to yellow	
Chroma	C*	Increases	Indicating an increase in biomass
Hue	H*	Increases in green/ yellow	Greener and yellower due to chlorophyll and other ancillary cell pigments

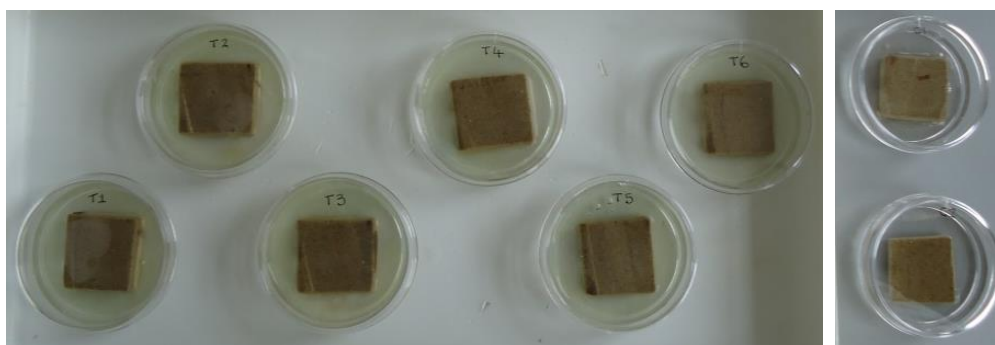
Table 9: Dungannon- General stone properties

Name	Origin and Age	Petrography	Examples of usage	Susceptibility	Image
Dungannon	Carboniferous Northern Irish (County Tyrone) sandstone from the Millstone Grit series.	<ul style="list-style-type: none"> <li>• Cream, white, grey or yellow in colour,</li> <li>• Fine- medium grained,</li> <li>• Granular texture, bedding, laminations</li> </ul>	Headline building, Belfast	<ul style="list-style-type: none"> <li>• Relatively durable</li> <li>• Vulnerable to surface delamination in polluted environments</li> <li>• Can undergo rapid soiling</li> </ul>	

(Ashurst and Dimes, 1977; BRE, 2000a; Curran et al., 2010)

Transparent lids were kept slightly ajar on all of the dishes throughout the experiment to reduce the likelihood of cross contamination between test tablets and controls (see Figure 33). All of the dishes were filled with 1.5 ml of distilled water (so that samples would have consistent access to water for moisture uptake, this volume of water was maintained throughout the experimental period). All samples were placed in the dishes of water for 30 minutes. This was taken to be the ‘baseline’ point for subsequent weight and colour measurements.

**Figure 33: Experimental set-up**



*Left: Test tablets, Right: Control tablets*

Six ‘test tablets’ were then inoculated with 1 ml weak (4 ml concentrated CCAP culture subcultured in 100 ml 3N-BBM+V stock medium) suspended solution of *Stichococcus bacillaris* Nägeli, 1849 (CCAP strain number 379/1A), herein referred to as *S. bacillaris*. Two ‘control tablets’ were treated with the same volume of autoclaved stock medium. Cultures and media were both obtained from the Culture Collection of Algae and Protozoa (CCAP, Argyll). Test and Control tablets were incubated in a MLR-352H Plant Growth Cabinet (Panasonic, UK) under ‘ideal’ conditions for growth (temperature  $20 \pm 1$  °C; relative humidity  $85 \pm 5$  %; photoperiod 16 hours light/ 8 hours dark) for a period of 35 days. Based on previous laboratory experiments (see Chapter 3) 35 days was decided as enough time to allow for development of a fledgling community and to prevent cross contamination of controls.

Samples were weighed at the baseline point (as defined above) and weekly thereafter. Tablets were removed from the dishes and placed on absorbent paper for 30 seconds (to dry any excess moisture) and weighed using a two-decimal place, bench-top balance (error  $\pm 0.001$ g). Weight was used as a proxy measure for moisture content. Digital photographs were taken at the baseline point and weekly after this to provide a visual

record of colour changes. Photographs were taken using a fixed tripod set-up, under consistent lighting conditions.

Colour change was measured at the baseline point (herein referred to as ‘target’ colour) and weekly after that, for five weeks. Colour change was measured using a CM-700d Spectrophotometer (Konica Minolta, Japan) which measures colour in CIELAB colour space using the colour variables:  $L^*$ ,  $a^*$ , and  $b^*$ . Total colour change was calculated using the  $\delta E^*2000$  measurement (see Equation 1), which provides a good representation of colour discrimination, as if observed by the human eye (Konica Minolta, 2007b), further details about the calculation can be found in Appendix 2. An average of 5 measurements was taken of the top surface of each tablet using the 7 mm Medium Area View (MAV) aperture, under standard illumination (D65: average daylight) conditions. Colour data was analysed using SpectraMagic™ NX colour data software (Konica Minolta, Japan). Only ‘Specular Component Excluded’ (SCE) data are presented here, as this measurement excludes gloss effects caused by surface water.

**Equation 1: CIE 2000 Colour difference formula**

$$\Delta E_{00}^* = \sqrt{\left(\frac{\Delta L'}{k_L \cdot S_L}\right)^2 + \left(\frac{\Delta C'}{k_C \cdot S_C}\right)^2 + \left(\frac{\Delta H'}{k_H \cdot S_H}\right)^2 + \left(R_T \left(\frac{\Delta C'}{k_C \cdot S_C}\right) \left(\frac{\Delta H'}{k_H \cdot S_H}\right)\right)}$$

Please see Appendix 2 for formula *components and calculation details*

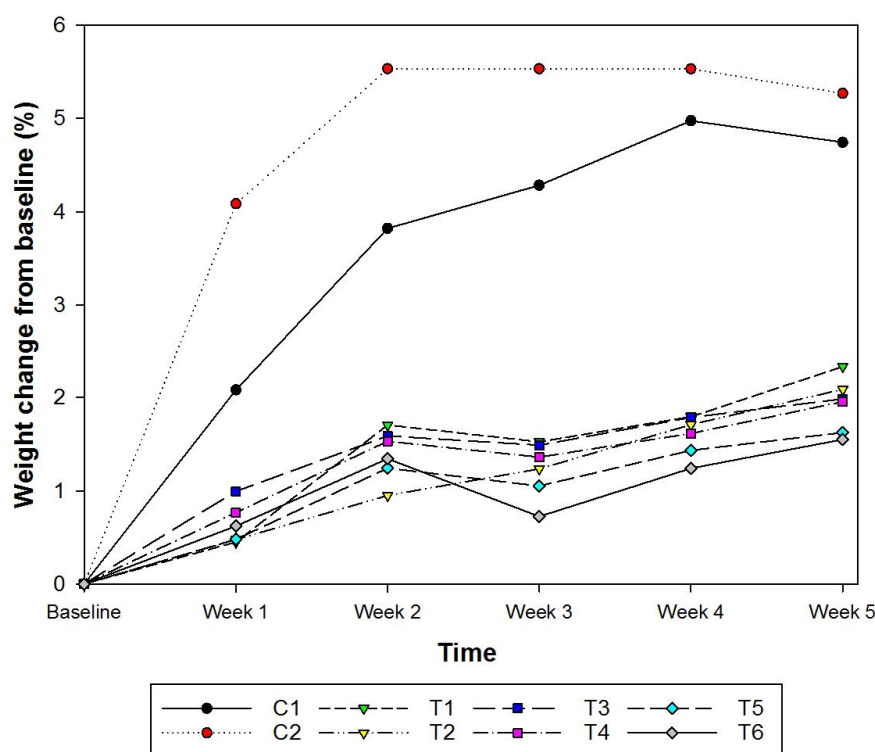
### 4.1.5 Experimental results

#### *Weight change as a proxy measurement for moisture movements*

The weight of all samples had increased by the end of the experiment, indicating moisture absorption over time. However, the trends over time and total weight change differed between control and colonised samples (see Figure 34). All colonised replicates behaved in a similar way with some variability occurring between individual replicates (see T1-T6, Figure 34). Control samples gained weight quickly, but overall weight did not alter as much beyond the 3<sup>rd</sup> week of incubation. Conversely, colonised samples gained weight more gradually over time. Control samples gained noticeably more weight by the end of the experiment (mean of 5 %) than colonised samples (mean of 2 %). We can therefore infer that surface colonisation reduces water absorption and/ or retention in this case.

However, only a limited number of control samples were tested here.

**Figure 34: Weekly weight change (%) from baseline**

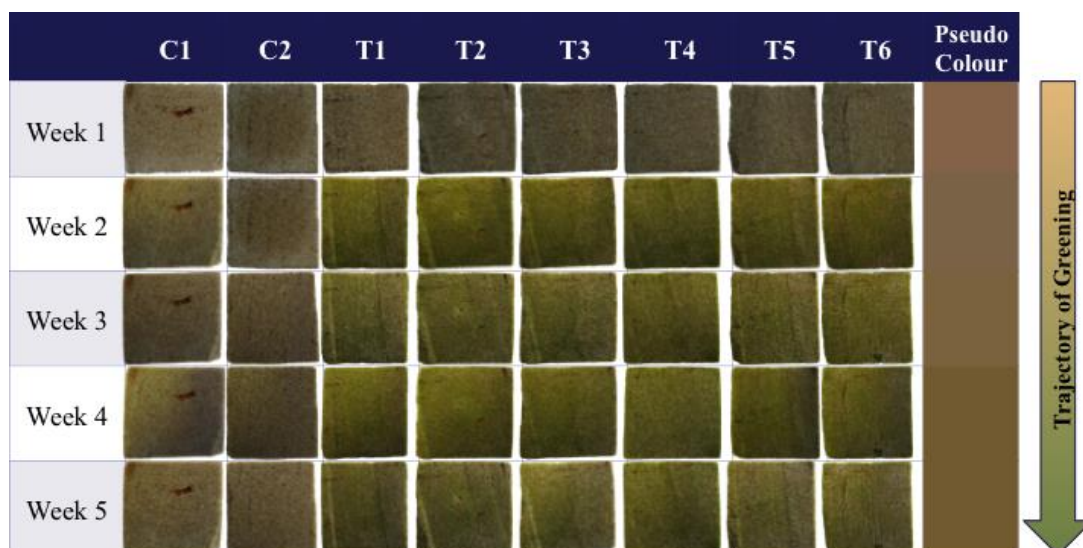


*Graph depicts change (%) in weekly weight of samples as calculated from baseline values  
C1-C2= Control samples, T1-T6= Inoculated tablets*

### Photography

Photography is hugely affected by ambient lighting conditions, as is human ability to perceive subtle changes in visual appearance. As Figure 35 demonstrates, visual greening of test tablets could be perceived in photos after one week of incubation as well as darkening of control tablets. Beyond one week however, there appeared to be little change in the visual appearance of the 'green' tablets. Whilst these photographs indicate greening, we cannot determine tangible information about changes in biomass from them.

**Figure 35: Photographic record of greening**



*Weekly photographs taken under constant lighting conditions.  
'Pseudo colour' represents the averaged colour measurements of all colonised 'test' samples*

**Figure 36: Averaged pseudo colour representation of colonised tablets**

L*= 51.89 a*= 5.37 b*= 19.23	L*= 51.8 a*= 5.28 b*= 19.21	L*= 50.76 a*= 5.46 b*= 19.30	L*= 47.23 a*= 1.88 b*= 26.37	L*= 47.22 a*= 2.27 b*= 25.7
<b>Week 1</b>	<b>Week 2</b>	<b>Week 3</b>	<b>Week 4</b>	<b>Week 5</b>

*'Pseudo colour' represents the averaged colour measurements of all colonised 'test' samples*

By measuring colour change of samples directly using a spectrophotometer, it is possible to formulate a colour representation of the measured sample. ‘Pseudo colour’ (see Figure 35) representations, as computed by colorimetric software, are objective so are not influenced by changing ambient lighting conditions that digital photography can be vulnerable to. In addition, pseudo colour is often better able to represent subtle changes in the hue of samples. Figure 36 illustrates this by depicting calculated pseudo colour (averaged for all colonised test tablets) each week. It allows easier differentiation of colour change over time than digital photographs. From this representation, we are able to see an apparent darkening and an increase in the yellow-brown hue of test samples over time. This is indicative of an increase in surface biomass. However, there appeared to be little change in pseudo colour between weeks four and five of incubation indicating that the level of biomass began to stabilise at this point.

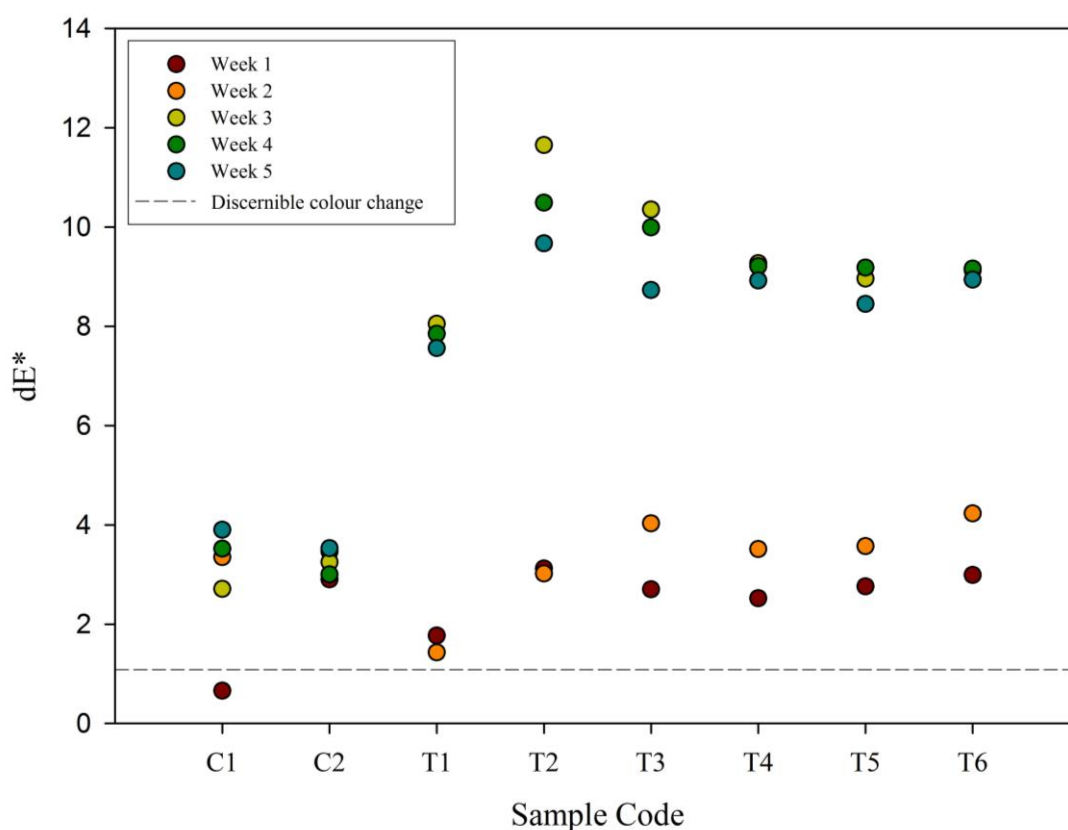
#### *Spectrophotometric assessment of colour change*

When considering the colour difference measurement  $\delta E^*2000$ , it is widely accepted that the standard figure for discernible colour change by eye is one CIELAB unit (see dotted line, Figure 37). All test samples and one control sample passed this threshold of discernibility after one week of incubation, demonstrating noticeable colour change. The second control passed this threshold after the second week of incubation. Total colour change in control samples averaged at 3 CIELAB units and little change was observed beyond the second week of incubation. Similarly, little discernible change was observed in photographic observation of control samples after this point (see Figure 35).

All inoculated ‘test’ samples demonstrated a similar level of total colour change to ‘control’ samples after two weeks of incubation. However, peak colour change in ‘test’

tablets was observed between week two and three of incubation (see Figure 37). Indeed, the highest  $dE^*$  value for four of these samples was recorded at three weeks of incubation. The average  $dE^*$  value for 'test' samples after five weeks of incubation (7 CIELAB units) was significantly greater (Two- tailed T-Test: 0.0007,  $P < 0.001$ ) than that of the control samples, indicating the apparent influence of algal colonisation on total colour change.

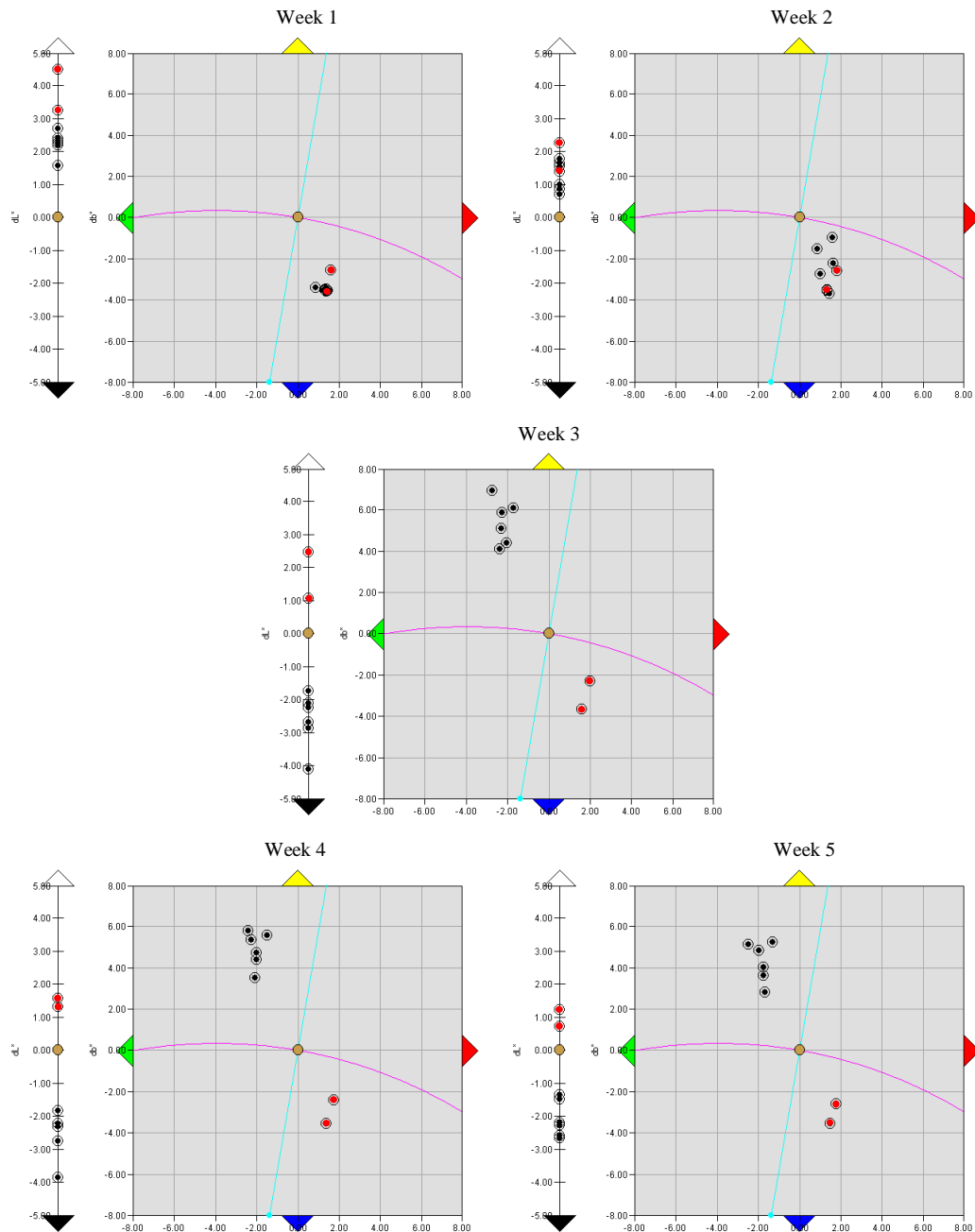
**Figure 37: Total colour change over time from the baseline point**



*Graph depicts total colour change ( $dE_{200}^*$ ) from the baseline point for each sample*

By plotting  $a^*$ -  $b^*$  (hue) and  $L^*$  (lightness) values in colour space, we can gain a more detailed view of colour change over time (see Figure 38).

Figure 38: Movement of samples over time through 2D CIE L\*a\*b\* colour space



Plots indicate directional colour change in reference to an averaged target colour of all samples at the baseline point (central 'brown' point indicates averaged pseudo colour).

Grey plots represent 2D  $a^*-b^*$  colour space where the  $x=da^*$  and  $y=db^*$  (blue line depicts the hue angle of the target, pink line depicts the chroma value of the target)

Vertical arrow plots represent change in Lightness.

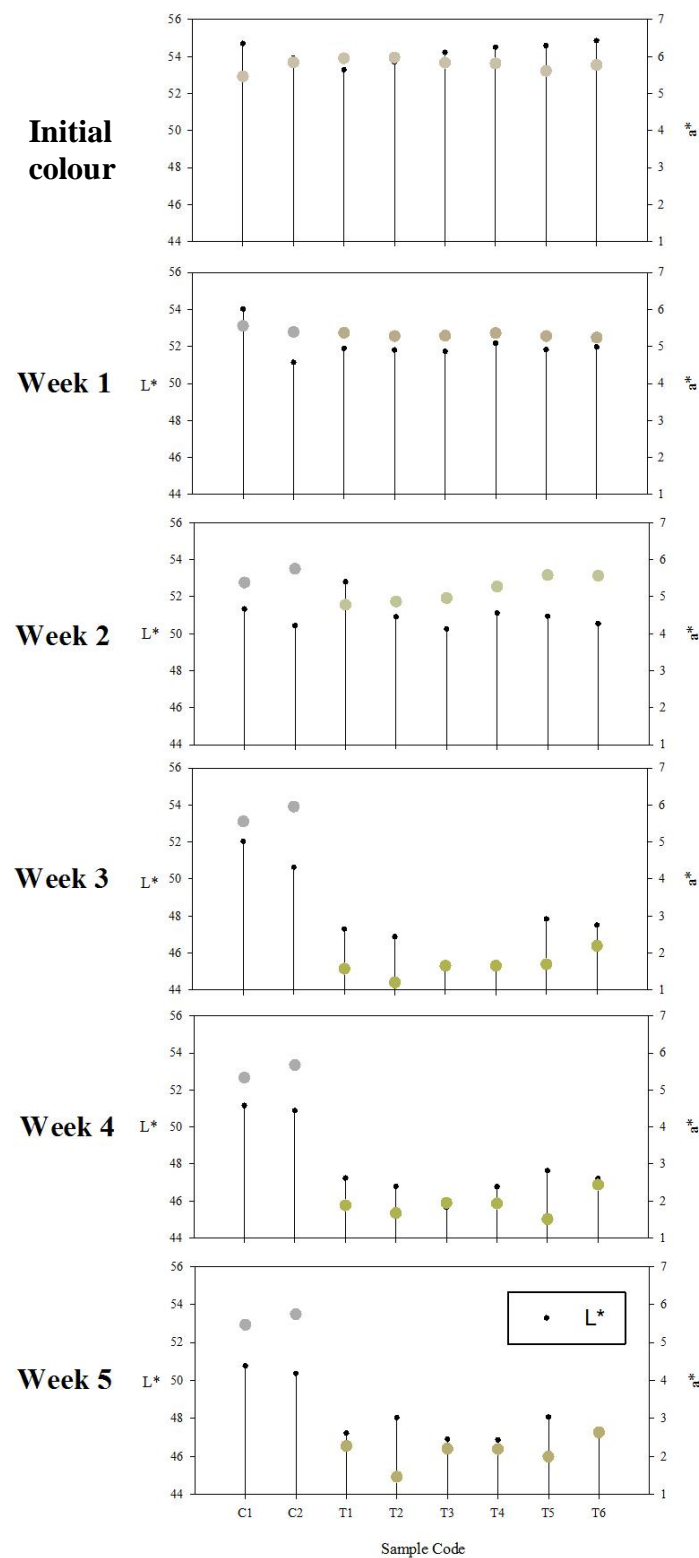
Circular red points represent 'controls' and black points represent inoculated 'test' tablets

All inoculated 'test tablets' exhibited a very similar trajectory of greening over time. Up to week one of incubation, 'control' and 'test' tablets demonstrated good correspondence in both hue and lightness. By week two of incubation, all samples exhibited a similar level of darkening but the majority of 'test' tablets began to show some yellowing.

By week three of incubation there was a clear distinction between the average hue and lightness of the two sample groups. 'Control' tablets did not demonstrate a significant change in either colour parameter beyond the second week of incubation. Conversely, 'test' tablets moved distinctly towards the yellow-green quadrant of colour space, over time (see Figure 38). This movement was proportional to an increase in the darkening of samples, demonstrating a possible link between biofilm induced surface colour change in  $a^*$  (red- green hue) and reduced lightness. To illustrate this point, Figure 39 compares change in lightness and greening over time.

The component of colour change that changed most in 'control' samples was lightness (see Figure 38 and Figure 39). Using weight data as a proxy for moisture content, we can make inferences about the relationship between surface darkening and moisture. Control samples became darker over the first two weeks of incubation (see Figure 38 and Figure 39, week 1-2) in line with a noticeable increase in weight (see Figure 34) and therefore, inferred moisture content. Beyond this point the surface lightness of control tablets did not change noticeably. All inoculated tablets exhibited progressive darkening over time.

**Figure 39: Changes in absolute values of lightness and greening over time**



*'L\*' represents lightness where a decrease in values indicates darkening over time and 'a\*' represents the 'a' axis of colour where a decrease in values indicates greening over time. The colour of 'Test' tablet symbols represents the average computed 'pseudo' colour for this sample group.*

Interestingly, the increase in darkening on test tablets was not linear over time. The peak values for darkening were recorded at week three of incubation in line with a peak in negative  $a^*$  values (see Figure 39), marking an increase in the green hue of samples. As Figure 39 demonstrates, there was good correspondence between levels of darkening ( $L^*$ ) and greening ( $a^*$ ) in all ‘test’ samples throughout the experimental period.

As discussed total colour change on colonised test samples peaked in the third week of incubation (see Figure 37 earlier). By looking at the constituent components of colour individually, we can attribute the peak in total colour change to corresponding peaks in the negative  $a^*$  (indicating greening), positive  $b^*$  (indicating yellowing) and negative  $L^*$  (indicating darkening) values. These changes in the hue and lightness of colonised samples demonstrate that algal greening is not a linear process over time but rather peak ‘greening-yellowing’ occurred during the third week of incubation after which the strength of these colours and the darkness of the surface decreased slightly (see Figure 39). These subtle fluctuations in the colour of colonised samples after week three of incubation were not discernible through human observation or analysis of digital photography (see Figure 35).

#### 4.1.6 Discussion

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There was limited intra-group variability in ‘control’ and ‘test’ samples over time. However, there was a significant difference between the total colour changes of the two sample groups (inter-group) by the end of the experimental period (see Figure 37). Indeed, ‘test’ samples exhibited an increase in total colour change over time in line with the development of a surface green algal biofilm, as observed through visual inspection and digital photography (see Figure 35).

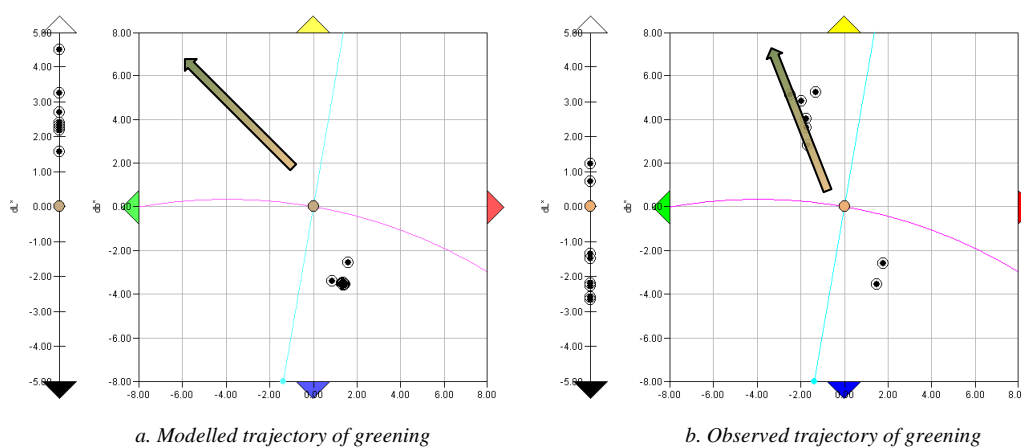
Lightness ( $L^*$ ) was a good indicator of weight increase in ‘control’ samples over time, and therefore inferred moisture contents. However, whilst ‘control’ samples gained more total weight gain than colonised ‘test’ samples (see Figure 34), the latter exhibited greater darkening over time demonstrating the confounding influence ‘algal greening’ has on lightness. It is possible that development of a surface biofilm may result in water retention at the stone surface/ subsurface (Smith et al., 2011) rather than throughout the block, which would not necessarily have an influence on (inferred) total moisture content of the sample. Therefore, an accumulation of surface moisture may result in darkening of the stone surface on algal covered samples.

With regards to colonised ‘test’ samples, the decrease in  $a^*$  values demonstrated an increase in the ‘green’ hue of the surface, as was hypothesised in Table 8. However, movement of colonised samples in colour space demonstrated that colonisation resulted in greater ‘yellowing’ of samples than anticipated (see Figure 40). In their study of granites, Prieto et al. (2002) noted samples became more green-yellow in line with an increase in the number of cyanobacteria cells. They also found that this effect was more pronounced when the organisms were kept in favourable conditions. In the documentation of green biofilms therefore, it appears that green and yellow hues are closely related, with the colour bias being towards yellowing. It is important to note that we were analysing a single-strain biofilm, therefore the yellowing observed may be due to the particular combination of pigments present in the cells of *S. bacillaris*.

As all photosynthetic algae possess different quantities of chlorophyll, pigments and other ancillary substances (McStay et al., 1996), if enough is known about the coloration of particular genera occurring on stone, it may be possible to attribute greening of building

facades to specific groups of microorganisms. For example, in their linked laboratory and field studies, Sanmartín et al. (2012) found changes in  $b^*$  values (yellowness-blueness) to be a good indicator of colonisation by phototrophic cyanobacteria. Their study demonstrates that shifts in the hue of samples over time may have particular importance with regards to identifying early colonisation.

**Figure 40: Testing the modelled trajectory of greening**



Peak colour change in the ‘test’ sample group was observed during the third week of incubation (see Figure 37). This corresponded with a peak in levels of both darkening (negative  $L^*$  values) and greening (negative  $a^*$  values), as depicted in Figure 39. If we assume a link between a peak in these components of colour change and biofilm health, we could relate that this point corresponds to the point of exponential growth or maximum algal yield.

Results demonstrated that there was good correspondence (see Figure 39) between levels of darkening ( $L^*$ ) and greening ( $a^*$ ) in all ‘test’ samples throughout the experimental period. Where specific information about the hue of a biofilm is not required, these results

highlight the potential use of lightness measurements as an independent indicator of surface colonisation (Young and Urquhart, 1999).

Whilst absolute biomass was not quantified in this experiment, comparative studies by Prieto et al. (2004) have demonstrated a good relationship between total colour change ( $dE$ ) and total cell aggregates. Therefore, it is possible that colorimetry could be used as an indirect indicator of the health of a biofilm. This has particular use where traditional methods of visual analysis (human observation or digital photography) may not be able to distinguish subtle changes in the appearance of a biofilm (see Figure 35). This may be due to changes in ambient light levels across seasons and substrate characteristics (e.g. if the lithotype is dark or affected by surface roughness); these are common confounding variables in field analysis of stone facades. For example in their study of cyanobacterial biofilms, Prieto et al. (2002) were able to correlate changes in the metabolic activity of cells with hue shifts. They found that movement in the green-yellow direction indicated healthy growth under moist conditions and a shift towards red-blue demonstrated stressed growth in conditions of enhanced desiccation.

#### 4.1.7 Conclusions

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This study has shown the advantages of portable spectrophotometry in the documentation of green algal biofilm development on sandstone in the laboratory environment. It is particularly useful where traditional photographic methods of observation and characterisation are unable to detect subtle changes in the appearance of a biofilm. Whilst this experiment looked at a single species biofilm on a relatively small sample set, the methods proposed have good potential to be tested further in both the laboratory and field. Part two of this chapter focuses on application of the methodologies to the field

environment.

Future work will compare the relative greening of a number of common green algal species to establish the influence of individual species on the components of colour.

## Part 2: Using spectrophotometry to monitor algal biofilm development in the field environment

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### 4.2.1 Introduction and summary

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Spectrophotometry has been successfully used to monitor green algal growth on sandstone under laboratory conditions. The aim of this experiment was to apply methods tested under laboratory conditions, to a field location. 10 x 10 x 2.5 cm blocks of Dungannon sandstone were exposed on a purpose-built test structure in Derrygonnelly, Northern Ireland for 30 months. Total colour change and the individual components of colour (lightness and hue) demonstrate that colour change does not follow a simple trajectory of change over time. In most cases, samples became yellower-greener over time as observed under laboratory conditions. However, the effect of *aspect* led to differences occurring between samples.

### 4.2.2 Materials and methods

---

Twelve blocks (10 x 10 x 2.5 cm) of Dungannon sandstone were cut, washed with deionized water and dried at room temperature for 24 hours prior to exposure. Samples were taken to the FSC Centre in Derrygonnelly ready for exposure (see Chapter 2, section 2.5). Three samples were screwed vertically to an MDF base (Figure 41) on each face of a purpose-built test structure (Figure 42). The structure has four facades orientated towards North East (NE), South East (SE), South West (SW) and North West (NW). Twenty-four colour measurements were taken of each sample's top face and averaged to calculate a 'mean target' point. This point acts as the baseline reference point for all samples.

**Figure 41: Dungannon samples exposed vertically on test structure**



**Figure 42: Test structure at Derrygonnelly, Northern Ireland**



Colour change was measured using a CM-700d Spectrophotometer (Konica Minolta, Japan) using a 7mm Medium Area View (MAV) aperture, under standard illumination (D65: average daylight) conditions. Colour data was analysed using SpectraMagic™ NX colour data software (Konica Minolta, Japan) as discussed in Part 1 of this chapter. Only ‘Specular Component Excluded’ (SCE) data are presented here, as this measurement excludes gloss effects caused by surface water.

Samples were first exposed on 30<sup>th</sup> April 2010. One sample from each face was removed for analysis at each of the three sampling periods (T1 after 6 months: 29/10/10, T2 after 18 months: 28/10/11 and T3 30 months after exposure: 26/10/12). Prior to removal, 24 readings of surface colour were taken of each sample in situ.

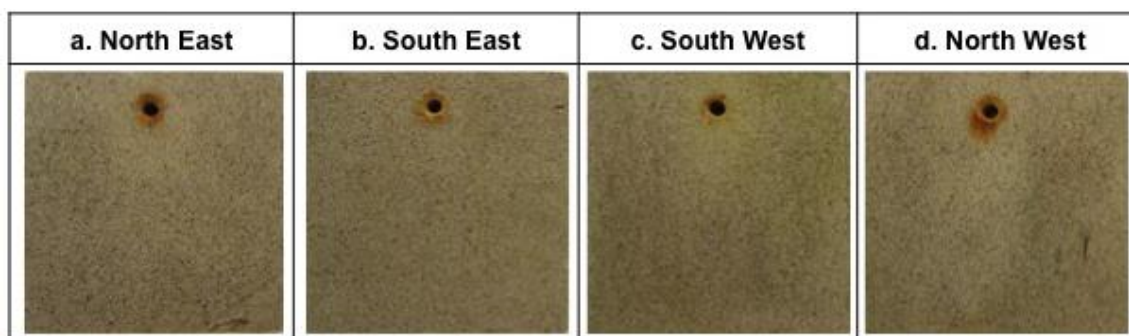
### 4.2.3 Results and Discussion

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Visual observation of samples at each time interval (T1, T2 and T3) highlighted patches of green growth as well as brown hued soiling on all blocks. By 30 months of exposure clear patches of green growth were visible, particularly on the South West face (see Figure 43c- top right corner). Dark brown streaking was visible on blocks exposed on all four faces but was most pronounced on the north North East and North West faces (see Figure 43a and d). Due to the patchy nature of soiling patterns, colorimetry was used to differentiate differences between blocks.

Generally, samples exhibited a similar trajectory of colour change over time as the samples under laboratory conditions, as presented in part 1 of this chapter (see Figure 40).

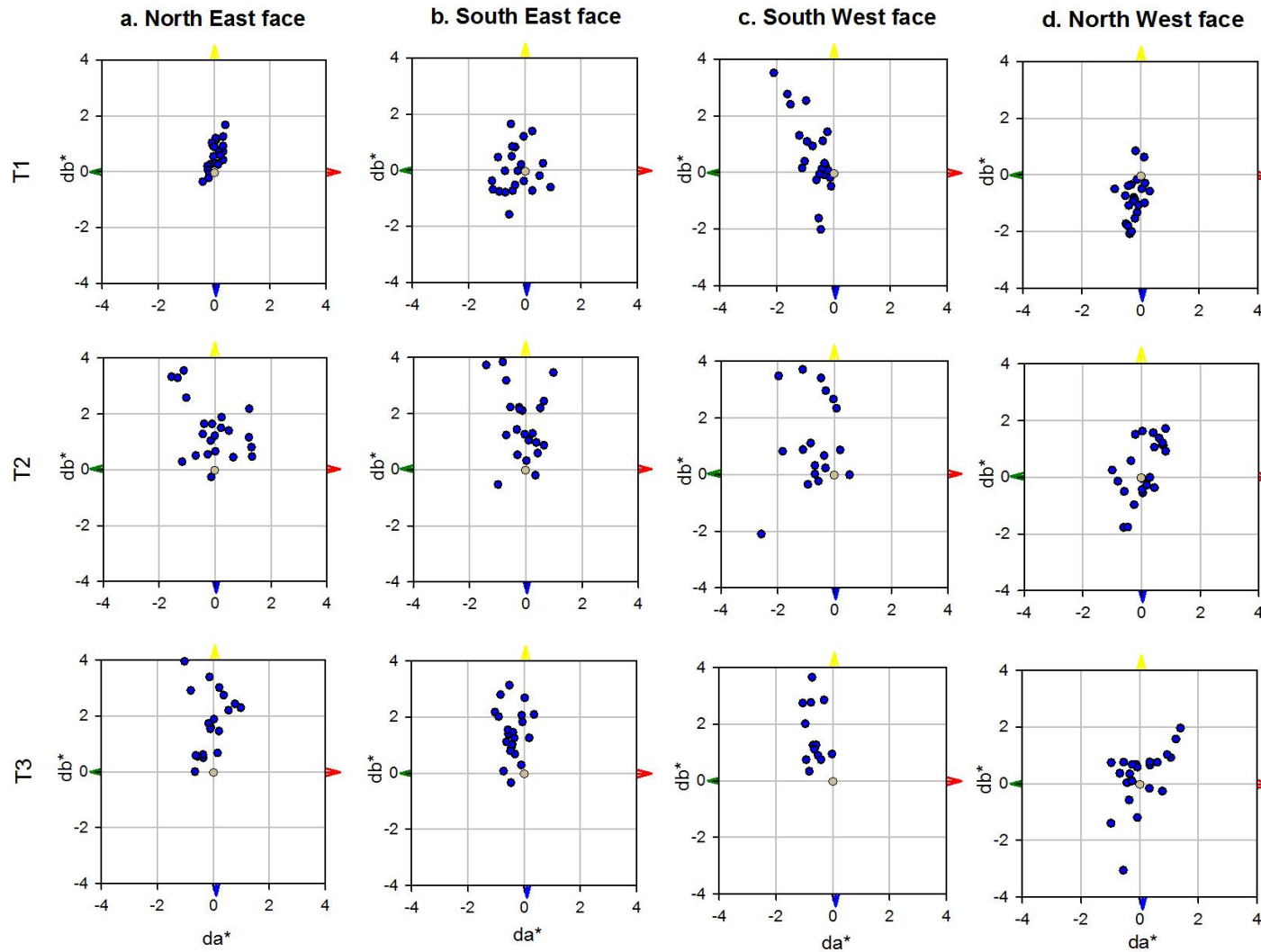
**Figure 43: Dungannon samples after 30 months of exposure**



At T1 there was least variability between individual surface readings on each sample (see Figure 44 T1), with readings clustered around the target point. Greater variability was observed thereafter. For the NE, SE and SW faces points clustered around the green-yellow quadrant of colour space after T2. The NW face displayed a slightly different trajectory of colour change with greater initial movement towards blue and red later. This might indicate growth of other more red hued microorganisms including cyanobacteria (such as the brown-red moisture-loving *Gloeocapsa*) or *Trentepohlia*. Red-brown hued growths have been observed on more shaded and therefore moist facades of buildings in Northern Ireland (see Chapter 7).

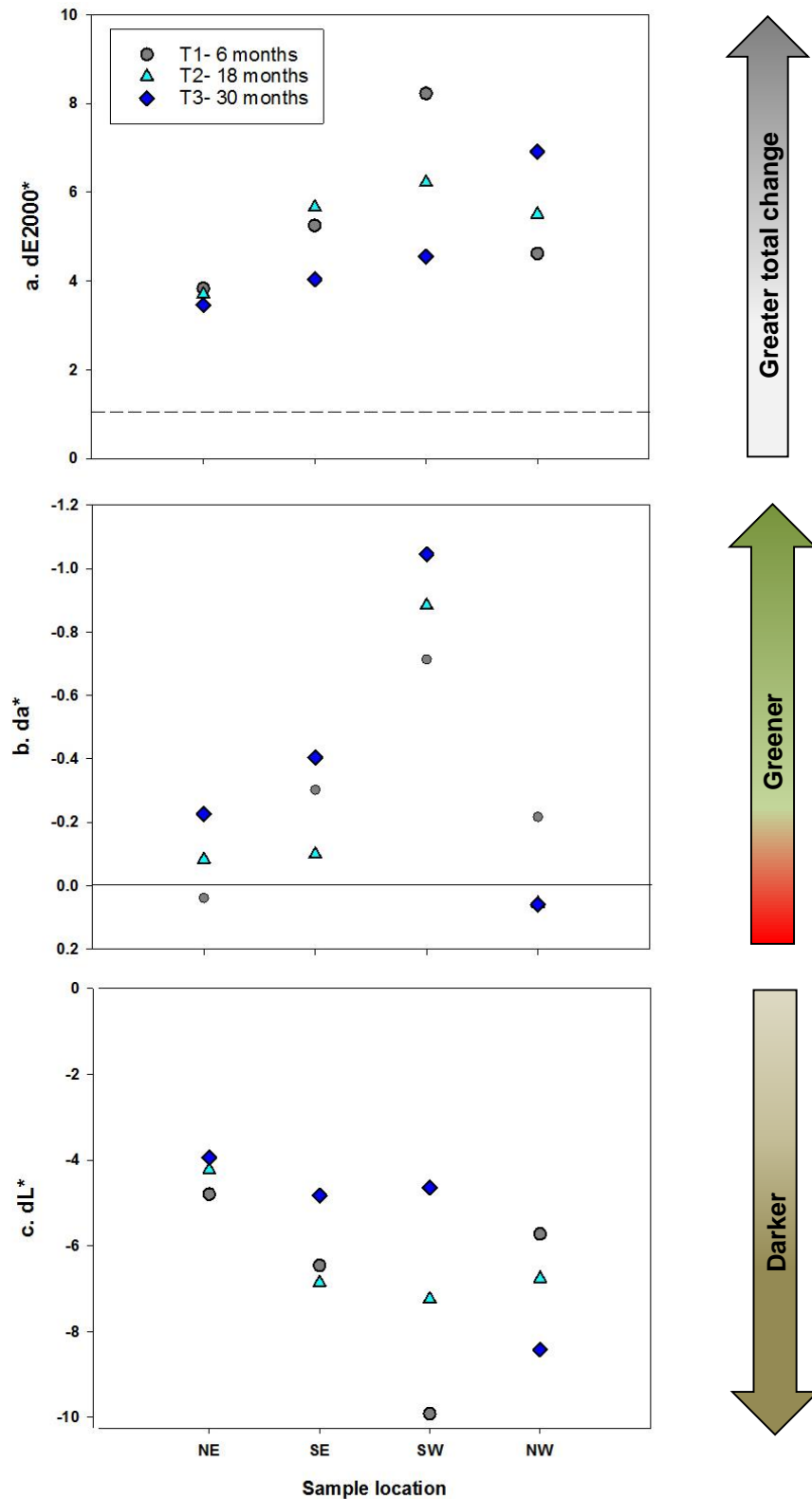
Figure 45 displays changes in three important variables of colour from the baseline point; total colour change (a.  $dE_{2000}^*$ ), greening (b.  $da^*$ ) and lightness (c.  $dL^*$ ). Total colour change is a useful parameter by which to compare the general influence of exposure time and aspect on colour. In all cases, the change that occurred surpassed the 1 CIELAB unit threshold of discernible colour change (as discussed in Part 1 of this chapter).

Figure 44: Movement of samples over time in 2D CIE  $a^*b^*$  colour space



Plots display the 24 colour measurements taken from each sample face at the time intervals T1 (6 months), T2 (18 months) and T3 (30 months). The central point (0,0) represents the averaged baseline target colour and blue points are the change in  $a^*$  and  $b^*$  from this baseline point

**Figure 45: Changes in the components of colour (total colour, greening and lightness) from the baseline point**



Each point represents the mean of the 24 colour readings taken of each sample face.  
**a:** Total colour change (dE2000\*)- higher values indicate greater total colour change from the baseline, dashed line represents discernible colour change,  
**b:** Change in greening (da\*)- negative values indicate greening  
**c:** Change in lightness (dL\*)- Lower values indicate darkening

South West facing samples exhibited the most change over the 30 months of exposure and the North East face the least (see Table 10). In reference to overall colour change, most change occurred within the first 6 months and least change in the final 12 months of exposure (see Figure 45a).

**Table 10: Average change in colour components across 30 months of exposure**

<b>Aspect</b>	<b>dE2000*</b>	<b>da*</b>	<b>dL*</b>
<b>NE</b>	3.7	-0.1	-4.3
<b>SE</b>	5.0	-0.3	-6.1
<b>SW</b>	6.3	-0.9	-7.3
<b>NW</b>	5.7	0.0	-7.0

*Data presented is the average of all data points across each of the sampling periods (T1, T2 and T3)*

Conversely, for the majority of aspects, most greening occurred in the final 12 months of exposure (Figure 45b) with South West facing examples exhibiting the most and the North West face the least greening over the 30 months of exposure (see Table 10). By the end of the experiment, North West facing samples had begun to show signs of reddening (see Figure 44d), as discussed previously.

Laboratory studies have demonstrated a good link between levels of darkening and greening, whereby greener values (higher negative  $da^*$  values) correspond with darker values (see Figure 39). A similar effect was found with field data. The darkness of samples generally increased over time (see Figure 45c) as might be expected due to samples absorbing and retaining more moisture over time leading to darkening of the surface colour.

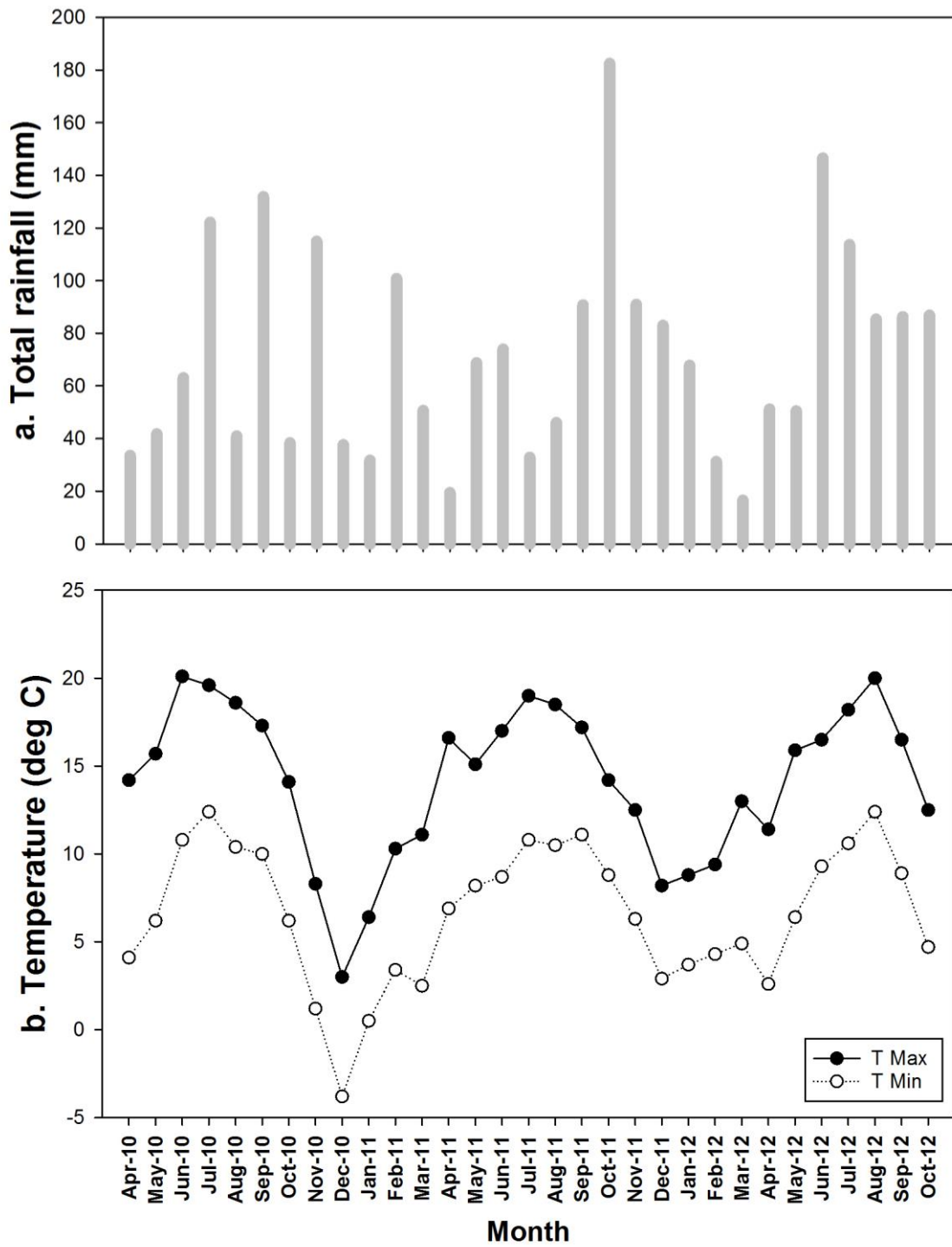
Two- factor ANOVA tests were run to compare the influence of aspect (NE, SE, SW and

NW) and time of exposure (T1, T2 and T3) on each of the three components of colour analysed here ( $dE2000^*$ ,  $da^*$  and  $dL^*$ ).

Exposure time did not have a significant influence ( $p < 0.05$ ) on any of the colour variables. This can in part be explained by climatic conditions. Whilst monthly averages of rainfall (see Figure 46a) indicated some peaks in rainfall in late summer/ early spring of 2010 and at a similar time in 2012, mean monthly values for the sampling periods T1, T2 and T3 all totalled around  $71 \pm 5$  mm (see Table 11). Temperature minima and maxima followed a similar seasonal pattern across the 30 months of exposure (see Figure 46b) apart from a particularly cool December in 2010 (average temperature  $-0.5$  °C). Temperature averages for the sampling periods T1, T2 and T3 (see Table 11) all occurred around  $11 \pm 3$  °C. It is likely that exposure time did not influence the degree of colour change observed on samples due to the minor variations in temperature and rainfall across the three sampling periods (T1, T2 and T3).

Aspect did not have a significant influence on Lightness ( $L^*$ ) or total colour change ( $dE2000^*$ ). However, aspect did have a clear influence on levels of greening ( $da^*$ ) observed (see Figure 45b), where the South West face was found to have significantly lower  $da^*$  values (indicating greening) relative to the other three aspects (see Table 12). This is contrary to the common assumption that greening of northern facades is generally faster and more widespread in comparison to other aspects. Indeed, in exposure trials across Northern Ireland Adamson et al. (2013a) found northern facing blocks became greener faster than south-facing ones. They attributed this to slower drying on northern faces leading to more moist and therefore favourable growth conditions for biological colonisation.

**Figure 46: Mean monthly rainfall and temperature at Lough Navar Field (LNF) station**



*Lough Navar is the nearest meteorological station (approximately 6km North West) to the Derrygonnelly field site. Data downloaded from the Met Office Historical Data, August 2015.*

**Table 11: Average temperature and total monthly rainfall for the sampling periods T1, T2 and T3 at Lough Navar Field station**

Time	Average temperature (°C)	Average rainfall (mm)
T1- 6 months	13.3	72.3
T2- 18 months	9.5	68.4
T3- 30 months	10.0	75.9

*Average temperature- calculated from the mean of monthly temperature minima and maxima values  
Average rainfall- this is the mean total for each month of the sampling period  
Raw data downloaded from the Met Office Historical Data, August 2015*

**Table 12: Two- Way ANOVA- Influence of aspect and time on  $Da^*$**

Source of Variation	SS	df	MS	F	P-value	F crit
Aspect	1.358322503	3	0.452774168	19.35533809	<b>0.001732541</b>	4.757062663
Time	0.048455888	2	0.024227944	1.035704071	0.410776722	5.14325285
Error	0.140356371	6	0.023392728			
Total	1.547134762	11				

In the case of our exposure trial it is likely that prevailing South Westerly conditions provided a good supply of direct rainfall, which in addition to the southern faces receiving greater sunlight, created localised conditions conducive to biological colonisation.

#### 4.2.4 Conclusions

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The field exposure trial in Derrygonnelly demonstrates that aspect influences the degree of soiling (organic and inorganic) observed on Dungannon sandstone blocks. Spectrophotometry was successfully used to quantify differences in the surface colour of blocks highlighting that the greatest total change ( $dE2000^*$ ) and the most greening (negative  $da^*$  values) occurred on South West facing samples. This is likely due to prevailing weather conditions providing a good supply of rainfall.

Conversely, northern facing blocks (particularly North-West facing) exhibited an increase in the red hue and greater darkening of the surface. This may be due to less frequent rain-washing of these faces and or the growth of more shade and damp-loving red/ brown hued cyanobacteria (as have been observed on building facades elsewhere in northern Ireland) or Trentepohlia. The study highlights that aspect has a clear influence on surface colour change and therefore biological colonisation. It emphasises the need for further field studies to explore the dynamic relationship between a sandstone structure (in terms of its geometry and orientation) and microclimatic conditions and the influence this has on biocolonisation of the surface.

Spectrophotometry has good application in both laboratory and field environments to monitor green algal biofilm development. In the laboratory experiment presented in part one of this chapter, the method was successfully used to monitor progressive greening of Dungannon sandstone tablets over 35 days of incubation in a growth cabinet. Peak greening ( $-da^*$ ) values of around -3 CIELAB units were recorded on inoculated tablets by the end of the experiment. In the field exposure experiment presented in part two, spectrophotometry successfully highlighted peak greening values on South West facing blocks of Dungannon. However, in comparison to incubated tablets, lower peak greening ( $-da^*$ ) values of around -2 CIELAB units were recorded on exposed blocks after 30 months. This demonstrates that values of greening recorded in laboratory experiments may not be directly comparable to field experiments due to accelerated incubation conditions and treatment with single, axenic cultures in the laboratory whereas field experiments rely on natural propagation of surfaces with mixed microbial populations. However, laboratory experiments with single- species treatment allow for more detailed

analysis of the growth patterns of individual species and their relationship with the host substrate. This is of particular importance as natural propagation of stone surfaces is often dominated by pioneering species of green algae. Furthermore, laboratory experiments highlight the bioreceptivity of Dungannon to colonisation by typical epilithic green algal species and give an indication of the likely trajectory of greening that would occur on samples exposed in the field environment over a more prolonged period.

# Chapter 5:

## Salts and algal greening: An experimental study of the influence of salts on the bioreceptivity of sandstone

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### Chapter Summary

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The stone surface is one of the harshest environments for biological growth. Yet recently, sandstone facades in wetter regions of the United Kingdom have experienced a significant increase in surface greening. In particular, there is a widespread phenomenon of patchy colonisation. Observations of building facades in these regions indicate that in some cases, this patterning is associated with salt efflorescence. These salts may occur naturally on the stone surface and within or may be deposited through atmospheric pollution, rainwater and sea water spray in coastal locations. This chapter addresses Objective 3 of the overall thesis (see section 1.9) by investigating the role of salts as an external factor affecting algal greening. Laboratory investigations tested the bioreceptivity of two commonly used building sandstones to colonisation by the freshwater alga *Stichococcus bacillaris*, under different concentrations of artificial sea water treatment. Moderately saline conditions can delay development of the biofilm by  $7\pm 1$  days and lead to distinctly patchy biofilm development. In the absence of salt treatment, blocks experienced rapid and heavy surface colonisation within 28 days. Healthy biofilm development resulted in a significant decrease in surface permeability. This demonstrates that graduated concentrations of sea salt treatment can result in similar patterns of patchy colonisation, to those observed naturally on building facades. This in turn, may affect the surface characteristics of the underlying material.

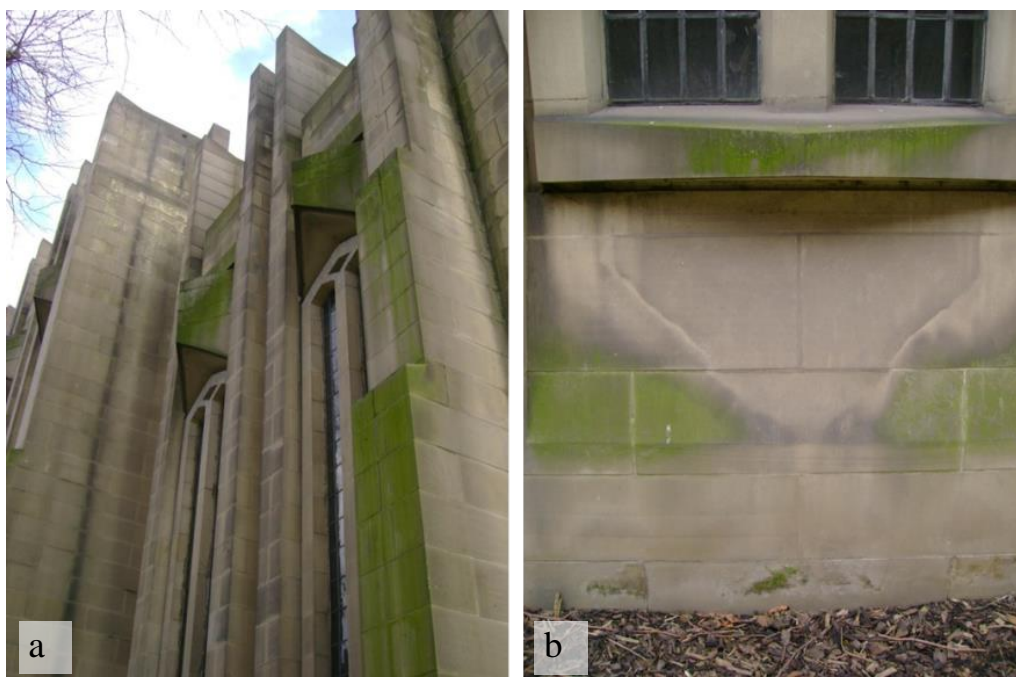
## 5.1 Introduction

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### *Patchy colonisation and observations from the field*

Observations of sandstone facades in the north-west of the UK have shown a wide-spread phenomenon of patchy algal greening. Previous laboratory experiments reported in Chapter 3 have demonstrated that patchy growth is due to single, or more likely, a combination of limiting factors to growth. Whilst moisture is often the primary limiting factor, a combination of naturally occurring salts within the stone or deposited from the environment (e.g. in coastal or polluted regions) can contribute to the variable patterning observed. A pilot study of Sheffield Cathedral (see Chapter 1, Figure 3) revealed distinct patterns of patchy colonisation in and around lines of salt efflorescence (see Figure 47b). This was particularly the case in areas of frequent moisture run-off and accumulation such as around windowsills, plinths and sheltered areas.

**Figure 47: Patterns of patchy colonisation, Sheffield Cathedral, Sheffield**



**a-** West face: Patchy colonisation along run-off lines and areas of moisture accumulation  
**b-** West face: Patchy colonisation in moderated zones, adjacent to areas of salt efflorescence

### *Salt as a limiting factor to growth*

Previous laboratory experiments reported in Chapter 3 have shown rapid algal colonisation of Peak Moor sandstone by *C. vulgaris* in optimum conditions of light, temperature and relative humidity. However, despite ideal conditions for growth, these experiments demonstrated uneven and patchy algal colonisation. Patchy colonisation is often seen on building stone in the natural environment (see Figure 47). Patchy colonisation observed both in the natural environment and in the laboratory is the function of one or a combination of limiting factors to growth. It is frequently debated whether green algal biofilms cause damage to, protect or have no impact upon the underlying stone substrata and in most cases it is not easy to distinguish their exact role, which may change over time (Viles and Cutler, 2012). Given this, understanding why a species colonises one area and not another (often adjacent area), is of key importance.

Salinity has been shown to have varying impacts on epilithic biofilms. For instance, May et al. (1999) documented that even under highly saline conditions, a high bacterial biomass was present on building stone in the Mediterranean. Conversely, Hayward (1974) found that salinity (in particular, sodium chloride treatment) limited the growth of the epilithic green alga *S. bacillaris* in culture. Whilst salinity did not curb growth all together, it resulted in larger cell and longer filament formation. The impact of salts and hypersaline conditions on algal biofilms present on stone substrates is less well understood. Salts are naturally present within a number of common building stones in the UK, are present in rainwater and are naturally deposited on the stone surface in coastal regions.

Chlorophyta must be well adapted to survive at the harsh stone- atmosphere interface. This means a species must be able to tolerate extremes of temperature, humidity, rainfall

and salinity. Indeed, whilst high concentrations of salts may deter algal colonisation, moderate levels have been shown to have a nutritive influence, thereby encouraging colonisation (Silva et al., 2000).

### *Experimental design – choice of algal species*

A number of species known to colonise sandstone monuments in the UK are able to tolerate extreme fluctuations in environmental conditions; thus making them well adapted to life on the stone surface. For example the green alga *Stichococcus* sp. has been documented on stone substrates from the warmer climates of the Mediterranean (Peraza Zurita et al., 2005) to the subzero, dry Antarctic (Meyer et al., 1988); as well as being a common constituent in green algal biofilms in the UK and Ireland (Young and Urquhart, 1998; Rindi and Guiry, 2002).

Species of *Stichococcus* have been documented to be growing on a range of stone monuments in Europe, particularly in the Mediterranean as mentioned (see Gómez-Alarcón et al. (1995); Macedo et al. (2009)). For example, growths of the species have been recorded in Italy (Tomaselli et al., 1999; Tomaselli et al., 2000; Zucconi et al., 2012), Spain (Gómez-Alarcón et al., 1995; Peraza Zurita et al., 2005), Germany (Häubner et al., 2006; Karsten et al., 2007; Gustavs et al., 2010) and Slovakia (Bohuslav, 2008) amongst others. Karsten et al. (2007) have demonstrated *Stichococcus*' general resilience under a wide range of ultra violet radiation treatments in comparison to other green algae. Przytocka and Marzena (1985) attribute its resilience to its filamentous nature, enabling it to attach quickly in a range of habitats.

*Stichococcus bacillaris* was chosen for this experiment because of its tolerance. *S. bacillaris* has been shown to be psychrotolerant and mesophilic (suited to temperate environments) in the natural environment, making it well suited to the British climate. For example, Kirst (1990) highlights its tolerance to saline conditions due to the strength of its cell walls. In addition, it has been shown to increase production of proline and sorbitol solutes under conditions of high salinity (Brown and Hellebust, 1978). These protective osmolytes enable microorganisms to remain metabolically active in the conditions of desiccation and high salt, typical of building stone (Gaylarde and Gaylarde, 2005).

This experiment monitors the growth of *S. bacillaris* (the most common, epilithic species of *Stichococcus*) and its impacts, on two commonly used building sandstones of the UK, when treated with different concentrations of salinity under controlled conditions in an environmental cabinet.

## 5.2 Aim and key questions

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The aim of the experiment was to investigate the role of external factors, specifically saline treatment, in the development of algal greening.

### **Research Questions:**

**R1.** How does salinity affect the appearance and morphology of algal biofilms?

**R2.** What are the mechanisms by which salinity affects colonisation? Do these relate to moisture regimes and water uptake?

**R3.** Are there notable differences in the impact of salt on algal growth between two types of building sandstone (Dungannon and St Bees)?

### 5.3 Materials and Methods

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#### *Stone preparation and salt treatment*

Thirty-two blocks of Dungannon (buff in colour, fine-medium grained) and St Bees (dull red in colour, fine-grained) sandstone were used in this experiment. Full details about the comparative properties of these stones can be found in Chapter 2. The stone was cut into 50 x 50 x 50 mm cubes and then gently washed in de-ionized water to remove any debris from the cutting process, autoclaved, dried and stored (see Figure 9, Chapter 2).

Three concentrations of autoclaved salt solution were used to replicate 0.1%, 1% or 10% strength North Sea water. For each concentration, the salt solution (Seawater Corrosion Test Mixture kit, product no. 331533P, VWR International Ltd., Poole) was weighed out and diluted with distilled water, as required. Full contents of the mixture can be found in Appendix 3. Solutions were bottled, heated in a water bath at 20°C for two hours and mechanically shaken intermittently to ensure all solute had dissolved before being autoclaved at 121°C for 20 minutes. Mixed solutions were refrigerated until required and brought to room temperature before use.

Blocks were totally submerged and soaked for 24 hours in either autoclaved de-ionized water or one of the three prepared salt solutions (see Table 13 for sample set-up). Three replicate blocks of each stone type were used per treatment, as well as 1 control per

treatment type. Control blocks were treated with salt solution but were not inoculated with green algae. All blocks were air-dried for 48 hours.

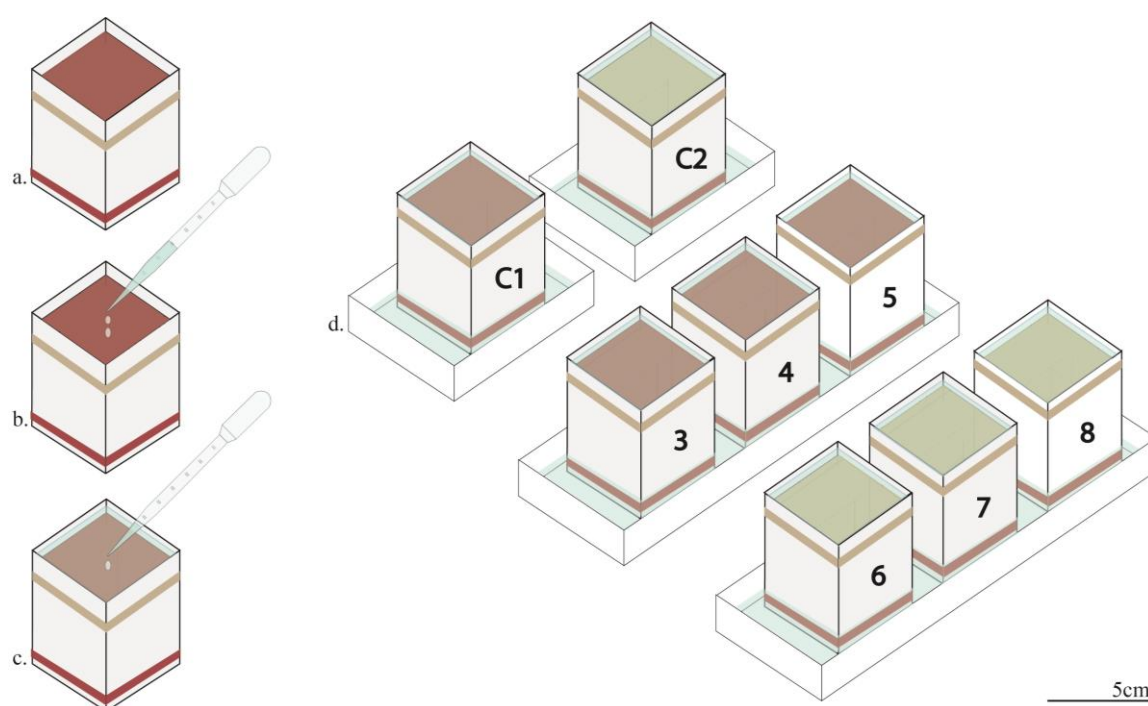
Table 13: Sample labelling

Salt treatment group	Sample code	Sample type	Stone type
0% (Distilled water only)	C1	Control	St Bees
	C2	Control	Dungannon
	R3	Replicate	St Bees
	R4	Replicate	St Bees
	R5	Replicate	St Bees
	R6	Replicate	Dungannon
	R7	Replicate	Dungannon
	R8	Replicate	Dungannon
0.1%	C9	Control	St Bees
	C10	Control	Dungannon
	R11	Replicate	St Bees
	R12	Replicate	St Bees
	R13	Replicate	St Bees
	R14	Replicate	Dungannon
	R15	Replicate	Dungannon
	R16	Replicate	Dungannon
1%	C17	Control	St Bees
	C18	Control	Dungannon
	R19	Replicate	St Bees
	R20	Replicate	St Bees
	R21	Replicate	St Bees
	R22	Replicate	Dungannon
	R23	Replicate	Dungannon
	R24	Replicate	Dungannon
10%	C25	Control	St Bees
	C26	Control	Dungannon
	R27	Replicate	St Bees
	R28	Replicate	St Bees
	R29	Replicate	St Bees
	R30	Replicate	Dungannon
	R31	Replicate	Dungannon
	R32	Replicate	Dungannon

In previous laboratory studies (see Chapter 3) we found application of waterproof tape effective in minimising water ingress and regress from the side faces of sample blocks. We also left a tape overhang on the top surface of blocks to minimise run-off of inoculant and water prior to and during the experiment.

Therefore each test block in this study was wrapped tightly on four faces (leaving a small 3mm overhanging lip) with white waterproof tape (UniBond extra strong power tape, Henkel Ltd, Cheshire) following sterilisation and air-drying. Sterile rubber bands were then applied to the top and bottom of blocks to ensure tape adhesion and to minimise water ingress from the side faces of each block (see Figure 48a).

**Figure 48: Treatment procedure and sample group set-up**



- a. Stone blocks were wrapped in tape on four side faces and tied with rubber bands at the top and bottom*  
*b and c. Inoculation: blocks were inoculated with 2ml of *S. bacillaris* suspended in growth medium*  
*d. Treatment group set-up: blocks were placed in sterile plastic trays filled with autoclaved distilled water*

Once prepared, all blocks were partially submerged in autoclaved de-ionized water (to a depth of one centimetre) for a period of 12 hours at room temperature. This was to ensure a base level of moisture to encourage development of fledgling algal cells. Blocks were then weighed, photographed and base measurements of surface moisture collected. This

formed the 'wet baseline' reference point.

### *Stone inoculation*

The top face of each replicate block was inoculated with 2 ml of *S. bacillaris* suspended in growth medium (see Figure 48b and c) according to the procedure outlined in Figure 12. Control blocks were not inoculated but treated with the same volume of autoclaved distilled water. Blocks were positioned in trays of autoclaved distilled water to allow a constant supply of moisture via capillary rise; water was refilled every two days.

The depth of water was always maintained at one centimetre. Blocks were placed in separate trays according to stone type. Control blocks were placed in individual trays to ensure sterility and minimise cross-contamination (see Figure 48d). Trays were placed in a climatically controlled MLR-352H Plant Growth Cabinet (Panasonic, UK) set to replicate temperate climatic conditions; constant relative humidity of  $85 \pm 5\%$ ,  $20 \pm 1^\circ\text{C}$  temperature and cycled between 8 hours of dark and 16 hours of illumination. Blocks were moved around in their trays and the position of each tray in the growth chamber was alternated daily. Trays were scrubbed of debris and any algal growth and sterilised weekly before refilling with water and replacing blocks. Samples were incubated for a period of 35 days.

### *Stone assessment*

The moisture contents of the stone blocks were assessed using two methods throughout the experiment (weight change and Protimeter) as in Chapter 3. Firstly, all samples were weighed (using a 2d.p balance with  $\pm 0.001\text{g}$  error) prior to initial algal treatment and subsequently every seven days under the same ambient laboratory conditions. For a

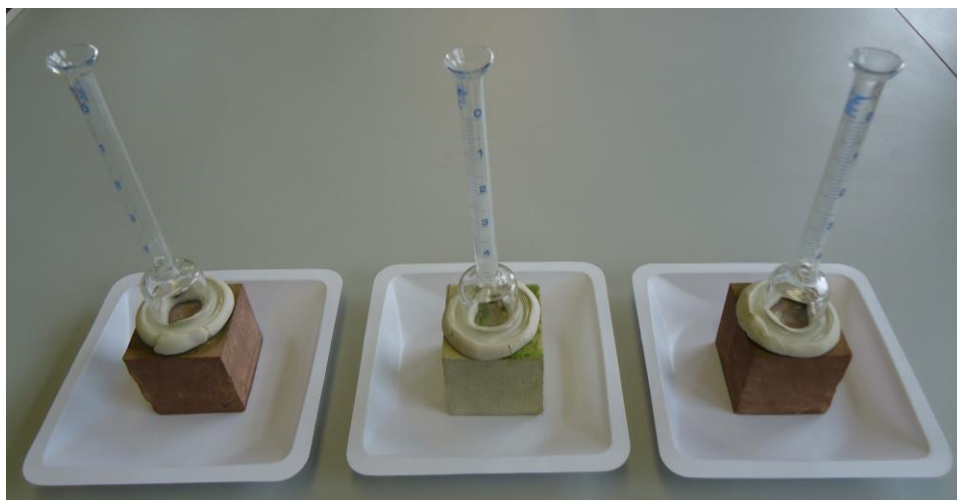
subset of samples (only those inoculated with pure water), surface moisture was also monitored weekly with a hand held moisture meter (Protimeter Surveymaster moisture meter, GE, London) in 'resistance' mode. Only untreated (0%) samples were measured as resistance is known to be affected by salinity (Eklund et al., 2011). For the Protimeter measurements, nine measurement points were taken across the top face as in previous laboratory experiments (see Figure 20).

In order to evaluate algal growth and other changes such as crystallisation of salts, the top exposed face of every sample was photographed (with a fixed tripod and constant light source set-up) every seven days. Photographs were used to quantify percentage algal cover and density of colonisation (used as proxies for biomass) using the method discussed in section 2.6, Chapter 2.

Water penetration (standard 10 minute vertical karsten tube tests, see Figure 49) was assessed at the end of the experimental period. Tape and rubber bands were removed from all samples. Samples were then dried in a drying cabinet at a constant 20° C for 24 hours before the first test. Water penetration capacity of the top, bottom and a single side face of all blocks was measured for comparison. Samples were dried in the manner above for 24 hours between each test.

Chip samples were taken from a subsample of blocks and prepared for anion extraction. Chips were removed, ground, immersed in water and the effluent filtered and analysed in a Dionex IC DX500 Chromatograph.

**Figure 49: Set-up of Karsten Tubes for permeability testing**



A chip was removed from the surface centre (*a*), surface outer edge (*b*) and the internal block centre (*c*). The subsample of blocks included two controls (one of each stone type) and two replicates (one of each stone type) for each treatment group, totaling 16 samples.

Optical microscopy (using a Leica MZ10F stereomicroscope) was conducted on a subset of samples (all controls and two replicates per treatment group) prior to inoculation and at the end of the experiment whilst samples were still moist.

### *Statistical analysis*

A range of statistical techniques was used to evaluate any significant differences in algal cover between stone types and treatment types over the course of the experiment, including T-tests, ANOVA and Mann Whitney U tests (depending on whether pairwise or multiple comparisons were needed, and whether the data were normally distributed or not). The details of tests used for each dataset and corresponding results tables are presented in the following section.

## 5.4 Results

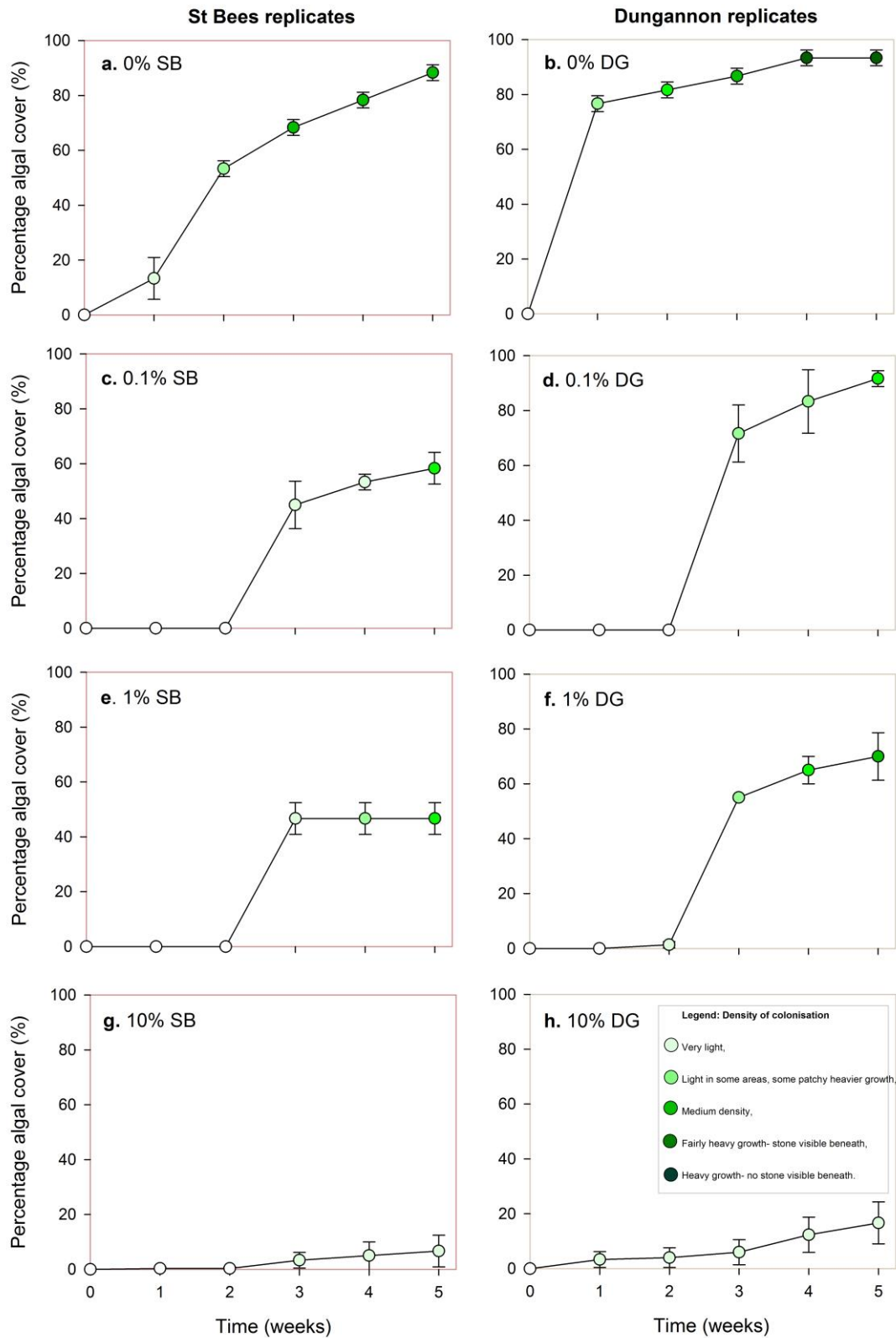
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### *How does salinity affect algal growth on Dungannon and St Bees sandstone blocks?*

Figure 50 illustrates the increase in biomass (as estimated by % algal cover) over time during the 5-week experiment. Untreated (0% group) samples of both stone types showed clear increases in percentage algal cover and density over time. In comparison to St Bees, Dungannon samples exhibited faster initial growth with almost 80% algal cover at the end of the first week (in comparison with less than 20% cover on St Bees). The difference in algal cover at week one between the two stone types was statistically significant (t-test:  $t(4) = -3.881$ ,  $p = 0.018$ ). However, by week five a similar level of around 90% algal cover at 'fairly heavy' density was observed on both stone types for the untreated (0% group) samples (see Figure 50a and b). This difference was not significant (Mann-Whitney test:  $U(4) = 1.000$ ,  $p = 0.200$ ).

For all samples treated with saline solution, initial algal growth was delayed by at least seven days (see Figure 50c-h) in comparison with the 0% group. The percentage cover and density of colonisation was inversely related to strength of saline treatment. That is, the higher the salt concentration the less algal biomass observed. Using algal cover data at week five, a two-way ANOVA test indicated significant interaction between treatment (salt concentration) and stone type (see Table 14). Therefore, to determine the pattern and magnitude of the interaction, pairwise comparisons were conducted (see Table 15). The difference in algal cover between untreated St Bees and Dungannon samples was not significant. However, significant differences did occur between stone types when treated with salt solution (see Table 15a). This indicates that salt treatment was more/ less effective in inhibiting growth dependent upon stone type.

**Figure 50: Averaged biomass change over time on St Bees and Dungannon replicates**



*Symbol positions indicate the averaged (mean) figure for algal cover for all treatment groups (0, 0.1, 1 and 10%). In all instances, controls remained free of colonisation so are not presented here. Error bars represent standard deviation from the mean. Symbol colours represent the average (mode) 'density of colonisation' for each sample group, per week.*

Table 14: Algal cover at week 5- ANOVA results

Two way ANOVA					
Normality Test (Shapiro-Wilk)	Passed			(P = 0.369)	
Equal Variance Test:	Passed			(P = 1.000)	
Source of Variation	DF	SS	MS	F	P
Treatment (% salt)	3	21061.458	7020.486	217.409	<0.001
Stone type	1	1926.042	1926.042	59.645	<0.001
Treatment (% salt x Stone type)	3	744.792	248.264	7.688	0.002
Residual	16	516.667	32.292		
Total	23	24248.958	1054.303		

ANOVA indicated significant interaction (0.002) between factors (stone type and salt concentration)

Table 15: Algal cover at week 5- all pairwise multiple comparison procedures (Tukey Test)

a. Stone type and concentration comparison- Dungannon vs. St Bees				
Concentration	Diff of mean	p	q	P
0%	5	2	1.524	0.297
0.1%	33.333	2	10.16	<0.001
1%	23.333	2	7.112	<0.001
10%	10	2	3.048	0.047

b. Salt concentration comparison for St Bees				
Concentration	Diff of mean	p	q	P
0 vs. 10%	81.667	4	24.892	<0.001
0 vs. 1%	41.667	4	12.7	<0.001
0 vs. 0.1%	30	4	9.144	<0.001
0.1 vs. 10%	51.667	4	15.748	<0.001
0.1 vs. 1%	11.667	4	3.556	0.096
1 vs. 10%	40	4	12.192	<0.001

c. Salt concentration comparison for Dungannon				
Concentration	Diff of mean	p	q	P
0 vs. 10%	76.667	4	23.368	<0.001
0 vs. 1%	23.333	4	7.112	<0.001
0 vs. 0.1%	1.667	4	0.508	0.984
0.1 vs. 10%	75	4	22.86	<0.001
0.1 vs. 1%	21.667	4	6.604	0.001
1 vs. 10%	53.333	4	16.256	<0.001

Two-way ANOVA indicated significant interaction (<0.002) between factors (salt concentration and stone type). Therefore Tukey Test conducted to explore pairwise comparisons.

For 0.1% and 1% treatment, Dungannon samples exhibited significantly more widespread and denser colonisation than St Bees samples ( $P < 0.001$ ). As Figure 50 demonstrates, at five weeks of incubation St Bees samples treated with 1% saline solution reached peak growth at around 50% surface cover with 'light, patchy growth' (see Figure 50e). For untreated samples, this compared with 90% cover at 'fairly heavy' density at the same point in time (see Figure 50a). When comparing the same treatment groups, there was a smaller difference of around 30% in Dungannon samples (see Figure 50b and f).

For the 10% treatment groups, the difference in algal cover between the two stone types was less significant ( $P = 0.047$ ), but still significant at the 0.05 the cut-off point. This might indicate a threshold effect whereby after a certain level of salt treatment, stone type becomes unimportant. This may be because the conditions become too inhospitable for algae to grow irrespective of substrate characteristics (see Figure 50g and h). For both stone types, colonisation remained light and patchy and reached a peak of under 20% cover by five weeks (see Figure 50g and h).

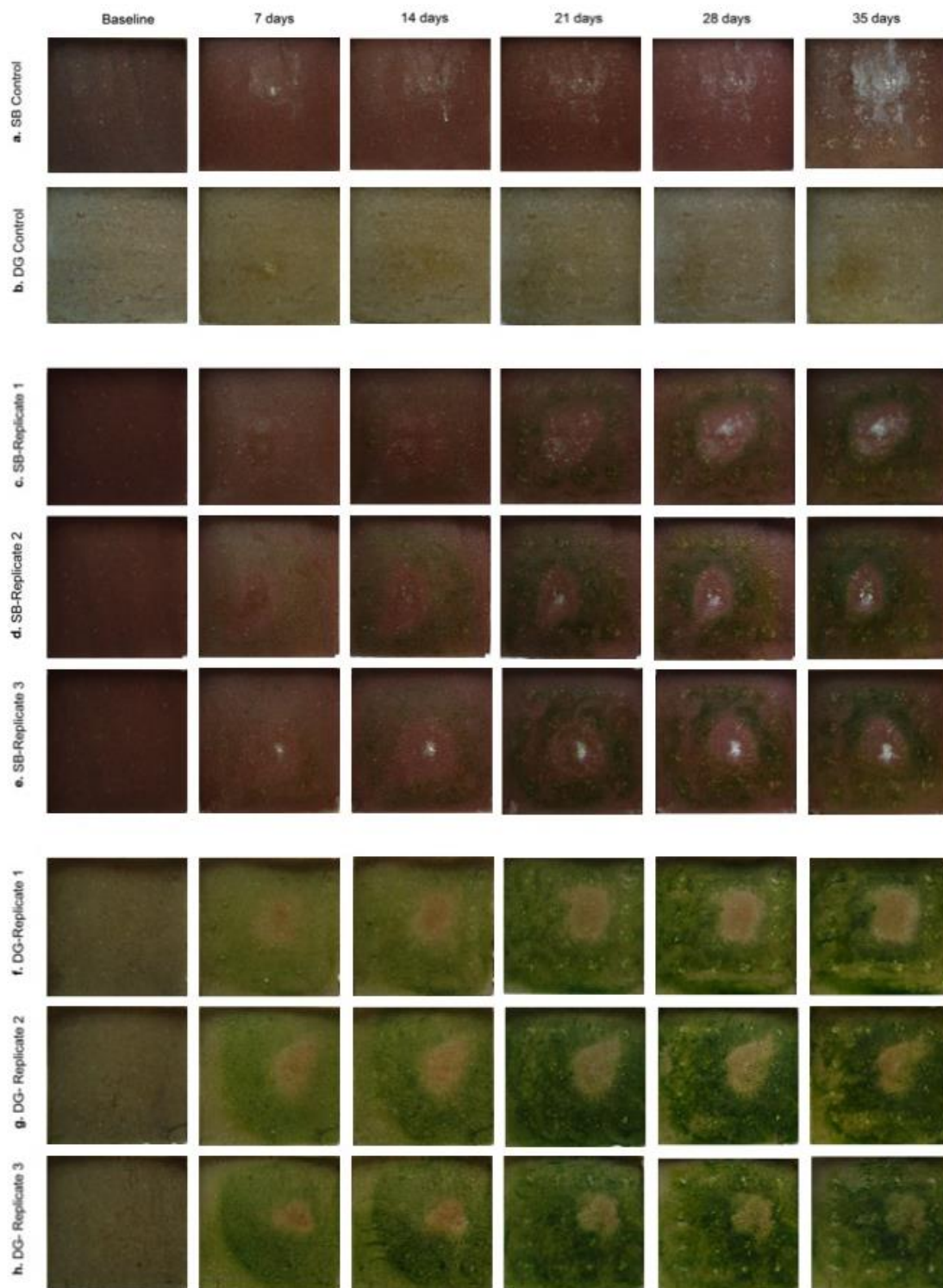
#### *How does salinity affect biofilm morphology on Dungannon and St Bees blocks?*

Figure 51 presents a summary of photographic observations of the appearance of control and 0% salinity groups over the course of the 5-week experiment. Figure 51a and b illustrate that all control samples remained free of algal colonisation over the entire experiment. However, mineral efflorescence was visible on the central surface of these samples within the first week of incubation. The volume of this efflorescence increased over time on both Dungannon and St Bees blocks but was much more visible on the latter (see Figure 51a and b).

Biofilm morphology (shape and texture) on inoculated (0% salt treatment) samples (see Figure 51) was similar to that observed in previous chapters, under ideal incubation conditions (see Chapter 3). Ring-shaped biofilms were observed on all blocks by seven days but were more pronounced on the lighter, Dungannon stone (see Figure 51f-h). In all cases, the central surface zone remained free of colonisation. This is likely due to reduced moisture movement in this zone and an accumulation of growth inhibiting minerals, deposited on the surface as efflorescence (see section 3.5, Chapter 3). The spread and density of algal growth was greater on Dungannon blocks than St Bees. Colonisation appeared more luxuriant and healthy. By 35 days of incubation, EPS and trapped moisture was visible on samples of both stone types but particularly on Dungannon samples (see Figure 51c-h).

Figure 52 illustrates the impacts that salt treatment had on biofilm morphology. Salt treatment had a clear inhibiting effect on the spread, density and overall health of biofilms. For both 0.1% and 1% salt treatment, growth was delayed by at least seven days in comparison to untreated samples (see Figure 52b and c). As on the untreated samples, concentric ring-shaped biofilms were observed on samples treated with 0.1% and 1% salt solution. The outline of these biofilms was much more pronounced with higher salt concentration (see Figure 52c). Biofilms on blocks treated with 1% salt solution appeared desiccated with visibly less deposition of EPS than blocks treated with no or lower concentrations of salt. A few areas of higher density colonisation were observed on these blocks, likely in more hospitable regions (e.g. around mineral grains, areas of greater surface roughness or pockets of lower salt deposition).












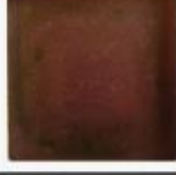




























**Figure 51: Change in visual appearance of 0% (no salt) treatment group**



*Photographs of the top face of samples taken at the 'wet baseline' point and subsequently every 7 days. Photographs taken under consistent light and with tripod set-up.*

*a and b: control samples  
c- h: treated sample replicates*

Figure 52: Biofilm development after 35 days of incubation (all groups)

	Sample code	7 days	14 days	21 days	28 days	35 days
<b>d. 10%</b>	R27					
	R30					
<b>c. 1%</b>	R19					
	R22					
<b>b. 0.1%</b>	R11					
	R14					
<b>a. 0%</b>	R3					
	R6					

*The repeat weekly photographs presented here are of a subsample of representative replicates within each treatment group*

Minimal growth was observed on samples of both stone types treated with 10% salt solution. Where growth was visible, this was restricted to the outer perimeter of blocks. Growth was more inhibited on St Bees blocks than Dungannon (see Figure 50g-h and Figure 52d).

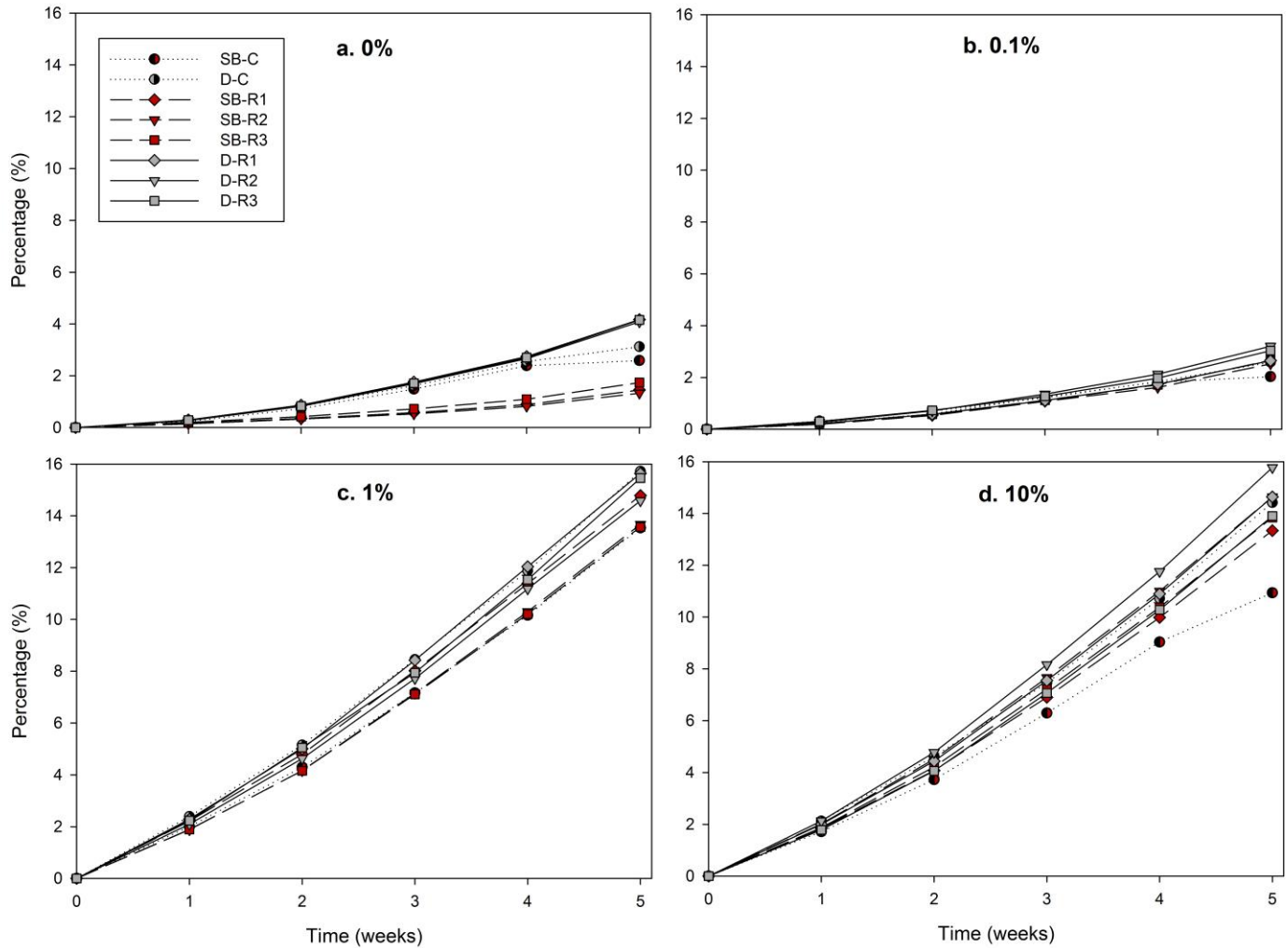
### *Weight*

Weight change was used as a proxy measurement of changing moisture contents of the blocks during the course of the experiment. Figure 53 summarises the results, showing that the weight of all blocks increased by an average of 4% over five weeks, indicating that all blocks absorbed moisture over time.

For the majority of sample sets (see Figure 53a-d), there was little observable difference in the pattern of weight gain between controls and algal-colonised replicates. This implies that algal cover does not significantly affect moisture uptake or retention. Therefore, controls and inoculated sample replicates were pooled for statistical analysis. ANOVA results indicated significant differences in weight gain between stone types and between samples treated with different concentrations of salt. The interaction of the two variables was not significant (when  $p = 0.05$ ) indicating that the effects of stone type and salt treatment on weight gain were independent from each other (see Table 16).

By week five of the experiment, Dungannon blocks had gained significantly more weight (an average of 3.2% more) than St Bees blocks (see Table 17a). This reflects differences in characteristics such as porosity between the two stone types (see section 2.2, Chapter 2).

Figure 53: Cumulative percentage weight change over time



*Weight change is calculated from the 'wet baseline' point and graphed cumulatively.*

Table 16: Weight at week 5- ANOVA results

Two way ANOVA					
Normality Test (Shapiro-Wilk)		Passed		(P = 0.249)	
Equal Variance Test:		Passed		(P = 0.488)	
Source of Variation	DF	SS	MS	F	P
Stone type	1	5.937	5.937	5.942	0.023
Salt concentration	3	1065.149	355.05	355.34	<0.001
Stone type x Salt conc.	3	1.664	0.555	0.555	0.65
Residual	24	23.98	0.999		
Total	31	1096.73	35.378		

ANOVA indicated significant differences in weight gain between the two stone types ( $p=0.023$ ). Salt concentration had a significant effect on weight gain ( $p<0.001$ ). The effect of stone type and salt concentration were independent from each other ( $p=0.65$ ).

Table 17: Weight at week 5- all pairwise multiple comparison procedures (Student- Newman- Keuls Method)

a. Stone type comparison- Dungannon vs. St Bees			
Diff of mean	p	q	P
0.861	2	3.447	0.023

b. Salt concentration comparison				
Concentration	Diff of mean	p	q	P
0 vs. 0.1	0.163	2	0.461	0.747
1 vs. 0.1	11.951	4	33.818	<0.001
10 vs. 0.1	11.268	3	31.883	<0.001
1 vs. 0	11.788	3	33.357	<0.001
1 vs. 10	0.684	2	1.935	0.184
10 vs. 0	11.105	2	31.422	<0.001

Pairwise comparisons conducted to determine direction of differences

a- Dungannon samples gained significantly more weight than St Bees,

b- Based on comparison outcomes, the order of weight gain for the different concentrations is:  
 $0\%=0.1\%<1\%=10\%$

Figure 53 also indicates differences in moisture changes between the different salt treatment groups. For example, the pattern of weight gain in samples treated with 0% salt solution was moderate and steady; this was echoed by samples treated with 0.1% (see

Figure 53a and b). Samples treated with 1% and 10% behaved in a similar way to one another with more rapid weight gain over time than samples treated with the lower concentrations (see Figure 53c and d).

Based on pairwise comparisons, the difference in weight gain between the two lower and two higher concentrations was significant where  $0\% = 0.1\% < 1\% = 10\%$  (see Table 17b). A threshold effect seems to occur at 1% where a lower concentration may lead to reduced weight gain and a higher concentration may result in no change.

### *Surface moisture*

Further information on moisture characteristics of the blocks within different treatment groups can be gained from Protimeter measurements. As measures of resistivity are affected by salinity (see section 2.6, Chapter 2) only data from the 0% treatment group and sterile (untreated controls) blocks are presented here (see Figure 54). The pattern of moisture distribution was broadly similar between controls (not inoculated with algae) and replicates (see Figure 54). Of the colonised replicate blocks (see Figure 54c-h), Dungannon blocks showed greater variability in moisture distribution than St Bees. However, a significant difference in average surface wetness between the two stone types was not detected (Two tailed T-test:  $t(4) = -1.775$ ,  $p = 0.151$ ).

After 5 weeks of incubation, the highest resistance readings for all blocks occurred in the central zone. This may be indicative of a central zone of greater surface wetness or may be due to an accumulation of salt. Mineral efflorescence was particularly visible on the darker St Bees samples (see Figure 55a and c-e).

Figure 54: Surface moisture distribution of the 0% (no salt) treatment group

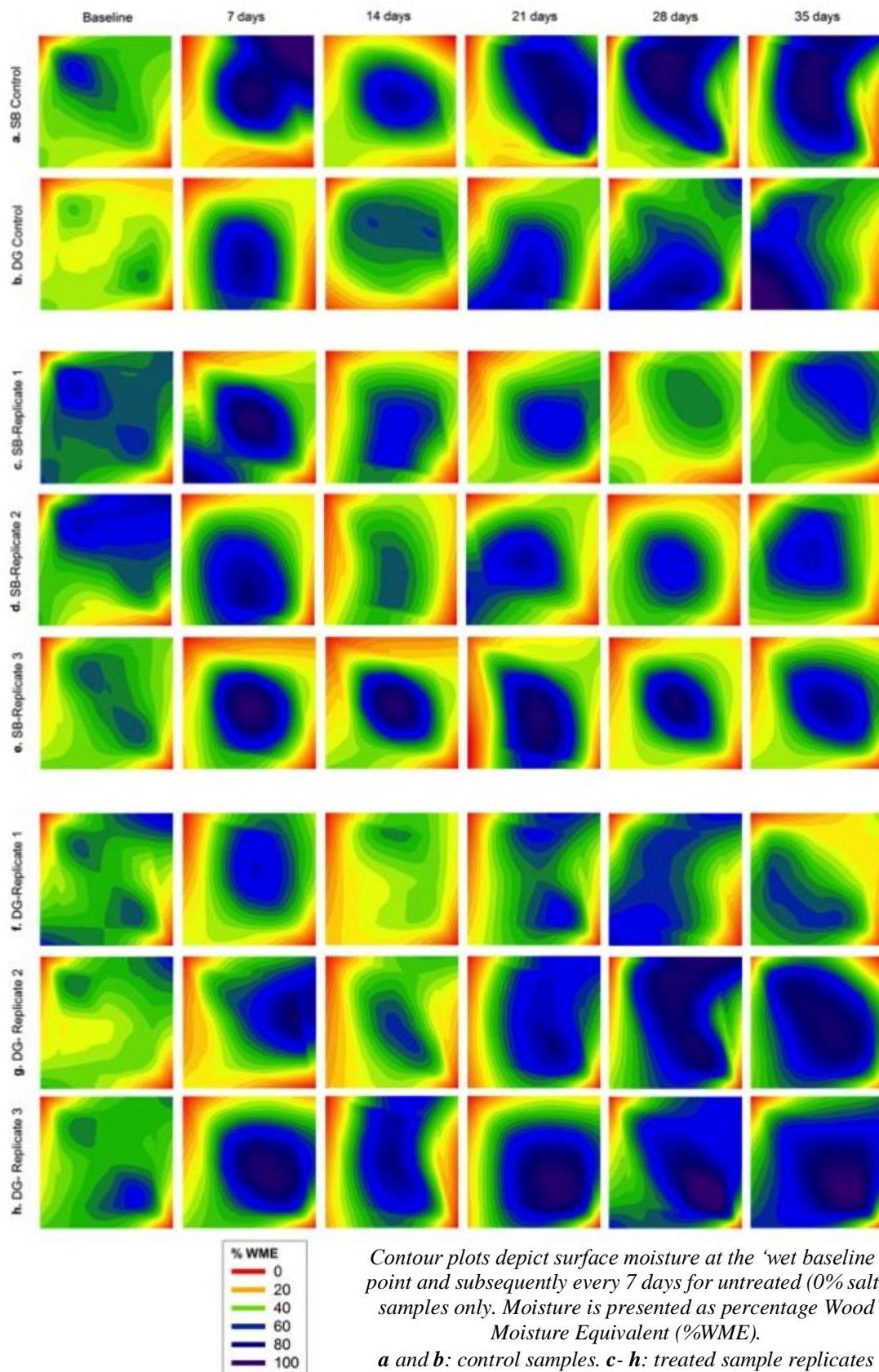
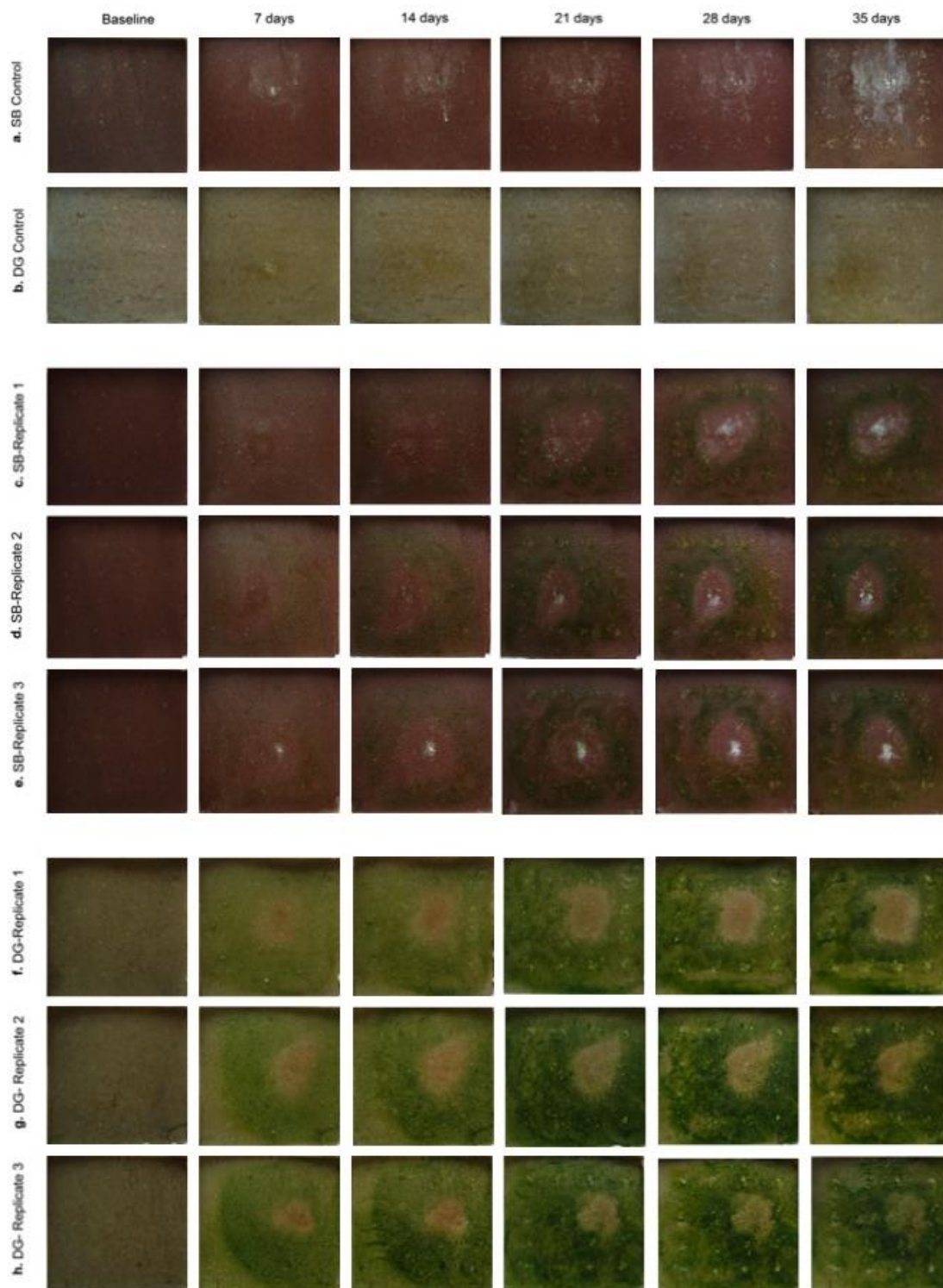


Figure 55: Change in visual appearance of 0% (no salt) treatment group



*Photographs of the top face of samples taken at the 'wet baseline' point and subsequently every 7 days. Photographs taken under consistent light and with tripod set-up.*

*a and b: control samples  
c- h: treated sample replicates*

Mineral efflorescence in the centre of the majority of blocks was probably due to the cycle of moisture upwelling and drying at the surface during incubation. Similar mineral deposits have been found in previous experiments (see Chapter 3).

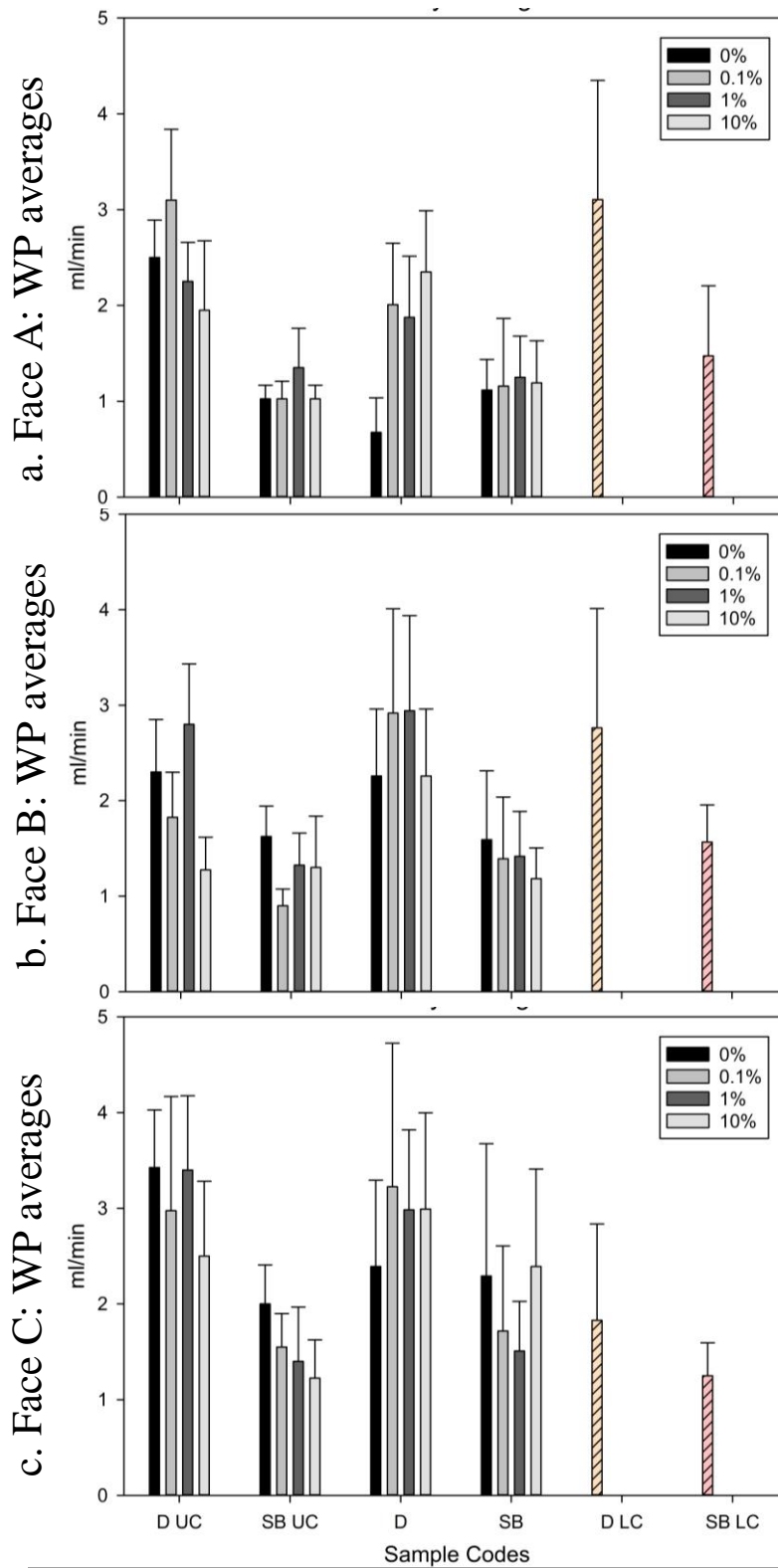
By week 5, patterns of colonisation could be roughly correlated with those of surface moisture distribution. Of the inoculated blocks (see Figure 55c-h), heaviest colonisation occurred in the moderate moisture region of 40-60% WME. Similar results have been found in previous experiments (Chapter 3).

### *Water penetration*

Vertical Karsten tubes were used to see if any differences in water penetration occurred between colonised and bare blocks and also, to compare differences in water penetration between the various salt treatment groups. The water penetration capacity of three faces (A-top exposed face, B- tape covered side face and C- bottom face) of each block was measured. Measurements were conducted at the end of the incubation period, after blocks had been dried (see Methodology section).

Figure 56 illustrates the water penetration characteristics of the different treatment groups. A three-way ANOVA test was run to assess whether permeability differed between stone type, salt concentration and block face using all data apart from laboratory controls (due to the uneven sample number). Whilst the normality test was not passed, balanced three-way ANOVA tests are rigorous (see Table 18). We have also adopted a conservative p-value of 0.01 in our analysis of pairwise comparisons to account for this.

Figure 56: Influence of salt concentration and algal greening on water penetration (WP)



Bar graphs display mean data and standard deviation for each sample group: **D UC** (Dungannon Untreated Control), **SB UC** (St Bees Untreated Control), **D** (inoculated Dungannon blocks), **SB** (inoculated St Bees blocks), **D LC** (Dungannon Lab Control) and **SB LC** (St Bees Lab Control)

Table 18: Water penetration variables- ANOVA results

Three way ANOVA					
Normality Test (Shapiro-Wilk)	Failed		(P < 0.050)		
Equal Variance Test:	Passed		(P = 0.499)		
Source of Variation	DF	SS	MS	F	P
Salt treatment	3	0.639	0.213	0.64	0.593
Stone type	1	14.806	14.806	44.498	<0.001
Face	2	11.919	5.96	17.911	<0.001
Salt treatment x Stone type	3	3.465	1.155	3.471	0.023
Salt treatment x Face	6	3.748	0.625	1.877	0.104
Stone type x Face	2	1.489	0.745	2.238	0.118
Salt treatment x Stone type x	6	1.326	0.221	0.664	0.679
Residual	48	15.971	0.333		
Total	71	53.364	0.752		

Significant interaction noted between factors, particularly salt concentration and stone type (0.023)

Stone type and face had significant p-values, but cannot be interpreted individually. However, significant interaction was detected between variables. Pairwise comparisons help to determine which interactions are significant (see Table 19a and b) and plotted data (averaged data for each sample group) to determine the direction of significance (see Figure 56).

Table 19: Water penetration- all pairwise multiple comparison procedures  
(Student- Newman- Keuls Method)

a. Block face comparison				
Face	Diff of mean	p	q	P
C vs A	0.984	3	8.36	<0.001
C vs B	0.357	2	3.034	0.037
B vs A	0.627	2	5.326	<0.001

b. Stone type and concentration comparison Dungannon vs. St. Bees				
Concentration	Diff of mean	p	q	P
0%	0.181	2	0.939	0.51
0.1%	1.294	2	6.732	<0.001
1%	1.208	2	6.284	<0.001
10%	0.944	2	4.912	<0.001

Three-way ANOVA indicated significant interaction between factors (salt concentration and stone type). Therefore S-N-K Method conducted to explore pairwise comparisons.

Regardless of stone type or salt treatment concentration, the absorption capacity of the top face (face A) of all blocks was consistently lower than both the side face (B) and bottom face (C) (see Table 19a and Figure 56).

Untreated (0% salt) and therefore, heavily colonised blocks of Dungannon had noticeably lower absorption rates than control blocks with no algal colonisation (see Figure 56a). In such instances, the biofilm may act as a barrier to water penetration, thereby reducing the loss of sub-surface moisture. It follows that this effect may be more pronounced on the more porous of the two stone types, Dungannon. In their survey of heritage structures in Belfast, Cutler et al. (2013b) found lower infiltration rates in green areas in comparison to un-colonised surfaces, however they did not note a consistent pattern when a larger number of blocks were studied.

Regardless of treatment face, a significant difference did not occur between the two stone types at 0% salt treatment. However, significant differences did occur between the stone types for blocks treated with the varying concentrations of salt solution (see Table 19b). Dungannon blocks had a higher water penetration capacity (see Figure 56), demonstrating that the effect of salt concentration varies between stone type. We also found that Dungannon blocks gained significantly more weight over time than St Bees blocks (see Table 17a and Figure 53a). Again, the higher porosity of Dungannon may be the contributing factor here.

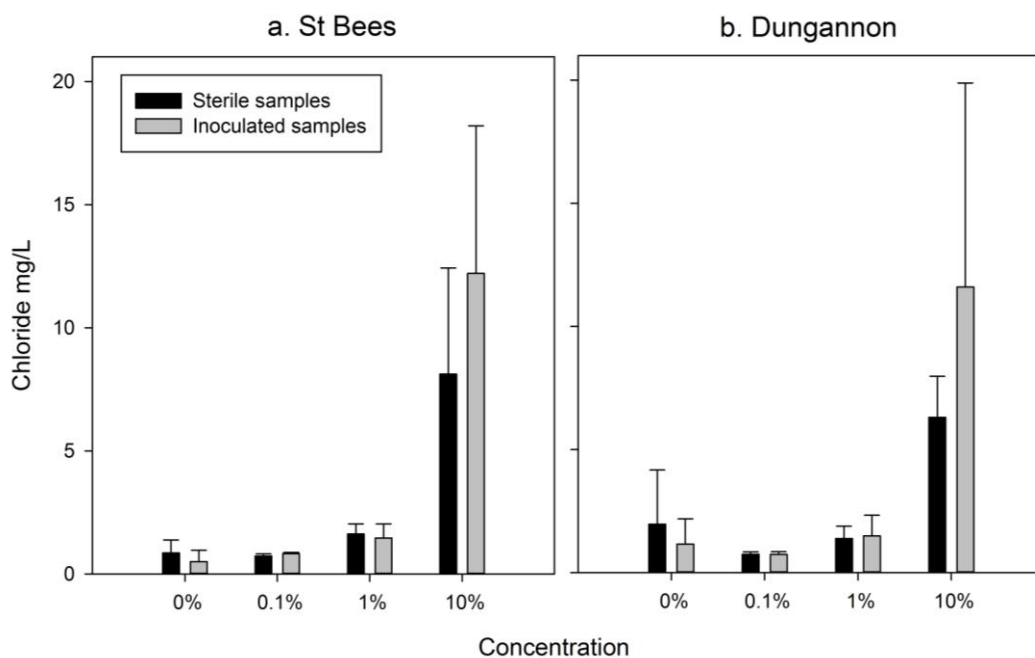
### *Dionex results*

Dionex measurements were carried out to determine the volume of salts stored on the surface and internally within colonised and uncolonised (bare) blocks treated with

varying concentrations of salt. A range of anions were extracted from prepared samples. Only Chloride (Cl) data is presented here as it is the primary constituent of the seawater salt mix used to treat samples. Chip samples were taken from three locations on a subsample of blocks, please refer to section 5.3 for details.

A significant difference was not found in Chloride concentration between sample positions (*a*, *b*, *c*) therefore data from all three points was pooled to enhance data resolution. The distribution of Chloride was broadly similar between St Bees and Dungannon blocks (see Figure 57). Indeed, a significant difference did not occur between stone types (see Table 20).

**Figure 57: Influence of inoculation method and salt concentration on Chloride (mg/l) levels observed**



ANOVA results indicated that inoculation method (whether blocks were inoculated or kept sterile) and salt concentration had a significant impact upon chloride levels (see Table 20). Pairwise comparisons were conducted for these variables (see Table 21).

Table 20: Total Chloride data- ANOVA results

Three way ANOVA					
Normality Test (Shapiro-Wilk)	Passed		(P < 0.322)		
Equal Variance Test:	Passed		(P = 1.00)		
Source of Variation	DF	SS	MS	F	P
Stone Type	1	5.654	5.654	8.052	0.066
Inoculation	1	20.271	20.271	28.866	0.013
Salt Concentration	3	1684.438	561.479	799.566	<0.001
Residual	3	2.107	0.702		
Total	15	1861.154	124.077		

Data from sampling points a, b and c was totalled for this analysis. ANOVA results indicated that inoculation method and salt concentration had a significant impact upon chloride levels observed

Table 21: Total chloride data- all pairwise multiple comparison procedures (Student-Newman-Keuls Method)

a. Inoculation method				
Face	Diff of mean	p	q	P
Inoculated vs. Sterile	2.251	2	7.598	0.013

b. Salt concentration comparison				
Concentration	Diff of mean	p	q	P
10 vs. 0.1	24.72	4	58.998	<0.001
10 vs. 0	23.635	3	56.41	<0.001
10 vs. 1	22.528	2	53.768	<0.001
1 vs. 0.1	2.191	3	5.23	0.068
1 vs. 0	1.107	2	2.642	0.159
0 vs. 0.1	1.084	2	2.588	0.165

Data from sampling points a, b and c was pooled for this analysis

A significant difference was found between inoculated and sterile blocks. Higher concentrations of chloride were observed in inoculated blocks than sterile blocks (see Figure 57 and Table 21) indicating that *S.baccilaris* itself or the growth medium it had been suspended in may have had an impact upon chloride levels.

Of the different salt concentrations, there was no difference in chloride concentration in blocks treated with 0, 0.1 and 1% salt solution (see Table 21b). However, significantly higher concentrations of chloride were observed in blocks treated with 10% solution, as might be expected (see Figure 57).

## 5.5 Discussion

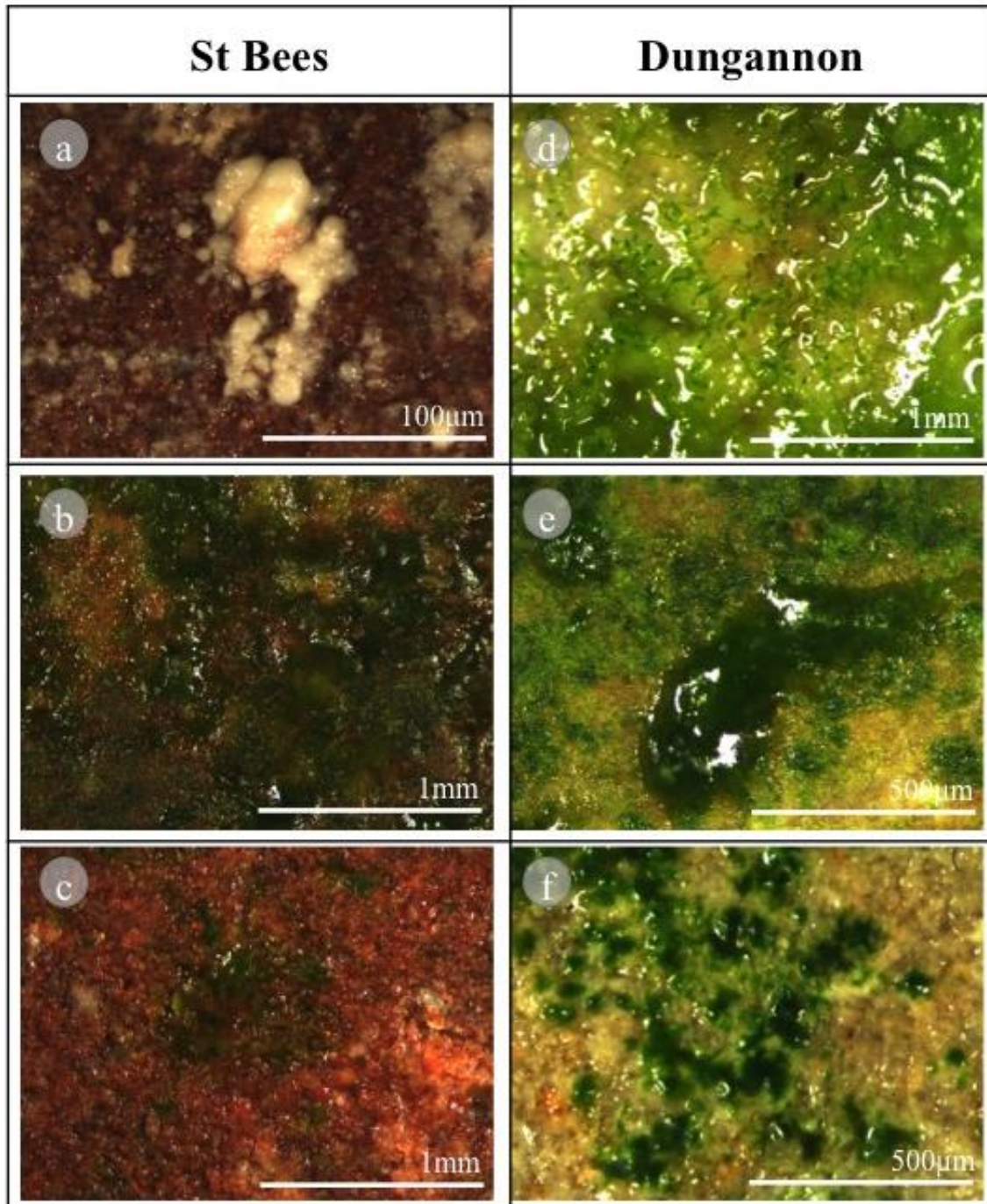
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### *R1. How does salinity affect the appearance and morphology of algal biofilms?*

Regardless of stone type, biofilm growth on all blocks treated with saline solution was delayed by at least seven days (see Figure 52b-d). In their study of salt shocked cells in culture, Ahmad and Hellebust (1993) found a significant increase in the doubling times for algae exposed to higher concentrations of artificial sea water and a marked decrease in total algal biomass with increased salinity. We found a similar effect whereby total biomass decreased in line with higher salinities, to the point of severely stunted and minimal growth at 10% treatment (see Figure 50g and h).

Growth of *S. bacillaris* on untreated (0% salt) blocks of both Dungannon and St Bees was healthy. Deposition of EPS was clearly visible after one to two weeks of incubation and biofilms appeared luxuriant and moist by the end of the experimental period (see Figure 58b and e). Our results also indicated that untreated (0% salt solution) and therefore heavily colonised blocks had a lower water penetration capacity than saline blocks with less or no surface colonisation (see Figure 56a).

**Figure 58: Micrographs of colonised samples at the end of the experiment**



- a. Sample 1 (0% salt): Visible mineral/ salt deposits on the top centre surface*  
*b. Sample 4 (0% salt): Close- up of thick EPS development in 'ring' of colonisation*  
*c. Sample 20 (1% salt): Clumped algal cells in grain space (ecological niches/ saline adaptation)*  
*d. Sample 14 (0.1% salt): Long algal filaments imbedded in EPS around mineral/ salt deposits, top centre*  
*e. Sample 7 (0% salt): Thick EPS deposition on surface*  
*d. Sample 22 (1% salt): Clumped algal cells in grain spaces (ecological niches/ saline adaptation).*

It is possible that this may be a bioprotective role of algal biofilms where lower absorption rates may reduce the volume of water entering the stone surface, thereby protecting the substrate from dissolution and decay. Garcia-Vallès et al. (2003) found lichen coatings of volcanic tuff in Turkey to have this effect.

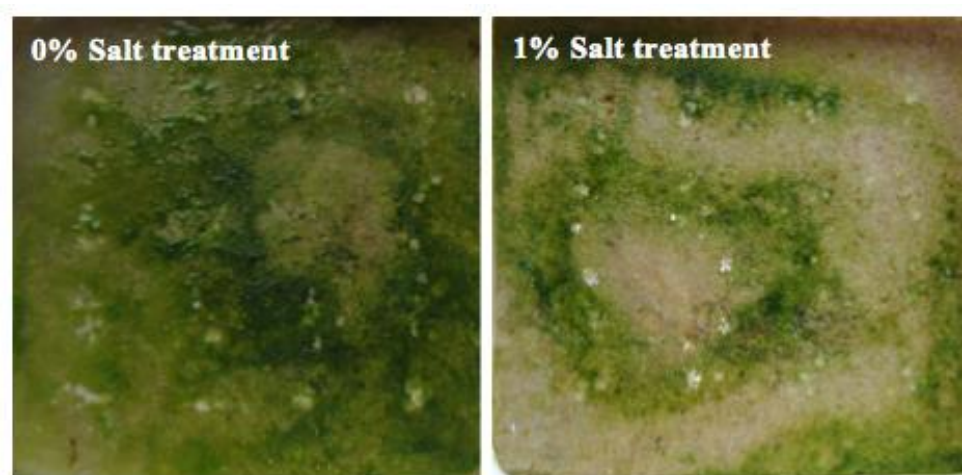
In non-saline and low salinity conditions, *S. bacillaris* cells appeared evenly spread and embedded in EPS on the surface of blocks (see Figure 58b, d and e). In these instances, it is likely that growth was dominated by single cells (Hayward, 1974). This was seen as a thin, but widely spread biofilm across the surface of blocks in the first two weeks of incubation (see Figure 51).

Biofilms appeared more desiccated with higher concentrations of salt treatment with a notable reduction in the deposition of surface EPS. In moderately saline conditions, longer filaments of *S. bacillaris* were observed (see Figure 58d). Studies have reported growth of longer filaments (of four to eight cells and up to 18) in salt-rich sea water medium (George, 1957; Hayward, 1974). As Figure 58c and f demonstrate, under conditions of higher salinity algal cells were found clustered in grain spaces and small recesses on the stone surface. Under conditions of stress, it is common for colonies to cluster in/ around these protective niches, which allow the accumulation of moisture and nutrients (Warscheid and Braams, 2000).

‘Ring-like’ patterns of colonisation (see Figure 59) were observed on all colonised blocks as seen in previous laboratory experiments (see Chapter 3). The location of these ‘rings’ of growth was closely related to a moderate zone of moisture corresponding to 40-60% WME (see Figure 54 and Figure 55). Algal colonies favoured this moderated zone of

frequent internal moisture movement (upwelling of moisture from the base of blocks via capillarity) and surface evaporation; forming rings of growth. The outline of these rings was more distinct with higher concentrations of salt treatment (see Figure 59- 1% treatment).

**Figure 59: Comparison of biofilm morphology between untreated and treated samples of Dungannon**



*Photographs highlight typical biofilm morphology on Dungannon samples treated with 0% and 1% saline solution after 35 days of incubation*

### ***R2. What are the mechanisms by which salinity affects colonisation?***

Studies have demonstrated the moisture retention capacity of sandstone due to the influence of salts on vapour pressure differentials and rates of evaporation, where higher salt content leads to greater moisture accumulation (McCabe et al., 2015). Indeed, where weight gain is used as a proxy for total moisture uptake, our results indicate that blocks treated with 1% and 10% saline solution accumulated significantly more moisture than sample groups treated with 0.1% and 1% solution (see Table 17b).

Our results also emphasise a clear link between salt concentration and algal cover, (see Figure 52 and Table 15) where higher concentrations of salt treatment have an inhibiting

affect on algal growth. Relating this to weight, water penetration and visual observations, we infer that high moisture volumes caused by salt- induced hygroscopy may have an inhibiting affect on algal colonisation. This is likely due to super-saturation of blocks reducing moisture movements within the stone interior and on the surface, thereby reducing the mobilisation of nutrients required for healthy growth.

Salts have been shown to alter population balance of bacteria and other soil microorganisms and cause displacement of minerals (Tresner and Hayes, 1970; Papida et al., 2000). Reduced moisture movements will also reduce the propagation capacity of cells on the stone surface, preventing the 'spread' of a surface biofilm.

As discussed, algal colonisation on blocks treated with high salt concentrations showed visible signs of desiccation and reduced production of EPS (see Figure 59). It is likely that the difference between the near neutral intracellular pH and that of the saline moisture available on and within the block resulted in osmotic stress. Hayward (1974) notes that high sodium chloride concentrations affect growth of *S. bacillaris* grown in liquid culture in two ways; either through osmotic stress or directly through sodium toxicity. Therefore, despite increasing block weights denoting increasing internal moisture over time, slow growth on these blocks was likely due to dehydration of the algal biofilm itself, which inevitably prevents or severely stunts growth (Madigan et al., 2009b).

In summary, we hypothesise that limited biofilm growth on saline blocks was due to one or a combination of three reasons. Moisture movement and therefore nutrient mobilisation was reduced in saline blocks due to hygroscopicity. Secondly, high salt contents in available moisture resulted in osmotic stress reducing algal metabolism and thirdly, the

direct effect of sodium toxicity inhibited cell growth (possibly through cell deformation/reduction in production of necessary metabolites for growth). This may go some way in explaining the cause for the distinct 'ring-like' patterns of growth mentioned earlier (see Figure 59) and for patterns of patchy colonisation observed on building facades (see Figure 47).

*R3. Are there notable differences in the impact of salt on algal growth between two types of building sandstone (Dungannon and St Bees)?*

Significant differences in algal cover did not occur between Dungannon and St Bees samples which were not treated with salt solution (see Table 15a). However, differences did occur between the stone types for those samples treated with differing concentrations of the saline mixture. In particular, notable differences in algal cover at week 5 of incubation occurred between the stones when treated with 0.1 and 1%, but the magnitude of differences was smaller when treated with 10% solution. This might indicate a threshold effect whereby after a certain level of salt treatment, stone type becomes unimportant. This may be because the conditions become too inhospitable for algae to grow irrespective of substrate characteristics.

The effect of salt in inhibiting growth was more pronounced on St Bees than Dungannon (see Figure 50). Studies of similar red carboniferous sandstones have highlighted their vulnerability to salt-induced decay, particularly by sulphate salts (Goudie et al., 1970; Kamh, 2005; Hyslop et al., 2006). Dionex results from this study did not reveal a significant difference between concentrations of Chloride in/ on St Bees and Dungannon blocks. However, photographic and visual observations noted a larger volume of deposited mineral efflorescence on control blocks of St Bees in comparison to Dungannon

(see Figure 51).

Whilst notable differences in the mobilisation of Chloride did not occur between the two stone types, it is possible that mobilisation and surface deposition of other salts may have varied between the two and these salts may have had a greater inhibiting effect on the growth of *S. bacillaris*. In addition, it is likely that petrographic characteristics of St Bees (such as its porosity, surface texture or colour) may have reduced its bioreceptivity and led to the differentiation in surface colonisation patterns observed.

When treated with salt, Dungannon samples were more bioreceptive than St Bees samples. This is likely due to the stone's primary/ intrinsic bioreceptivity; that is, its initial potential to become colonised based on its unaltered properties (Guillitte, 1995). For instance, untreated samples (no salt) of Dungannon gained significantly more weight than St Bees samples (see Figure 53a) and had a significantly higher water penetration capacity (see Figure 56). The magnitude of these differences was often affected by the concentration of salt treatment. However in general, Dungannon's comparative ability to absorb and store a greater volume of moisture made it more bioreceptive than St Bees. Given the interesting differences between the two stone types studied here, a more detailed comparative study of the bioreceptivity of these sandstones is proposed.

## 5.6 Conclusions

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Samples treated with de-ionized water alone (0% salt) experienced rapid and extensive colonisation by *S. bacillaris* over five weeks of incubation. Biofilms appeared healthy, moist and a rich layer of EPS was detected on both Dungannon and St Bees samples after

five weeks of incubation. Heavily colonised blocks also had a lower surface water penetration capacity than blocks with limited or no surface colonisation.

Clear ring- like patterns of colonisation were observed on all blocks treated with 0, 0.1 and 1% ASW (Artificial Sea Water). These ‘rings’ were most likely due to patterns of moisture upwelling and surface evaporation, corresponding to 40-60% WME. We have found similar results in previous laboratory experiments (see Chapter 3).

Salinity reduced the bioreceptivity of all blocks. Saline treatment delayed colonisation of blocks by at least seven and up to 14 days on samples treated with higher concentrations of up to 10% ASW as well as reducing overall biomass. Growth of *S. bacillaris* on saline blocks appeared desiccated with markedly less EPS deposition than untreated (0%) samples.

Salinity reduced the bioreceptivity of St Bees more than Dungannon, which remained the more receptive stone to colonisation by *S. bacillaris*. The magnitude of differences in bioreceptivity were most pronounced under moderate saline conditions (0.1 and 1%) where St Bees samples exhibited significantly less biomass than Dungannon. However, under conditions of high salinity (10%), there was limited colonisation on either stone type.

The results presented in this chapter demonstrate the influence of salinity on sandstone bioreceptivity, and how it varies between different types of sandstone. They also indicate that algal biofilms can significantly decrease surface permeability on some sandstones.

We propose further study of the sandstones used here to better determine and compare the intrinsic controls on bioreceptivity.

# Chapter 6:

## Comparing the bioreceptivity of different sandstones to single and mixed green algal cultures

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### Chapter Summary

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This chapter addresses Objective 4 of the overall thesis (see section 1.9) to investigate the role of inherent factors in the development of algal greening by comparing the intrinsic bioreceptivity of three widely used British building sandstones, Dungannon, St Bees and Peak Moor. Each stone type underwent the same exogenous treatment conditions with single (*Stichococcus bacillaris*, *Chlorella vulgaris* and *Desmococcus olivaceus*) and mixed green algal strains (mixed culture of the three individual strains tested) and was exposed under the same environmental conditions in a controlled growth chamber. Stone samples were exposed for a period of 42 days (six weeks). Sample weight, colour and appearance were monitored prior to exposure, during and following experimentation.

Of the axenic algal strain treatments, *S. bacillaris* exhibited greatest biomass on all stone types tested, contrasting with *C. vulgaris* which failed to grow successfully beyond three weeks of incubation. Growth patterns of *D. olivaceus* and the mixed culture were similar on all stone types. The intrinsic properties of each stone type were found to influence the rate and extent of algal greening observed with different properties such as stone texture and colour, favouring colonisation and production of EPS.

## 6.1 Introduction

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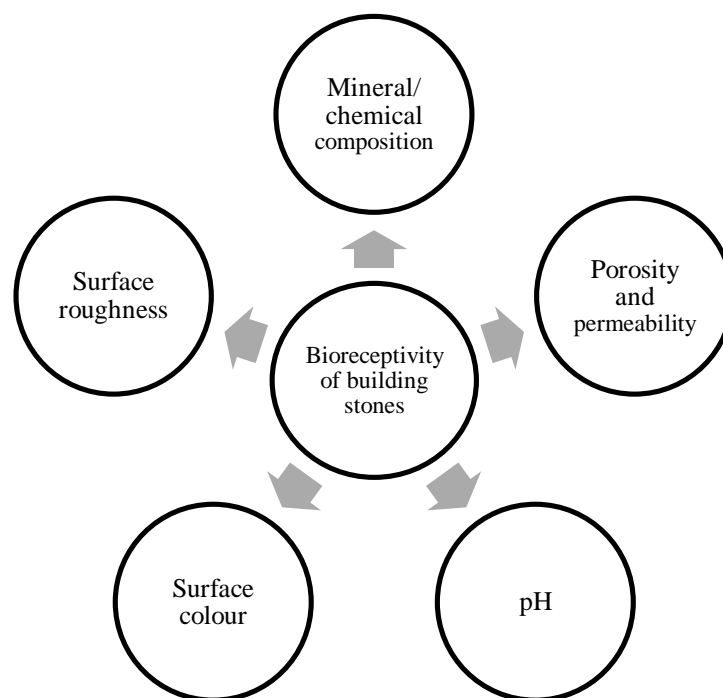
### *Bioreceptivity*

Within the scope of bioreceptivity studies of building stones are widely used sub-terms such as *primary*, *secondary* and *tertiary* bioreceptivity (Miller et al., 2012), these are defined and discussed in greater detail in section 1.5.

Here we look at the *primary bioreceptivity* of three sandstones to green algal colonisation. That is, the initial bioreceptivity or first stage of ecological colonisation of fresh, previously untreated and sterile stone. We also concentrate on the *intrinsic bioreceptivity* of test stones, which is the influence of inherent characteristics of the stone, which control its bioreceptivity (see Figure 60). It is likely that the amount of biological growth observed on a stone, is variable dependent upon its intrinsic properties and exposure conditions (Miller et al., 2010).

When considering building stone and sandstone's, inherent characteristics such as the chemical or mineral composition, porosity, permeability, pH, surface colour and surface roughness (see Figure 60) control the ability of the surface to become colonised and the rate at which this occurs.

**Figure 60: Inherent factors that control the bioreceptivity of building stones**



Surface characteristics play an important role in the total volume and diversity of surface biomass. For instance, biomass will increase as surface roughness increases (Macedo et al., 2009). The rougher a surface, the greater the surface area and consequently the more pockets or niches available for colonisers to adhere to the surface or for water and or nutrients to accumulate, thereby promoting further growth. In their study of a range of building stones for example, Tiano et al. (1995) recorded most widespread microbial growth on limestones with both high surface roughness and porosity. Similarly, in their study of a range of building stone substrates, Tomaselli et al. (2000) found that the combination of high surface roughness and porosity promoted most widespread microbial colonisation.

The texture of a building stone can also determine the diversity and ecology of microbial flora. Indeed, coarse-grained stones may promote temporary or initial colonisation due to high permeability whereas fine-grained stones may favour longer term colonisation due

to their ability to bind humidity (Warscheid et al., 1991).

The mineral or chemical composition of a surface can catalyse and stunt growth of microorganisms. This may be due to the presence of preferential minerals or nutrients for growth or the growth-enabling properties offered by a particular mineral, such as high porosity. For example, preferential growth of some microorganisms is known to occur on stone rich in clays, feldspars (Warscheid et al., 1991) and ferruginous minerals (Warscheid and Braams, 2000). Indeed, Welton et al. (2003) observed strong etching on carbonates and feldspars in their study of mineral chips submerged in a mixed green algal culture. However, Tomaselli et al. (2000) note that whilst some cyanobacteria species prefer calcareous or siliceous substrates for growth many green algae such as *Chlorella* sp. are ubiquitous and occur on a range of substrates with a wide mineralogical diversity.

Moisture availability is argued by many to be the most influential factor controlling the amount of biological soiling observed on substrates (Hoffmann, 1989; Tomaselli et al., 1999; Crispim et al., 2003; Gorbushina, 2007). Therefore, an increase in porosity can enhance water retention, which can catalyse the colonisation of a surface and improve its bioreceptivity. It is well established that a combination of high porosity and open capillarity can lead to rapid colonisation of stone surfaces (Guillitte and Dreesen, 1995; Miller et al., 2010). In early studies for example, Guillitte and Dreesen (1995) noted very low primary bioreceptivity of the limestone Petit Granit in comparison to a range of other building materials (limestone, concrete, mortar and brick) due to its particularly low macroporosity. Similarly, in their study of ceramic roofing tiles, Gazulla et al. (2011) noted that altering clay mixtures to produce roofing tiles with lower porosities significantly reduced the bioreceptivity of these samples to growth of the cyanobacteria, *Oscillatoria* sp. However, very high porosity may increase moisture evaporation from the

stone surface (Guillitte and Dreesen, 1995), thereby reducing the bioreceptivity of the material in the longer term.

Another factor determining the timing and rate of surface colonisation is pH. In their study, Tiano et al. (1995) found that stone surface pH values of between 6.2 and 6.5 promoted most microbial growth whereas higher values had a negative influence. When considering concrete, Govin et al. (2013) found that a decrease in pH (due to carbonation) favoured colonisation by the ubiquitous green algae, *Klebsormidium flaccidum*. In their study of the colonisation kinetics of green algae, Giovannacci et al. (2013) hypothesised that irrespective of porosity and roughness values, concrete tiles sustained least growth in comparison to limestone, clay and glass due to alkaline compounds and calcite crystals present in the matrix. For the majority of microorganisms and the neutrophilic green algae discussed here, the external pH range required to sustain growth is between pH 5-9 (Konhauser, 2007).

Building stones are often selected due to their aesthetic qualities, including colour. However, base colour may determine the spread and diversity of microbial colonisation on a substrate. Colour can influence the intrinsic bioreceptivity of a stone in two ways, by influencing the amount of light that penetrates the surface and secondly by influencing surface temperature (albedo effect).

Light is better able to penetrate at depth in lighter coloured stones than darker ones, particularly in the case of moist stone surfaces. In their study of the bioreceptivity of different cements, Manso et al. (2014) found lighter coloured mixtures more bioreceptive to growth of *Chlorella vulgaris* than those with a darker base colour. In another study, McGreevy et al. (2000) recorded significantly higher maximum surface temperatures for

stones such as Scrabo with a lower albedo (darker in colour) than lighter coloured stones such as chalk. This is due to the ability of lighter coloured stones to better reflect light and therefore heat. Latitude can influence to what degree albedo affects bioreceptivity. Whilst green algal cells are able to survive in wide temperature ranges, optimum growth occurs between 20-23°C (Häubner et al., 2006). In temperate environments, darker stones may be better able to absorb heat and therefore sustain the moderate, warm temperatures required for optimum growth. Conversely in higher and lower latitudes, thermal extremes caused by rapid cooling and heating of surfaces (due to their albedo) may render some stone surfaces too hostile to host some microbial species.

Inherent factors such as those presented in Figure 60, are likely to have a significant influence on the bioreceptivity of building stones. To study this influence, we have conducted an experiment to compare the inherent bioreceptivity of three commonly used British building sandstones to single and mixed green algal treatments.

## 6.2 Aim and objectives

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### *Aim*

The aim of this chapter is to investigate the role of *inherent factors* in the development of algal greening by comparing the bioreceptivity of three sandstones (Dungannon, St Bees and Peak Moor), as monitored by changes in weight, appearance and colour.

Previous experimental work (reported in Chapter 4. I) demonstrated that colonisation of stone samples by *S. bacillaris* resulted in noticeable surface colour change. Colonisation resulted in both darkening ( $L^*$  scale) and greening ( $a^*$  chromatic scale) of the surface.

When plotting the movement of samples through colour space, a strong colour bias was found towards the yellow end of the chromatic spectrum, but it is unclear whether other algal species have the same impacts.

### *Research Questions*

**R1.** What are the differences in the rate and nature of colonisation on three test sandstones when inoculated with *S. bacillaris*, *C. vulgaris*, *D. olivaceus* and a mixed culture and grown under the same environmental conditions? (as assessed by weight change, visual and microscopical observations and overall colour change).

**R2.** How does the nature of colonisation on each each sandstone vary in terms of individual components of colour, as monitored by spectrophotometry?

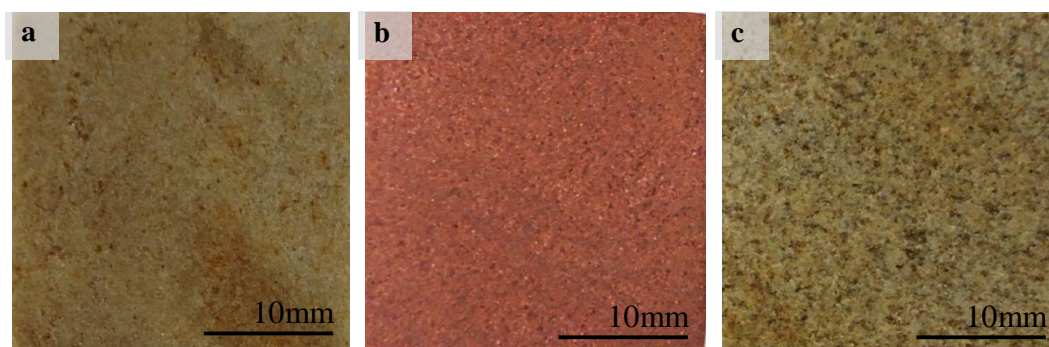
## 6.3 Materials and methods

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### *Stone types*

This experiment compares the intrinsic bioreceptivity of three commonly used building sandstones in the UK and Northern Ireland. All three stones are known for their durability and wide use in load-bearing masonry as well as cladding. The sandstones used include Dungannon, St Bees and Peak Moor (see Figure 61). The stones have been used for experimentation throughout the wider EPSRC ‘greening’ project and within the scope of this thesis (see Chapter 2, section 2.2). Their appearance and characteristics typify the kinds of ‘blonde’ (buff) and ‘red’ sandstones used for construction in northern cities such as Glasgow (Hyslop and Albornoz-Parra, 2009) as well as in Edinburgh and Belfast (see Chapter 1, Figure 1).

**Figure 61: Photographic comparison of the three stone types used for experimentation**

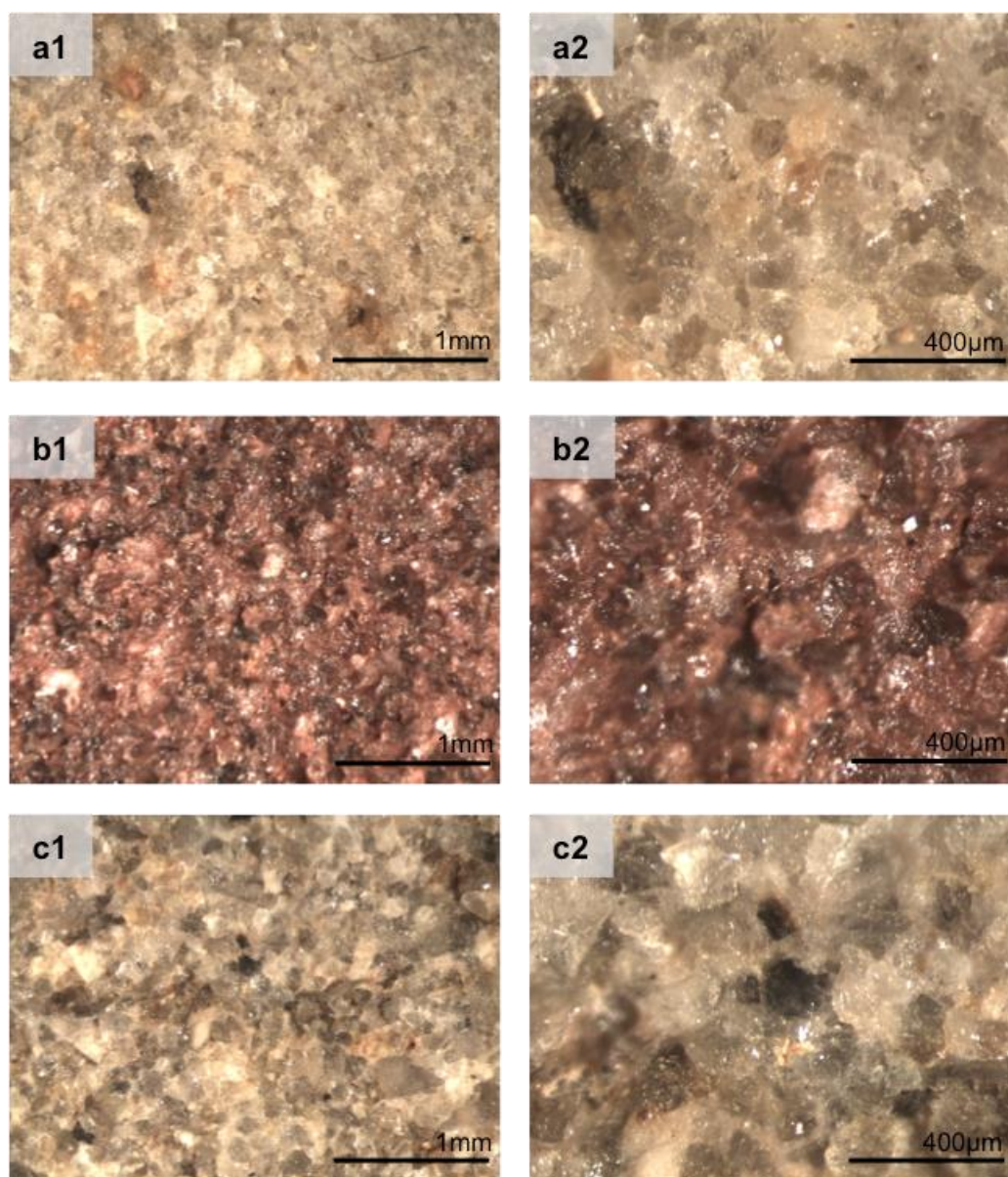


*a- Dungannon, b- St Bees, c- Peak Moor under baseline 'wet' conditions*

Dungannon is a grey-buff coloured, fine-grained stone (see Figure 61a and Figure 62a) known to contain laminations of silt and clay (Cutler et al., 2013a). St Bees is a dark terracotta coloured, fine-grained stone (see Figure 61b and Figure 62b) with fine laminations of kaolinite (Clark, 1995), iron oxide and mica (Hyslop et al., 2006). Of the three stones, Peak Moor grains have the widest diameter (see Figure 62 c2). Peak Moor is a yellow-buff coloured, medium grained stone (see Figure 61c and Figure 62c) with fine iron intrusions (Blockstone, 2008). General properties of each stone type can be found in Chapter 2, section 2.2.

### *Monitoring biomass*

Chapter 4 discusses the different methods available for mapping and quantifying biomass in laboratory and field settings. These include fluorescence measurements of chlorophyll *a*, fluorescein diacetate hydrolysis, changes in colour and direct cell counts.

**Figure 62: Comparative micrographs of the three test sandstones**

*Micrographs of a- Dungannon, b- St Bees and c- Peak Moor samples under baseline 'wet' conditions*

Previous experimental work (see Chapter 4, part I) has established a simple methodology to monitor green algal colonisation by measuring total colour change ( $dE_{2000}^*$ ) and change along the red-green chromatic scale ( $da^*$ ) combined with proxy measurements of biomass (percentage algal cover and density). Here we use the methodology previously established, supplemented with an increased number of replicates and controls in the

overall design of the experiment. This has been teamed with additional microscopical observations of surface change caused by the different treatment types.

### *Experimental design*

Sample groups A-D were inoculated with green algae and are herein referred to as the ‘inoculated’ groups (see Table 22). Each stone group was treated with either a single or mixed culture of green algae. A full description of each algal strain and its metabolic properties are provided in the Chapter 2, section 2.3.

**Table 22: Summary of treatment groups and replicate set-up**

Category	Group Code	Treatment type	Number of replicates per stone type		
			Dungannon	St Bees	Peak Moor
Inoculated samples	A	Stichococcus bacillaris	8	8	8
	B	Chlorella vulgaris	8	8	8
	C	Desmococcus olivaceus	8	8	8
	D	Mixed strain culture	8	8	8
Wet controls	E	3N BBM+V cabinet controls	8	8	8
	F	Sterile water cabinet controls	8	8	8
Dry controls	G	Untreated cabinet controls	8	8	8
	H	Untreated laboratory controls	8	8	8

Four sample groups were isolated as controls. Of these four groups, group E and F received sterile treatment and were kept under wet conditions in a growth cabinet (herein referred to as ‘wet controls’). The role of wet controls was to monitor that the growth cabinet environment remained sterile as well as to allow for a reference point of comparison for inoculated samples.

Conversely, groups G and H (herein referred to as ‘dry controls’) were not treated. Group G was incubated in a growth cabinet whilst group H was kept in a drying cabinet under

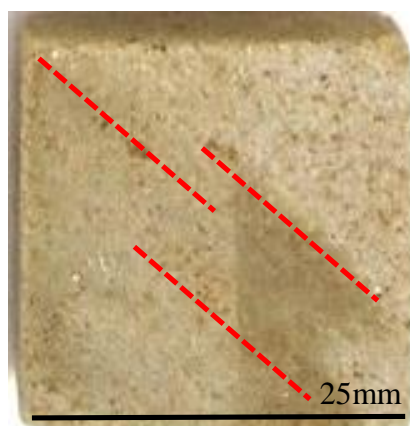
stable conditions. The role of group G was to monitor the influence of environmental conditions in the growth cabinet (temperature and RH in particular) on untreated samples. Group H was kept under constant conditions to monitor changes in sample weight and appearance caused by intrinsic stone properties.

### *Experimental set-up*

Each sample group was composed of twenty-four 25 mm x 25 mm x 5 mm tablets of sandstone. Stone was cut with consideration of bedding planes. Where possible, all samples were cut and aligned consistently so that laminations lay diagonally across the surface (see Figure 63). Each tablet (herein referred to as a 'sample') was prepared (sterilised and dried) according to the standards outlined in Chapter 2 (see Figure 9: Standard stone preparation protocol). A baseline assessment of each sample in groups A-F was carried out following the protocol outlined in Chapter 2 (see Figure 10: Standard baseline assessment protocol). Samples were soaked in sterile water for 30 minutes, removed, placed on absorbent paper for 30 seconds and assessed (weight, photography and colour change). Comparatively, baseline assessment of control groups G and H was conducted whilst samples were dry. For all samples, the 'baseline' point would act as a reference point for subsequent photographic, weight and colour measurements.

Following baseline assessment, all samples were placed in individual, sterile 5 cm diameter plastic petri dishes (Sterilin, South Wales). Transparent lids were kept slightly ajar on all of the dishes throughout the experiment to allow for respiration whilst minimising the likelihood of cross-contamination between test samples and controls.

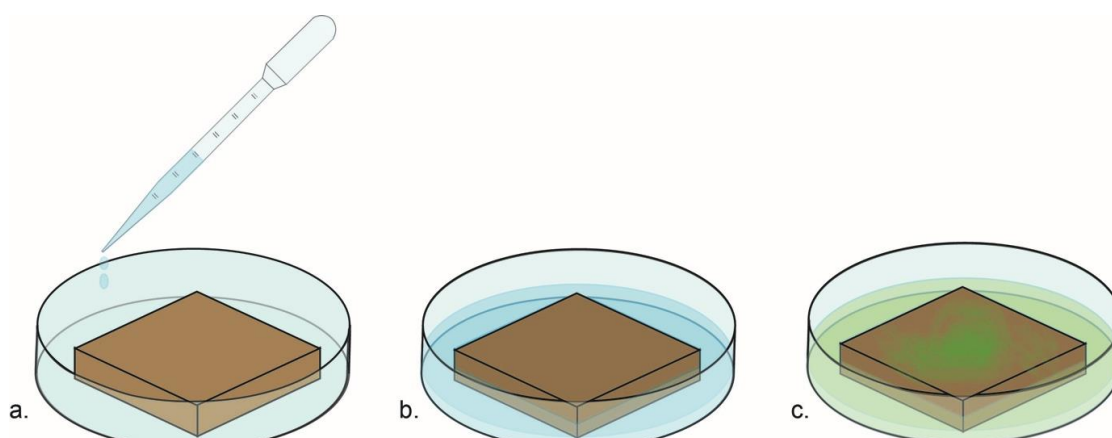
**Figure 63: Sample Dungannon experimental tablet**



*Samples cut and aligned consistently so that where possible, laminations (c1mm clear layers, see red dashed lines and section 2.2 for further details) lay diagonally across the surface.*

All sample dishes in groups A-F were filled with 1.5 ml of distilled water (so that samples would have constant exposure to moisture uptake) prior to inoculation (test samples) or sterile treatment (wet controls). The volume of water in each dish was checked every 12 hours and maintained throughout the experimental period (see Figure 64b). Water was always topped up with a sterile pipette, directly into each dish so as to avoid ‘washing’ of the stone surface (to prevent algal colonies being disturbed).

**Figure 64: Experimental set-up**

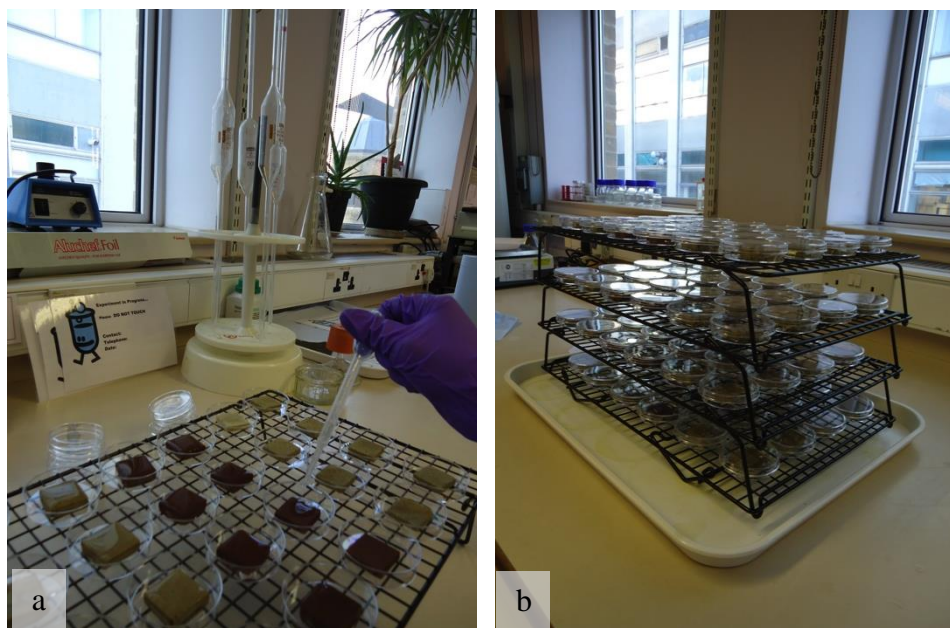


*a. Test sample in petri dish, b. Test sample partially submerged in sterile water, c. Inoculated sample after 42 days of incubation*

The samples in groups A-G were placed on coated metal wire racks and stacked in a MLR-352H Plant Growth Cabinet (Panasonic, UK). Samples were incubated under temperate environmental conditions (temperature  $20 \pm 1^\circ\text{C}$ ; relative humidity  $85 \pm 5\%$ ; photoperiod 16 hours light/ 8 hours dark) for a period of 42 days.

Approximately every 12 hours during the experimental period, samples were moved around on their racks, the racks themselves were rotated and the order of stacking changed to ensure all samples received as equal exposure to light and ventilation as manually possible (see Figure 65 for rack set-up).

**Figure 65: Set-up of sample groups on a stacked wire rack system**



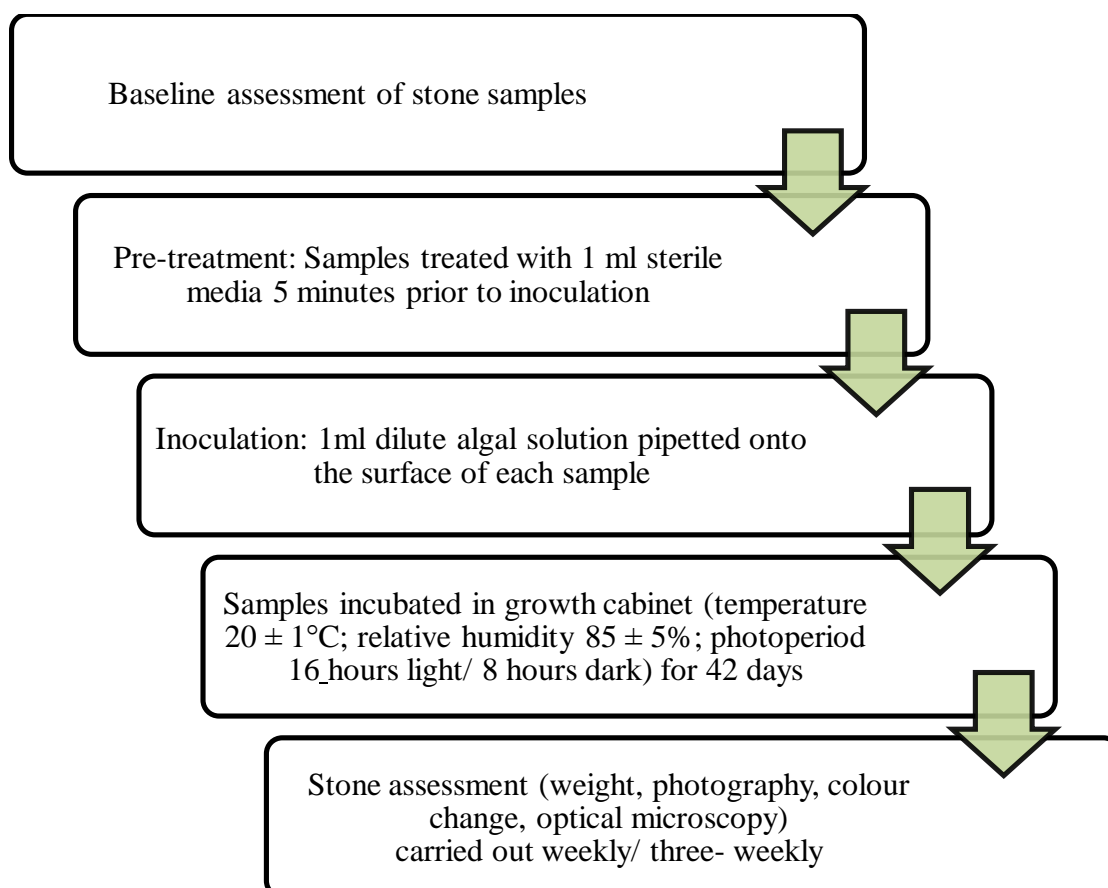
*a- Inoculation of stone samples set in petri dishes, b- Stacked rack set-up*

### *Inoculation procedure*

Sample groups A, B, C and D ('inoculated samples') were inoculated with green algae, the experimental process is summarised in Figure 66. All samples within these groups were first treated with 1 ml sterile medium (3N-BBM+V) five minutes prior to

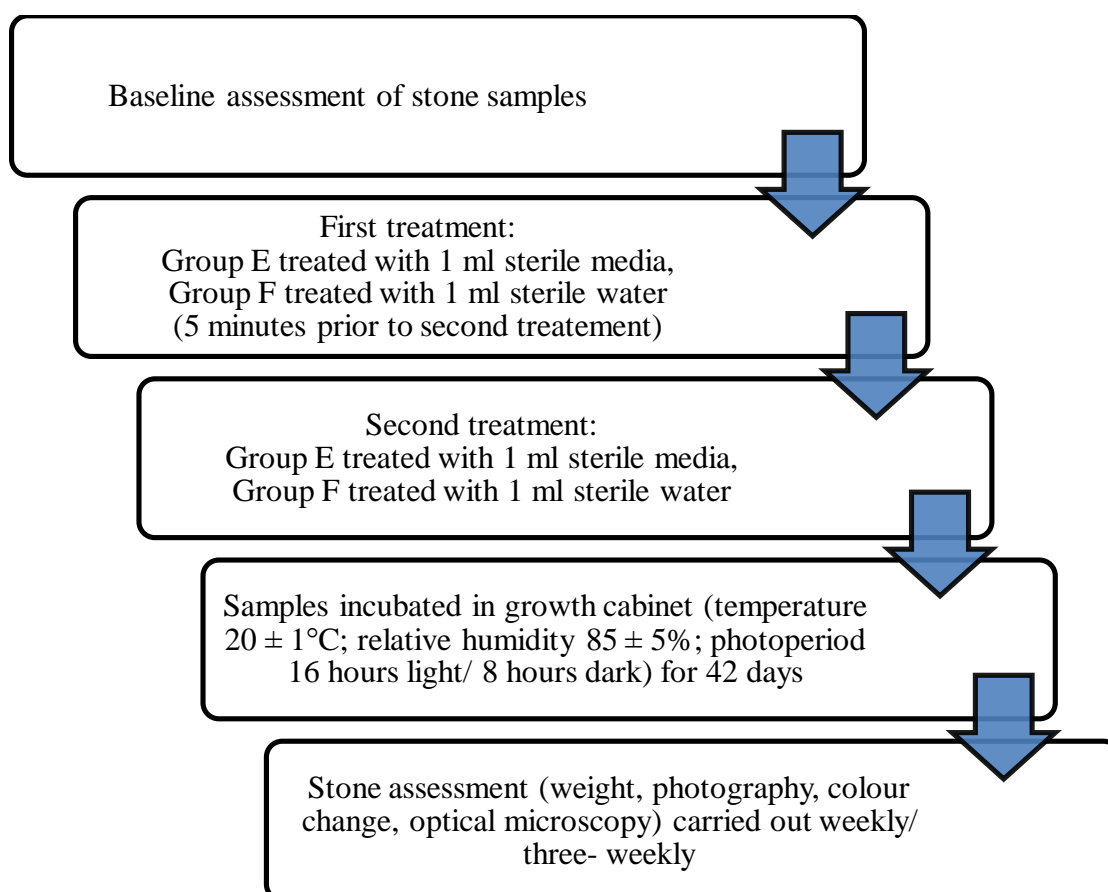
inoculation so as to provide enough minerals to catalyse initial growth. Groups A, B and C were treated with diluted single strain cultures of *Stichococcus bacillaris*, *Chlorella vulgaris* and *Desmococcus olivaceus* respectively. All cultures and media were obtained from CCAP (Argyll). The methods and concentrations of dilution are detailed in Chapter 2, section 2.3. Sample group D was treated with a mixed culture of the three strains listed. The mixed culture was formed by adding 1 ml concentrated solution of each strain to 75 ml 3N-BBM+V medium. This was mechanically stirred and then mixed using a bench-top shaker at 80rpm for 30 minutes prior to use. One milliliter of prepared dilute algal solution was pipetted onto the top surface of each test tablet, whilst in its petri dish (see Figure 33a). The lids of these dishes were then replaced and all samples placed in the growth cabinet for incubation.

**Figure 66: Inoculated samples-  
Summary of experimental process**



Control sample groups E and F ('wet controls') were kept in petri dishes filled with distilled water, but kept isolated to prevent algal contamination. The experimental process is summarised in Figure 67. All samples in Group E were first treated with 1 ml sterile medium (3N-BBM+V) and group F with 1 ml sterile water. After five minutes, samples were retreated with a further 1 ml of the appropriate treatment. All controls were then incubated. Groups E and F were kept under the same conditions in the growth cabinet as inoculated groups A- D.

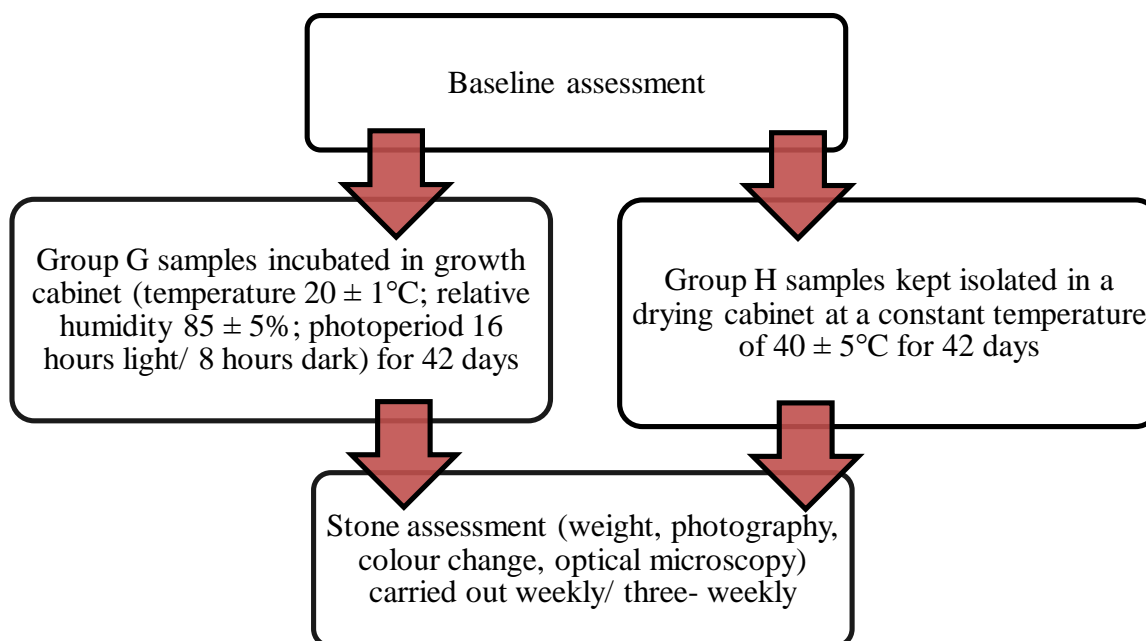
**Figure 67: Wet controls-  
Summary of experimental process**



Every seven to fourteen days group E and F petri dishes were sterilised and filled with fresh sterile water to try and minimise cross-contamination between inoculated samples and wet controls.

Conversely, groups G and H were kept as ‘dry controls’ for the duration of the experiment. The experimental process is summarised in Figure 68. These groups were not subject to the baseline assessment procedure outlined in Figure 10, to avoid any moisture influence. Instead, baseline readings were taken immediately after samples were removed from storage (see Figure 9). Group G was kept in the growth cabinet under the same ambient conditions as the other incubated groups whilst group H was kept isolated in a drying cabinet at a constant temperature of  $40 \pm 5^\circ\text{C}$ .

**Figure 68: Dry controls-  
Summary of experimental process**



### *Stone assessment*

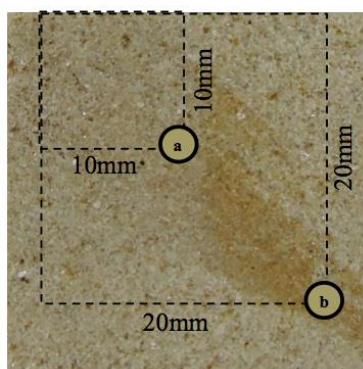
Samples were weighed at the baseline point (as defined above) and weekly after this. Samples were removed from their respective petri dishes and placed on absorbent paper for 30 seconds (to dry any excess moisture) and weighed using a three-decimal place,

bench-top balance (error  $\pm 0.001\text{g}$ ). Weight was used as a proxy measure for moisture content.

Digital photographs were taken at the baseline point and weekly after this. Photographs were taken of all samples using a fixed tripod set-up, under consistent lighting conditions in a photographic light box. These photographs were used to approximate biomass (percentage cover and density of growth) using the methods discussed in the Methodology chapter.

A Leica MZ10 F Stereomicroscope was used to observe and photograph colonisation at the micro scale. Microscope images were taken of a subset of samples from each group at the wet baseline point, after 21 days and again after 42 days of incubation to observe growth progress. The subset included samples 1, 9 and 17 from each group (A-H). Two, 2 mm diameter points were photographed (point 'a' and 'b') on each sample (see Figure 69). Each point was photographed at two magnifications (3.2 and 8).

Colour change was measured at the beginning of the experiment (herein referred to as 'target' colour) and weekly after that, for six weeks. Target colour was measured by averaging the surface colour of all samples within each sample group, whilst in a dry, sterile state. A 'dry' baseline measurement of *each* sample was then recorded.

**Figure 69: Microscope sampling points**

Colour change was measured using a CM-700d Spectrophotometer (Konica Minolta, Japan). An average of five measurements was taken of the top surface of each tablet using the 7 mm Medium Area View (MAV) aperture, under standard illumination (D65: average daylight) conditions. Colour data was analysed using SpectraMagic™ NX colour data software (Konica Minolta, Japan). Only ‘Specular Component Excluded’ (SCE) data are presented here, as this measurement excludes gloss effects caused by surface moisture.

Inoculated samples and wet controls were then subject to the baseline assessment protocol (see Figure 10) at which point, an additional ‘wet’ baseline measurement of each sample in these groups was recorded.

### *Statistical analysis*

Weight (g), algal cover (%) and colour ( $dE2000^*$  and  $da^*$ ) datasets were not distributed normally (established using Shapiro-Wilk tests) and this could not be corrected for by transformation. Therefore, non-parametric Kruskal-Wallis tests (one-way, based on ranks) were performed to test for significance. As interaction between the two factors

(stone type and treatment type) was not tested, a conservative  $p$  value ( $p < 0.05$ ) was assumed in all cases to reduce the likelihood of a type I error.

For weight and algal cover datasets, testing was used to measure the significance of differences between baseline readings and after six weeks of incubation. As colour change was expected to be variable over time, testing was used to measure the significance of differences in  $dE2000^*$  and  $da^*$  between baseline readings and after three *and* six weeks of incubation.

## 6.4 Experimental results

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The results of weight change (section 6.4.1, below), changes in surface biomass (section 6.4.2) and change in surface colour (section 6.4.3) over the six weeks of experimentation are presented here. For each variable (such as weight change) the results of statistical analysis (Kruskal-Wallis testing) are presented first, followed by a discussion of the observed data trends over time. Movement of samples through colour space is discussed last.

### 6.4.1 *Weight change as a proxy measurement for moisture movements*

Weight change is used here as a proxy for moisture content, the assumption being that the greater the weight gain, the more moisture retained by a sample. With regards to influence of stone type, overall trends indicate that (regardless of treatment type) compared to baseline weights, Peak Moor samples gained significantly less weight than Dungannon and St Bees samples by the end of the experimental period, there being no significant difference between the latter two (see Table 23a). When testing the influence of treatment type (regardless of stone type), dry control groups H and G gained significantly less

weight than all other treatment groups (see Table 23b). This indicates that there was no significant difference in the weight gained by ‘inoculated samples’ (groups A-D) and ‘wet controls’ (E and F).

**Table 23: Testing the significance of differences in weight change (from the baseline) after 6 weeks of incubation**

<b>Factor</b>	<b>Results</b>
<b>a. Influence of stone type</b>	Peak Moor < Dungannon = St Bees
<b>b. Influence of treatment type</b>	H = G < E = B = D = A = F = C

*Tests (Kruskal-Wallis) measure difference between baseline weights (wet baseline for groups A-F, dry baseline for G and H) and end weight after 42 days of experimentation. All tests conducted at  $p < 0.05$ .*

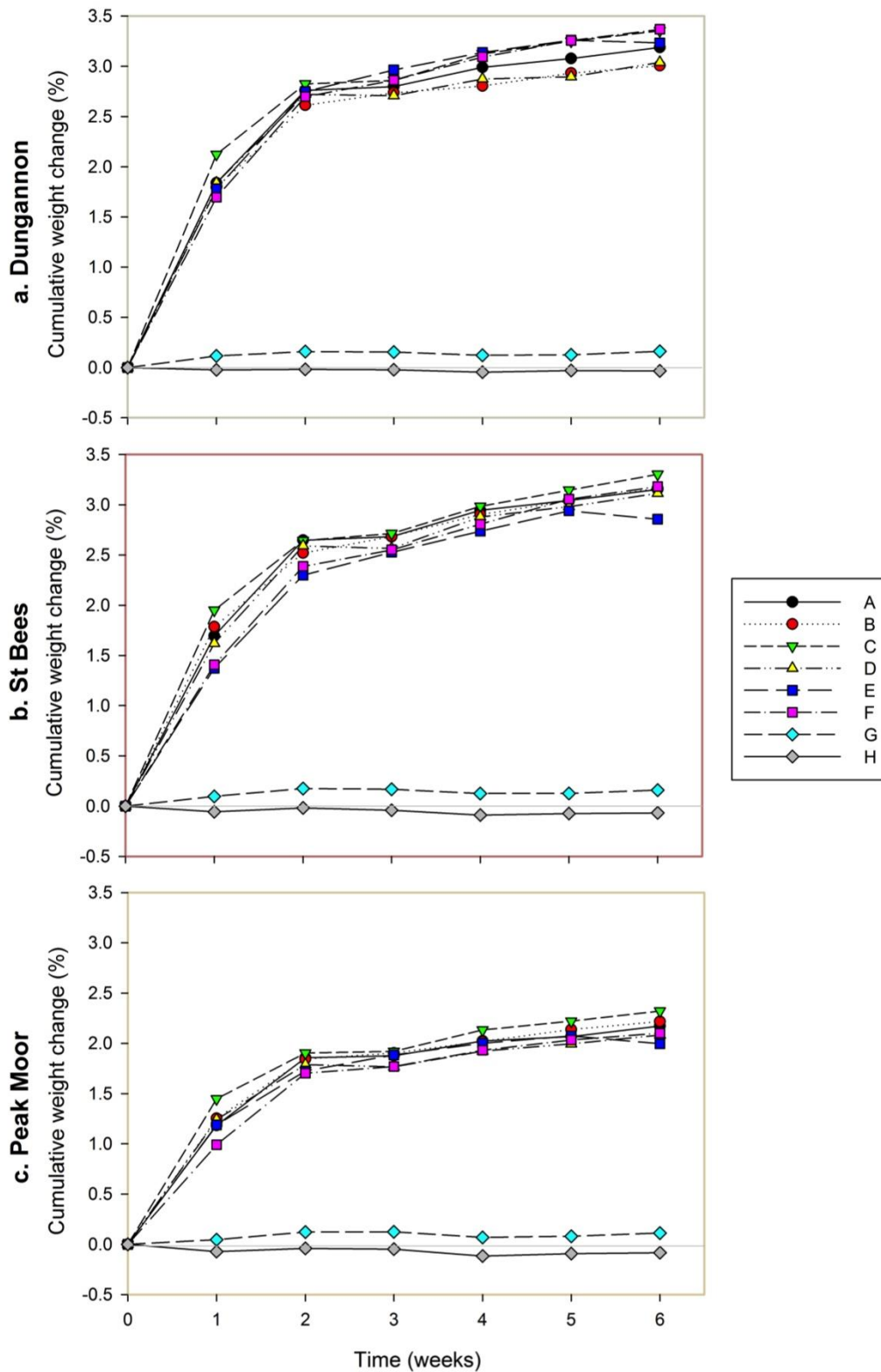
*< And > indicate degree and direction of difference,  
whereas = indicates no statistical difference between variables.*

#### *Observed trends in sample groups A-F*

Of the Dungannon samples, those inoculated with green algae (A, B, C and D- see Figure 70a), seemed to have gained less weight than the wet controls (E and F).

For St Bees and Peak Moor samples, treatment groups A-F all gained weight over time, indicating no discernible influence of type of treatment (including algal inoculation or sterile treatment) on the amount of weight gained. For all samples, greatest weight gain (from the baseline) was experienced in week one (see Figure 70). Weight gain stabilised by week three for the majority of samples, after which there was a gradual increase in weight gain.

Figure 70: Cumulative weight change over time



Weight change was calculated from the baseline point ('wet baseline' for groups A-F and 'dry baseline' for groups G and H). Symbols represent the average (mean) weight change per week, of each sample group (containing 8 replicates). See Table 22 for treatment group (A- H) set up.

There was no observable difference between total weight gained by Dungannon samples and St Bees samples (for both stone types, each treatment group gained an average of around 3% weight after 42 days), and the trajectory of weight gain was similar for both. However, Peak Moor samples gained the least weight in all instances of treatment (each treatment group gained an average of around 1.9% weight, after 42 days- see Figure 70c). Under all treatment types, Peak Moor samples behaved more similarly to one another than Dungannon and St Bees samples. The latter two groups displayed a greater degree of variability between treatment types.

#### *Observed trends in dry control groups G and H*

As would be expected, irrespective of stone type, group G samples all gained a small amount of weight (on average, 1.1 % after 42 days of incubation), which fluctuated slightly from week to week (see Figure 70). This was owing to the influence of fluctuating RH conditions ( $85 \pm 5$  %) in the growth cabinet. Conversely, group H samples (irrespective of stone type) all lost around 0.1 % weight after 42 days. This was due to the consistent drying conditions of the drying cabinet ( $40 \pm 5^{\circ}\text{C}$ ) and low, consistent relative humidity.

#### *6.4.2 Biomass change over time*

Biomass (percentage cover and density of colonisation) was approximated using the methodology described in Chapter 2, section 2.6. After 42 days of incubation, all samples within groups A-D exhibited growth (see Figure 71). Conversely, control groups E-H exhibited no growth at all and are therefore not discussed here.

Figure 71: Bi-weekly photographic record of surface change

a. Dungannon Samples					b. St Bees Samples					c. Peak Moor Samples					
	Baseline	Week 2	Week 4	Week 6		Baseline	Week 2	Week 4	Week 6		Baseline	Week 2	Week 4	Week 6	
Inoculated Samples	A1					A9					A17				
	B1					B9					B17				
	C1					C9					C17				
	D1					D9					D17				
Wet Controls	E1					E9					E17				
	F1					F9					F17				
Dry Controls	G1					G9					G17				
	H1					H9					H17				

*A subsample (the first replicate of each treatment group) of tablet photographs is presented here. Photographs were taken under controlled conditions (consistent light and tripod set-up).*

Where stone type was considered (regardless of treatment type), there was no overall trend or significant difference in algal cover (%) between Peak Moor, Dungannon or St Bees samples (see Table 24a). However, a clear difference was found between treatment types (see Table 24b). After 42 days of incubation, all samples (regardless of stone type) inoculated with *C. vulgaris* (group B) exhibited less widespread growth than those samples inoculated with the mixed culture (group D) and with *D. olivaceus* (group C). A significant difference in algal cover between groups C and D did not occur. Group A samples (inoculated with *S. bacillaris*) exhibited the most significantly widespread colonisation of the four groups after 42 days.

**Table 24: Testing the significance of differences in algal cover (%) between sample groups after 6 weeks of experimentation**

<b>Factor</b>	<b>Results</b>
<b>a. Influence of stone type</b>	<b>Peak Moor = Dungannon = St Bees</b>
<b>b. Influence of treatment type</b>	<b>B &lt; D = C &lt; A</b>

Tests (Kruskal-Wallis) measure difference in algal cover (%) between samples after 42 days of experimentation.

All tests conducted at  $p < 0.05$ .

< And > indicate degree and direction of difference,  
whereas = indicates no statistical difference between variables.

#### *Influence of treatment type on biomass*

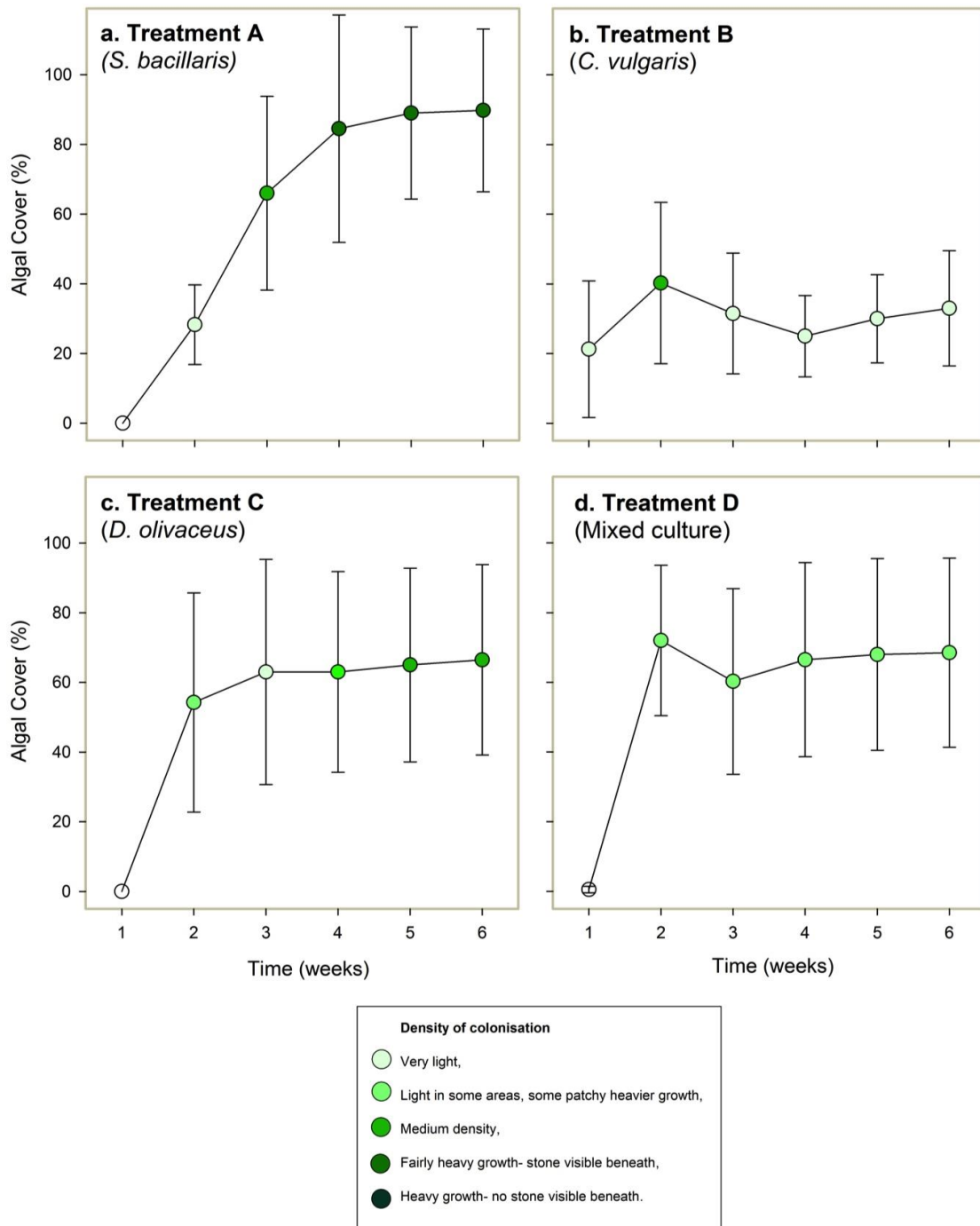
For all stone types, samples treated with *S. bacillaris* (group A) displayed the most healthy and uniform growth, with biomass increasing consistently over time (see Figure 72a, Figure 73a and Figure 74a). Peak growth occurred between three to four weeks of incubation after which it levelled off. After 42 days of incubation, samples exhibited an average of 80 % cover at fair to heavy density. This was significantly more than for other treatment groups (see Table 24b).

Biomass change was less linear for samples inoculated with *C. vulgaris* (group B). For all stone types, these samples exhibited early signs of colonisation within seven days of incubation and peak growth at 14 days (average of 45 % cover at light density). After this point, colonisation began to die back regressing to around 20 % cover at light density between 21-28 days. This is likely due to depletion of nutrients available from the initial algal treatment. Interestingly, on Dungannon and St Bees samples algal cover began to increase after this point, recovering to 40 % after 42 days but remaining at around 10 % on Peak Moor samples. Recovery may have been due to enhanced production of EPS after 28 days as well growth stabilising under reduced nutrient conditions. A similar pattern of early peak growth followed by decline and stabilisation was seen in earlier laboratory experiments using *C. vulgaris* (see Chapter 3, section 3.5).

Biomass increased over time on all samples inoculated with *D. olivaceus* (group C). However, the overall pattern of growth was more variable than for group A samples. Irrespective of stone type, all group C samples exhibited a decrease in biomass at 21 days of incubation, in line with a slight decrease in weight at this point in time (see Figure 70). All samples however recovered growth, which averaged at 70 % cover at medium density by 42 days of incubation.

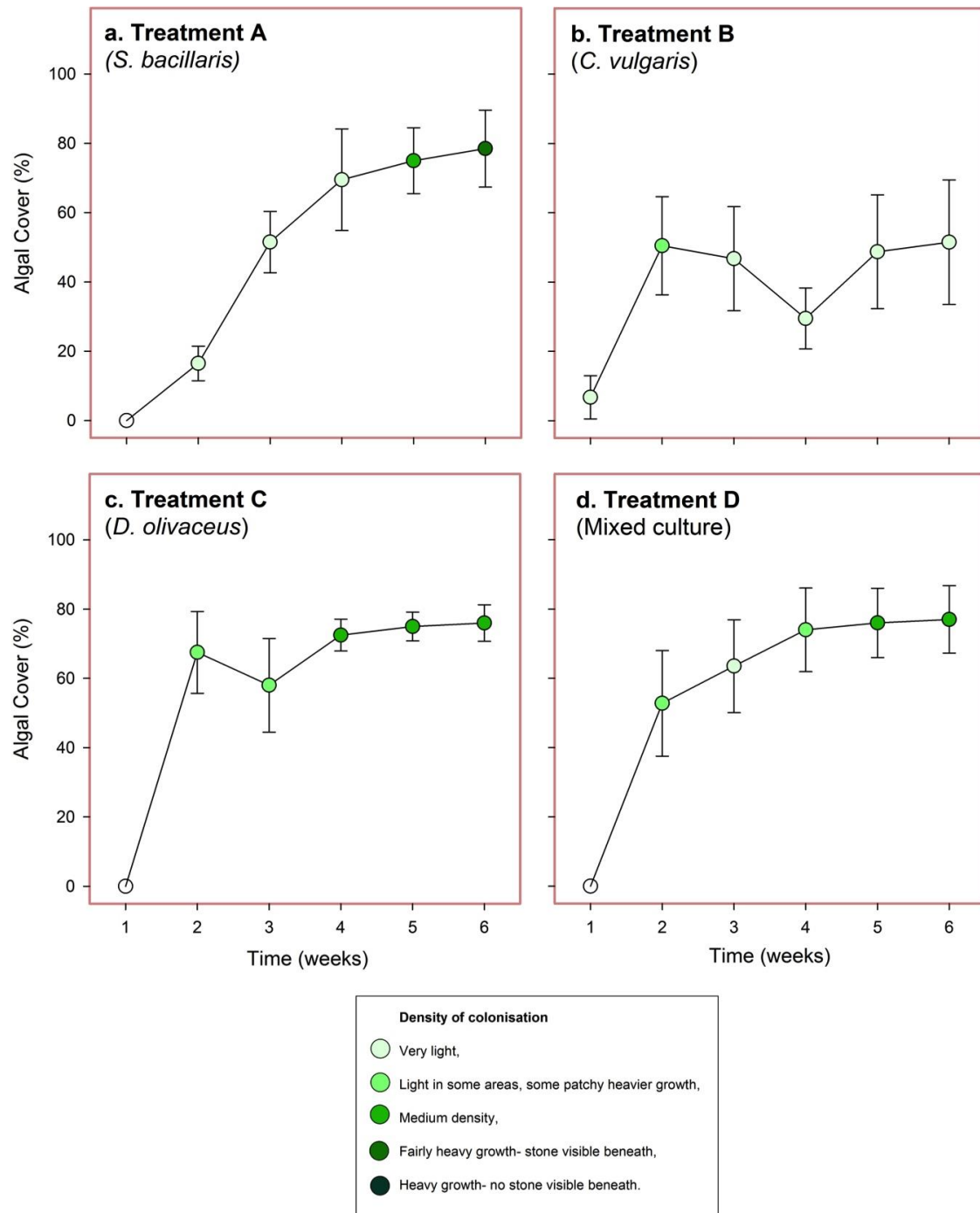
A statistical difference did not occur in total algal cover (irrespective of stone type) between samples inoculated with the mixed culture (group D) and *D. olivaceus* (group C). The pattern of growth over time between the two groups was also similar (decreasing at and subsequently recovering after week three). The trajectory of growth for groups C and D was very similar for all stone types (see Figure 72c and d, Figure 73c and d and Figure 74c and d for comparison).

Figure 72: Biomass change over time on Dungannon samples



Symbol positions indicate the averaged (mean) figure for algal cover for sample groups A, B, C and D.  
 Groups E, F, G and H remained free of colonisation so are not presented here.  
 Error bars represent standard deviation from the mean.  
 Symbol colours represent the average (mode) 'density of colonisation' for each sample group, per week.

Figure 73: Biomass change over time on St Bees samples

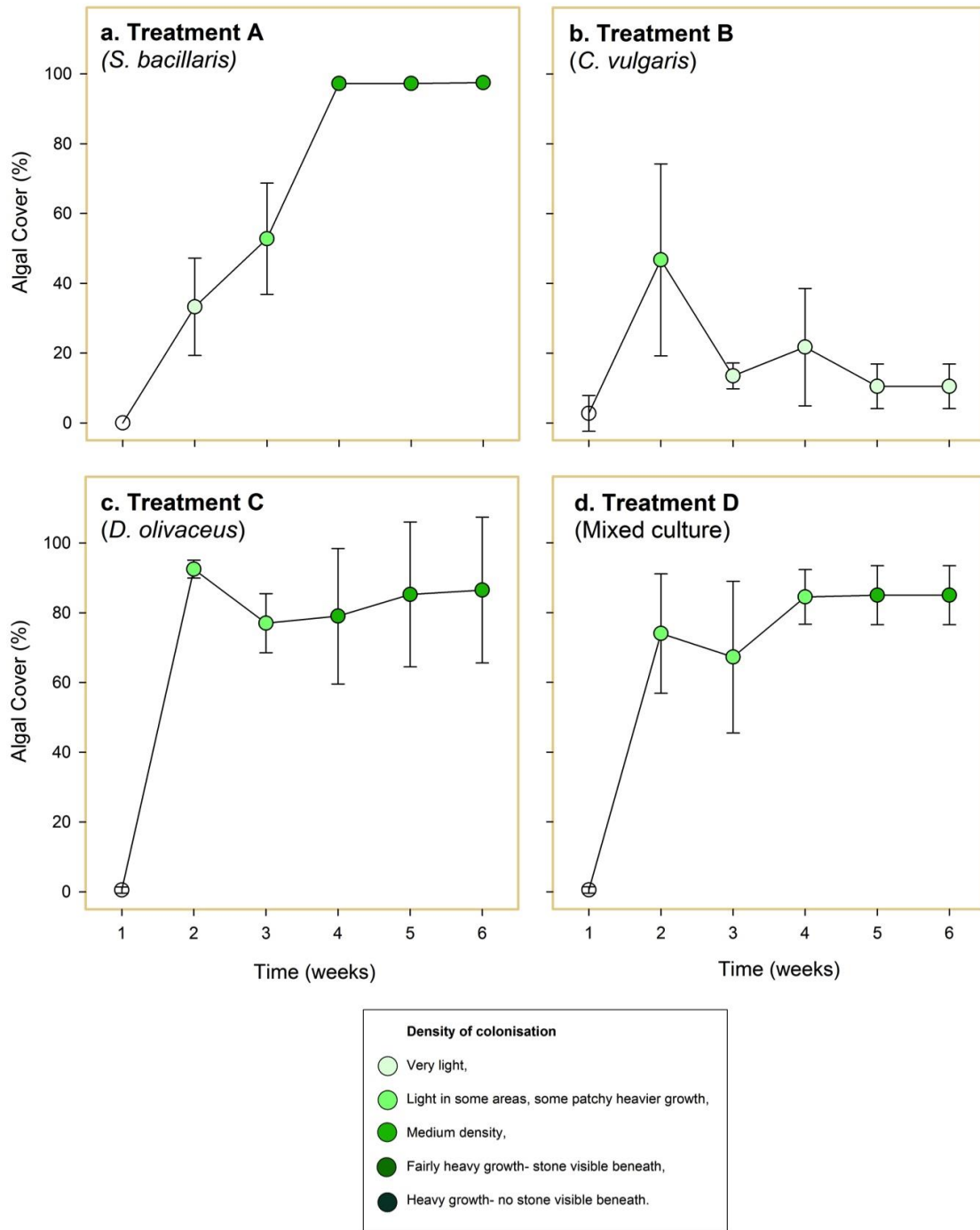


Symbol positions indicate the averaged (mean) figure for algal cover for sample groups A, B, C and D.  
Groups E, F, G and H remained free of colonisation so are not presented here.

Error bars represent standard deviation from the mean.

Symbol colours represent the average (mode) 'density of colonisation' for each sample group, per week.

Figure 74: Biomass change over time on Peak Moor samples



Symbol positions indicate the averaged (mean) figure for algal cover for sample groups A, B, C and D.  
 Groups E, F, G and H remained free of colonisation so are not presented here.  
 Error bars represent standard deviation from the mean.  
 Symbol colours represent the average (mode) 'density of colonisation' for each sample group, per week.

### *Detailed observed temporal trends in all groups*

#### **7 days**

After seven days of incubation, only group B (*C. vulgaris* treatment) replicates displayed any clearly visible colonisation of the surface (see Figure 72b, Figure 73b and Figure 74b) with samples of all stone types exhibiting around 10- 20 % cover with very light density. On replicates within groups C (*D. olivaceus* treatment) and D (mixed strain treatment) however, there appeared to be some very light colonisation, indicative of fledgling algal colonies.

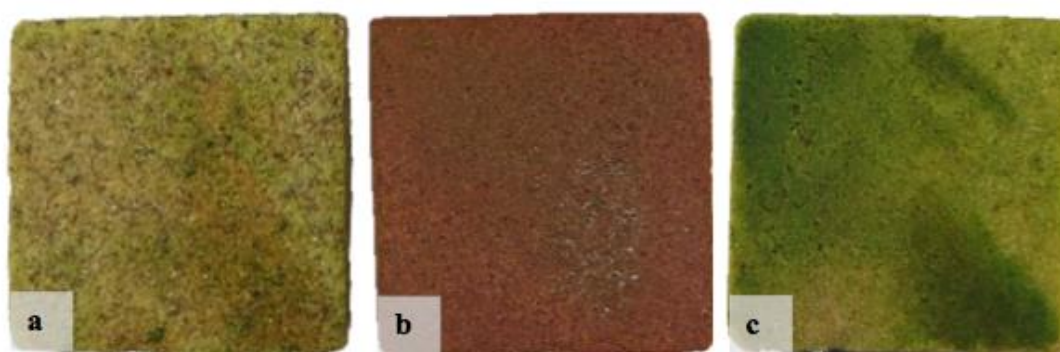
#### **14 days**

After 14 days of incubation, group A (*S. bacillaris* treatment) samples also began to exhibit colonisation. Low-density colonisation was apparent across the top surface of most blocks but was more profound on the lighter, buff coloured sandstones (the same pattern was apparent in group C, see Figure 71a and b). Algal colonies were clustered around the perimeter of sample tablets with some, sporadic colonisation of central regions.

Within group B, the majority of samples now exhibited widespread growth with some samples experiencing heavier growth in patches. This growth was not limited to the peripheries, but appeared more spatially centred. With regards to group C, some widespread colonisation was visible on samples (particularly, C1 and C5) by this point. On these samples, early colonisation appeared to be clustered around the outer perimeter, where denser growth was visible (C2, C17, C19 and C20). The pattern of growth favoured here was for initial colonisation of the surface peripheries, after which colonisation spread inwards towards the central zone. Within this group, colonisation appeared clustered and

in some cases, appeared to avoid laminations within the stone surface (C2, C20). After 14 days, group D samples exhibited a similar level of colonisation to group C. In particular, there was evidence of more ‘clumpy’ colonisation and EPS formation on Peak Moor samples in comparison to the other two, finer lithotypes (see Figure 75a).

**Figure 75: Photographic comparison of selected samples**



*a: Sample D19 (Peak Moor) at 14 days- EPS clumps, visible colonisation in grain spaces  
b: Sample C15 (St Bees) at 28 days- Heavy colonisation, EPS deposition and moisture retention at surface  
c: Sample A2 (Dungannon) at 35 days- growth concentrated around/ on clay and silt laminations (see section 2.2)*

### **21 days**

After 21 days of incubation, colonisation still appeared patchy on group A samples. There was little increase in the spread of colonisation, but there appeared to be an increase in the density of growth in areas already colonised, with evidence of some clumped colonies. Interestingly and in comparison with group C, colonisation was more visibly clustered around laminations on group A, Dungannon samples (A3, A4 and A8). This might indicate that more favourable conditions or minerals for growth might be available here, thereby enhancing growth of *S. bacillaris*. There was also some clumping of biomass around grain spaces on coarser, Peak Moor samples (A22).

Between 14 and 21 days of incubation, the spread of colonisation on group B samples decreased, demonstrating a degree of recession or dying back of biomass. In particular

(aside from some minor colonisation visible in grain spaces), very little to no colonisation of Peak Moor samples was visible (see Figure 74b). Comparatively, the pattern of growth on group C samples, was one of light but widespread colonisation, with some heavier colonisation on isolated tablets of Dungannon (C1, C3 and C5) and St bees (C9 and 15). Growth was generally more even and widespread on Peak Moor samples, which appeared more receptive to growth of *D. olivaceus* generally. After 21 days of incubation, most samples within group D demonstrated an increase in the spread of colonisation. In some cases, clustered growth in surface micro-fissures was evident (D3). Dungannon samples exhibited the greatest variability within group D (see error bars, Figure 72d), with denser growth on some tablets (D4 and D5) in comparison to little to no colonisation on others (D3) other than in marginal areas (see point above). Some clumps of EPS and clustered algal colonies were visible on Peak Moor tablets (D19 and D24).

### **28 days**

After 28 days of incubation, within group A most Dungannon samples exhibited dense, patchy colonisation. Colonisation was widespread across the surface of these blocks, but heavy in peripheral areas. There was also noticeable clustering of colonisation parallel to or around diagonal laminations (A1, A2, A3 and A6). St Bees tablets within this group exhibited widespread, light colonisation with two tablets only (A11 and A14), exhibiting particularly dense growth. Group A Peak Moor samples exhibited widespread but relatively even colonisation. On samples where there was patchier colonisation (A17 and A24) this was concentrated in the outer perimeter of the sample surface. Group B samples continued to experience a decrease in biomass spread and density. On group B Dungannon samples, where some colonisation remained, this was concentrated within the central zone of sample surfaces.

After 28 days, Group C Dungannon samples exhibited little further spread of colonisation. However, areas of growth did appear denser and there was visible deposition of EPS globules (C2 and C3). This sub-group exhibited quite a lot of intra- group variability, with some samples exhibiting medium density growth with others little to no growth at all. There was noticeable EPS deposition on St Bees samples (visible globules and mucous-like residue) and heavy colonisation on some samples (C11, C14, C15 and C16- Figure 75b).

Peak Moor group C samples appeared very moist at the surface, again with visible deposition of EPS globules (C20 and C23). Group D Dungannon samples exhibited more widespread colonisation after 28 days, with denser patchy growth in the peripheral areas of some samples (D8, top left corner). A similar pattern was observed for St Bees samples (D9, D10 and D13). Peak Moor samples appeared very moist at the surface, especially samples D17 and D19. Dense colonisation, particularly in grain spaces, was observed on these samples.

### ***35 days***

After 35 days of incubation, medium to heavy colonisation was visible on all group A samples. Total biomass on some of these samples was sufficient to obscure the underlying stone surface. On group A Dungannon samples (see Figure 75c), this colonisation was concentrated around laminations consisting of fine clay/silt minerals (see Table 4 and section 2.2).

Generally, group B samples experienced only a slight increase in biomass between 28-35 days of incubation. An increase in the spatial spread of colonisation could be observed on St Bees samples in particular, with samples appearing more moist at the surface (B15). However, little new growth was observed on Peak Moor samples despite samples also appearing more moist.

By this point in time, group C Dungannon samples exhibited relatively widespread growth with some maintained patches of colonisation in the peripheries. Interestingly, there appeared to be less favourability for laminations than with other algal treatments. St Bees samples exhibited a small increase in surface biomass. Those samples that were already sustaining dense patchy colonisation (particularly C1 and C15) appeared very moist with very visible EPS deposition at the surface. Peak Moor samples also appeared moist at the surface with enhanced EPS deposition on certain samples (C20, C23 and C24).

After 35 days of incubation, there was only a minor increase in biomass on Group D Dungannon samples. Biomass was spread quite evenly in this subgroup with no obvious preference for the peripheries or laminations. No significant change in St Bees samples was observed, though they appeared slightly 'drier' on the surface. Conversely Peak Moor samples appeared much more moist with globules of growth intermingled with EPS beginning to appear on some samples (D19 and D20). Again, colonisation did not appear to show any preference for peripheral areas or laminations.

***42 days***

Between 35 and 42 days of incubation, very little change was observed in both the density and spread of colonisation on the majority of samples in all groups. This appeared to be the period in which growth became stagnant with no major loss or increase in biomass on samples.

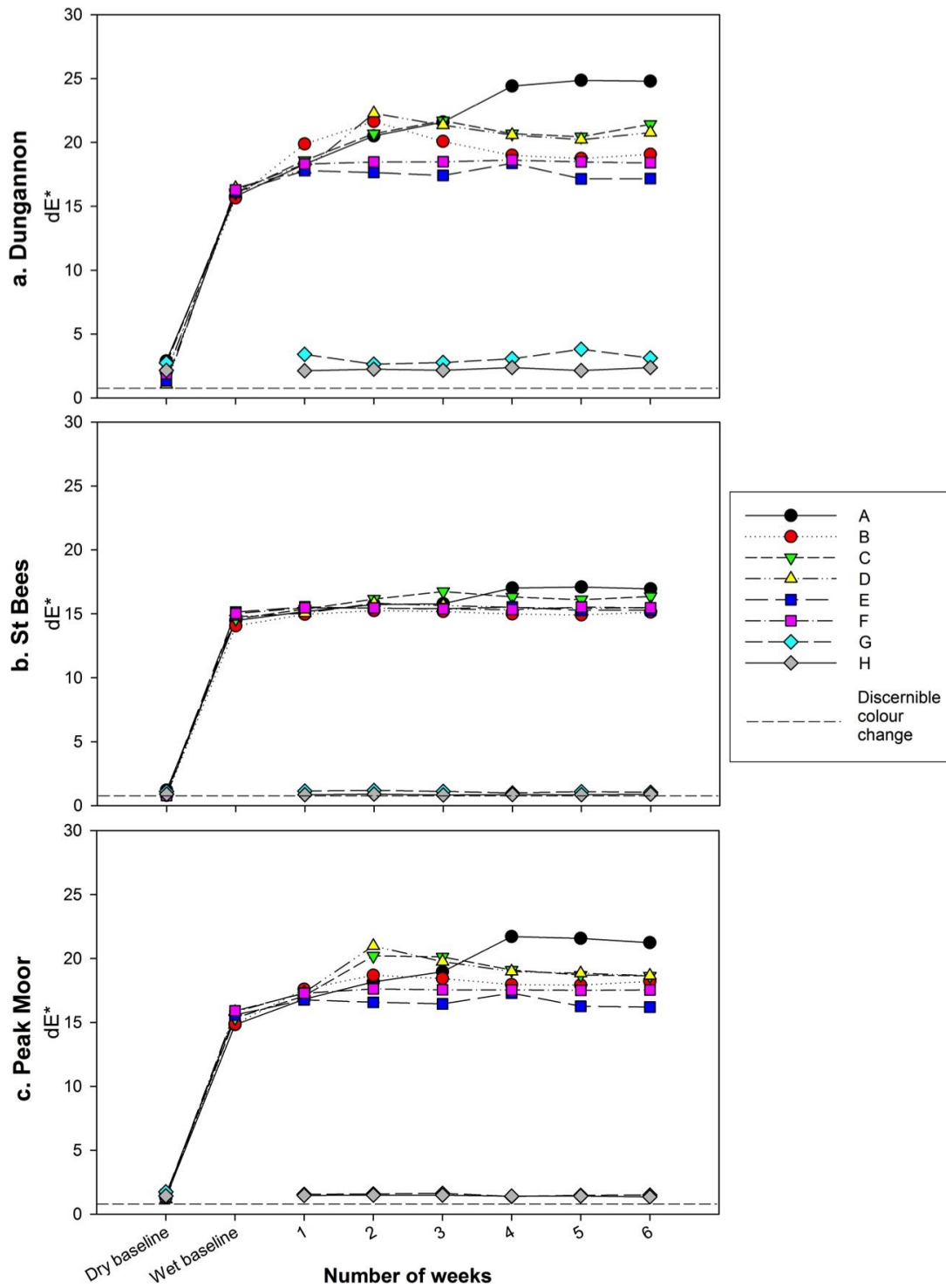
### *6.4.3 Spectrophotometric assessment of colour change*

#### *a. Total colour change (dE2000\*)*

*dE2000\** represents total change in colour from the target point to the dry baseline point, wet baseline point and weekly thereafter (see Figure 76) and is calculated as described in Chapter 4, section 4.1.4. Target points were calculated by averaging the dry colour for each sample group. Therefore ‘dry baseline’ measurements indicate the variability in total colour within each sample group. Most variability was observed in Dungannon (owing to differences in clay laminations within individual samples) and least in St Bees (which is known to be of consistent, homogeneous appearance) samples.

A significant difference was not detected in total colour change between Peak Moor and Dungannon samples at both three and six weeks of incubation. St Bees samples exhibited significantly less total colour change however, than the buff coloured stones (see Table 25). It is possible that less variability between treatment groups was observed on St Bees samples than the buff coloured stones because St Bees is a much darker stone, on which differences in total colour change are less measurable by the device used.

Figure 76: Total colour change (dE2000\*) over time



Data points represent the average (mean) colour change of each sample group (A-H) over time, categorised by stone type. Colour change (dE2000\*) is measured against the target measurement for each sample group. The dry baseline reading of each group is a measure of the inter-group variability in dE2000\* (as the target was the averaged colour for the whole group).

NB. Groups G and H were not subject to 'wetting' prior to the experiment.

See Table 22 for treatment group (A- H) set up.

**Table 25: Testing the significance of differences in total colour change (dE2000\*) between sample groups after 3 and 6 weeks of experimentation**

Factor	Results	
	21 Days	42 days
a. Influence of stone type	St Bees < Peak Moor = Dungannon	St Bees < Peak Moor = Dungannon
b. Influence of treatment type	H = G < E = B = D = A = F = C	H = G < E = B = D = A = F = C

*Tests (Kruskal-Wallis) measure difference in dE2000\* from the baseline to after 21 and 42 days of experimentation. All tests conducted at  $p < 0.05$ . < And > indicate degree and direction of difference, whereas = indicates no statistical difference between variables.*

Indeed, visual observations of biomass change indicate a similar level of spread and density of algal growth on St Bees samples (see Figure 73) as Dungannon (see Figure 72) and Peak Moor (see Figure 74) samples.

Dry control groups G and H experienced significantly less change in total colour than all other treatment groups. There was no difference between wet control and inoculated groups. This indicates that the main cause for the discernible difference between groups was due to inoculated groups and wet control groups being partially submerged in water, leading to significant and measureable darkening.

#### *Observed trends in total colour change*

Regardless of stone type, dry controls (G and H) displayed much less change in total colour than all other treatment types and the level of change was stable over time (see Figure 76). For all stone types, wet controls (E and F) exhibited the most noticeable change in colour after baseline wetting treatment. After this point, total colour fluctuated within the region of  $\pm 5$  colour space units (change was more variable on the buff coloured stones).

St Bees samples exhibited the least variability between treatment groups and overall, the least total colour change in comparison to Dungannon and Peak Moor samples (see Figure 76b). Conversely, Dungannon samples exhibited the most variability in colour change. Early change (around weeks one to two) was most noticeable for samples treated with *C. vulgaris* (group B), after which total colour change reduced, levelling off in line with wet control groups. This follows patterns observed in biomass change over time for group B (see Figure 72b).

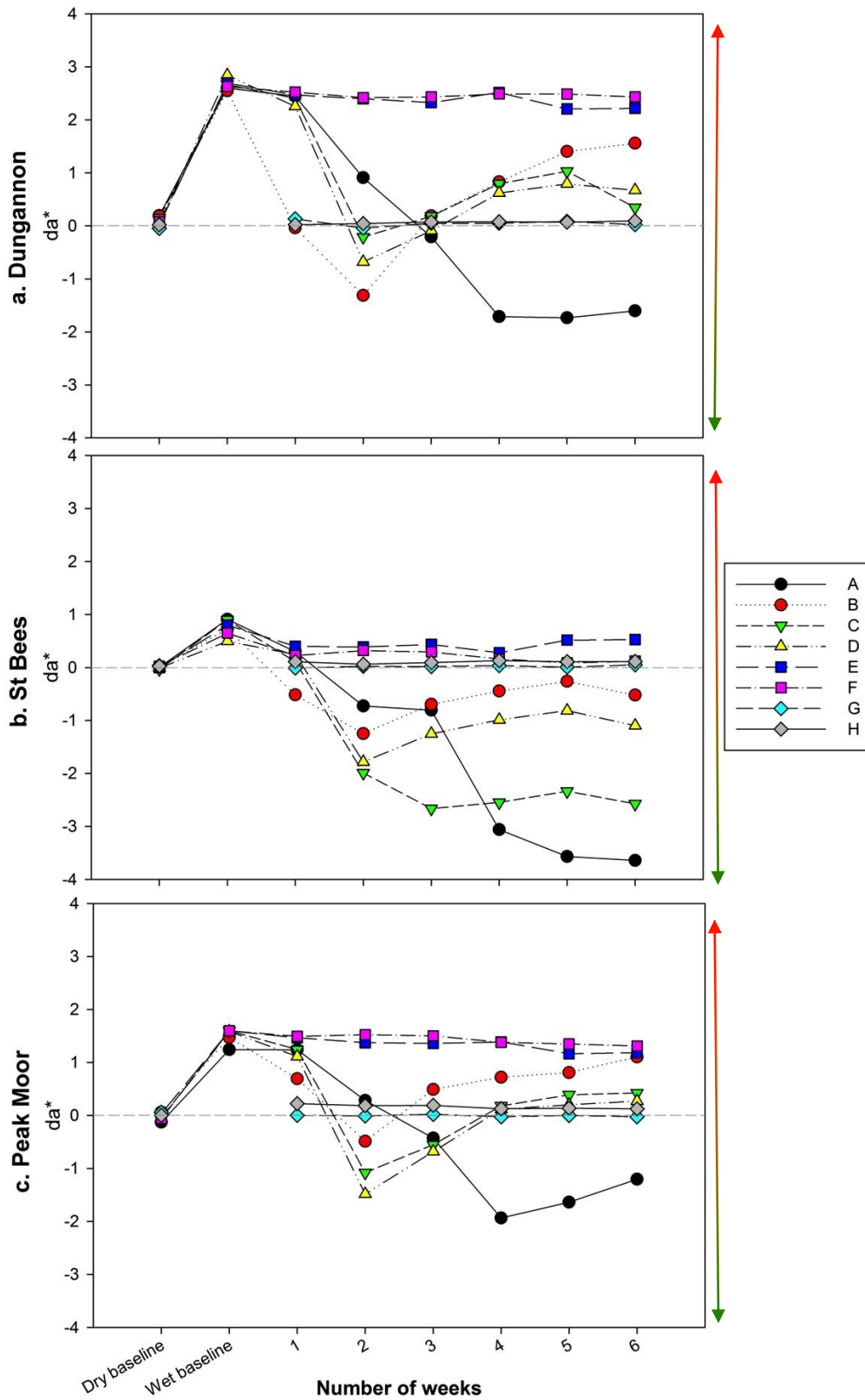
For both Dungannon and Peak Moor samples, treatment groups C and D exhibited a similar trajectory of colour change. Peak colour change was observed at two weeks of incubation after which change began to slowly decline to around 18-20 colour space units for both stone types.

For both buff coloured stones, treatment with *S. bacillaris* (group A) yielded the most colour change. Whilst initial change (within weeks one to three) was in line with the other algal treatments, later and more profound growth of the species resulted in much greater change amounting to around 22-25 colour space units.

#### *b. Greening of samples over time (da\*)*

The  $a^*$  colour scale represents colour hues varying from red to green, where a decrease in  $a^*$  values is indicative of greening. Change in  $da^*$  values from the target point to the dry baseline point, wet baseline point and weekly thereafter are presented in Figure 77. After 21 days of experimentation, St Bees samples had become significantly greener than Peak Moor and Dungannon samples, between which there was no difference (see Table 26a).

Figure 77: Change in 'greenness' (da\*) of samples over time



Data points represent the averaged (mean) change in 'greenness' (a\*) of each sample group (A-H) over time, categorised by stone type. 'da\*' represents the 'a' axis of colour where a decrease in values indicates greening over time. Greenness (a\*) is measured against the (dry) target measurement for each sample group.

NB. Groups G and H were not subject to 'wetting' prior to the experiment.

See Table 22 for treatment group (A-H) set up.

**Table 26: Testing the significance of differences in greening (da\*) between sample groups after 3 and 6 weeks of experimentation**

Factor	Results	
	21 Days	42 days
a. Influence of stone type	St Bees > Peak Moor = Dungannon	St Bees > Peak Moor > Dungannon
b. Influence of treatment type	A = B = C = D = G = H > E = F	A = C = D = G = H > B = E = F

*Tests (Kruskal- Wallis) measure difference in da\* from the baseline to after 21 and 42 days of experimentation.*

*All tests conducted at  $p < 0.05$ .*

*< And > indicate degree and direction of difference,*

*whereas = indicates no statistical difference between variables.*

With regards to influence of treatment type (see Table 26b) after 21 days, groups E and F (wet controls) appeared significantly less green than all other groups.

After 42 days, St Bees samples had become most green (in comparison to the original target), followed by Peak Moor samples. Dungannon samples appeared significantly less green than the other two stone types. At this point, treatment groups B (*C. vulgaris*), E and F (wet controls) appeared significantly less green than all other treatment groups.

Regardless of time period, St Bees samples exhibited the most significant greening in comparison to the other stone types (see Table 26a). This is likely due to the base red colour of the substrate, which would make any change in colour on the 'green' scale more profoundly visible and therefore, detectable by the spectrophotometer device.

Indeed, photographic evidence (see Figure 71b) and in particular, biomass data (see Figure 72, Figure 73 and Figure 74) did not indicate that St Bees samples had a significantly greater level of biomass than either Dungannon or Peak Moor samples.

Furthermore, the least difference in  $da^*$  figures was recorded between control groups and inoculated groups for St Bees (see Figure 77b) than for Dungannon or Peak Moor (see Figure 77a and c, respectively).

#### *Observed trends in greening of samples over time*

Dry controls (groups G and H) exhibited very little change in the  $a^*$  axis (see Figure 77). Of the dry cabinet controls (group H), Peak Moor samples became slightly redder during drying (see Figure 77c). All samples appeared most 'red' at the wet baseline point. For Dungannon and Peak Moor samples, wet controls did not change considerably from this peak point. St Bees controls (both wet and dry) did not exhibit as much variability as Peak Moor and Dungannon samples (see Figure 77b). Control samples increased in 'redness' by a maximum of one colour space unit, or exhibited very minimal change.

For all stone types, samples treated with *S. bacillaris* (group A) exhibited most greening. In the case of the buff coloured stones, greening was not linear over time but peak greening of around -2 colour space units occurred at week four of incubation (see Figure 77a and c). After this point, greening decreased slightly. This decrease in colonisation was also observed in the photographic record (see Figure 71).

For all stone types, samples treated with *C. vulgaris* (group B) demonstrated the earliest signs of greening (see Figure 71a and Figure 77) in the first week of incubation. For these samples, peak greening was noted in week two after which some colonisation died back resulting in an observed increase in  $da^*$  figures. Regarding the buff coloured stones, after two weeks of incubation samples treated with *D. olivaceus* (group C) and the mixed culture (group D) behaved in a similar way to group B samples (see Figure 77a and b).

St Bees samples treated with *S. bacillaris* exhibited a more linear trajectory of greening over time than growth of the species on the buff stones (see Figure 77b). An increase of greening of around 0.5 colour space units was measured weekly with ‘most profound’ greening occurring between weeks three and four. St Bees samples treated with *C. vulgaris*, *D. olivaceus* and the mixed culture all exhibited peak greening at week two or three of incubation. Conversely, those samples treated with *S. bacillaris* (group A) demonstrated greater total, but more steady greening peaking at week six.

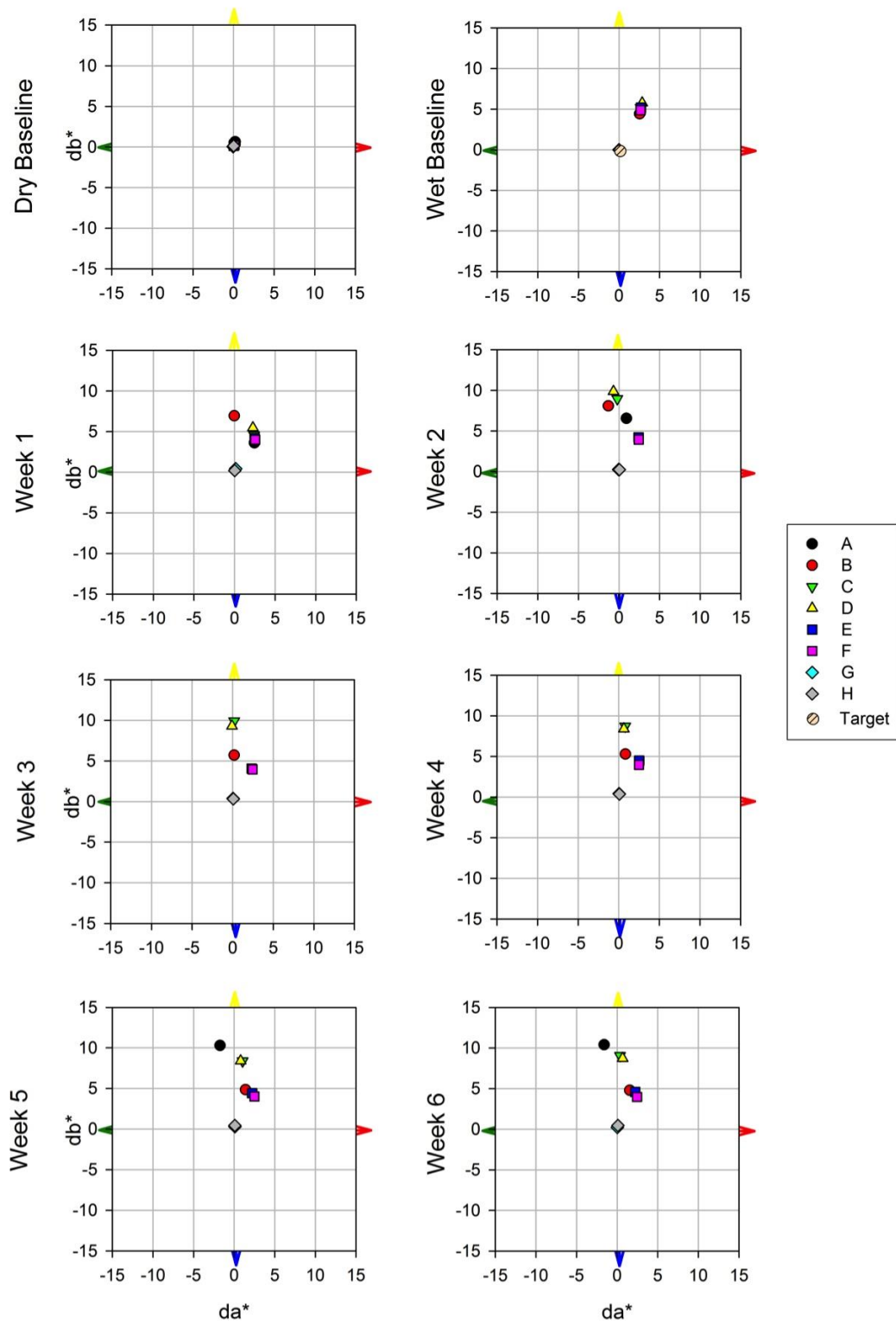
#### *c. Movement of samples through 2D CIE L\*a\*b\* colour space over time*

For all stone types, dry control samples (groups G and H) did not exhibit much movement in colour space over the six weeks of experimentation, indicating little change in their colour over time (see Figure 78, Figure 79 and Figure 80).

For the buff coloured stones (see Figure 78 and Figure 80), all samples became more red and yellow after baseline wetting treatment. Wet controls (groups E and F) did not move considerably from this position during experimentation. For both Dungannon and Peak Moor, inoculated samples (groups A-D) displayed a very similar trajectory of greening over time (see Figure 85).

As discussed, Figure 77 indicates that for both Dungannon and Peak Moor, peak greening and therefore, colonisation was detected for inoculated groups B, C and D around week two of incubation.

Figure 78: Movement of Dungannon samples through 2D CIE L\*a\*b\* colour space over time



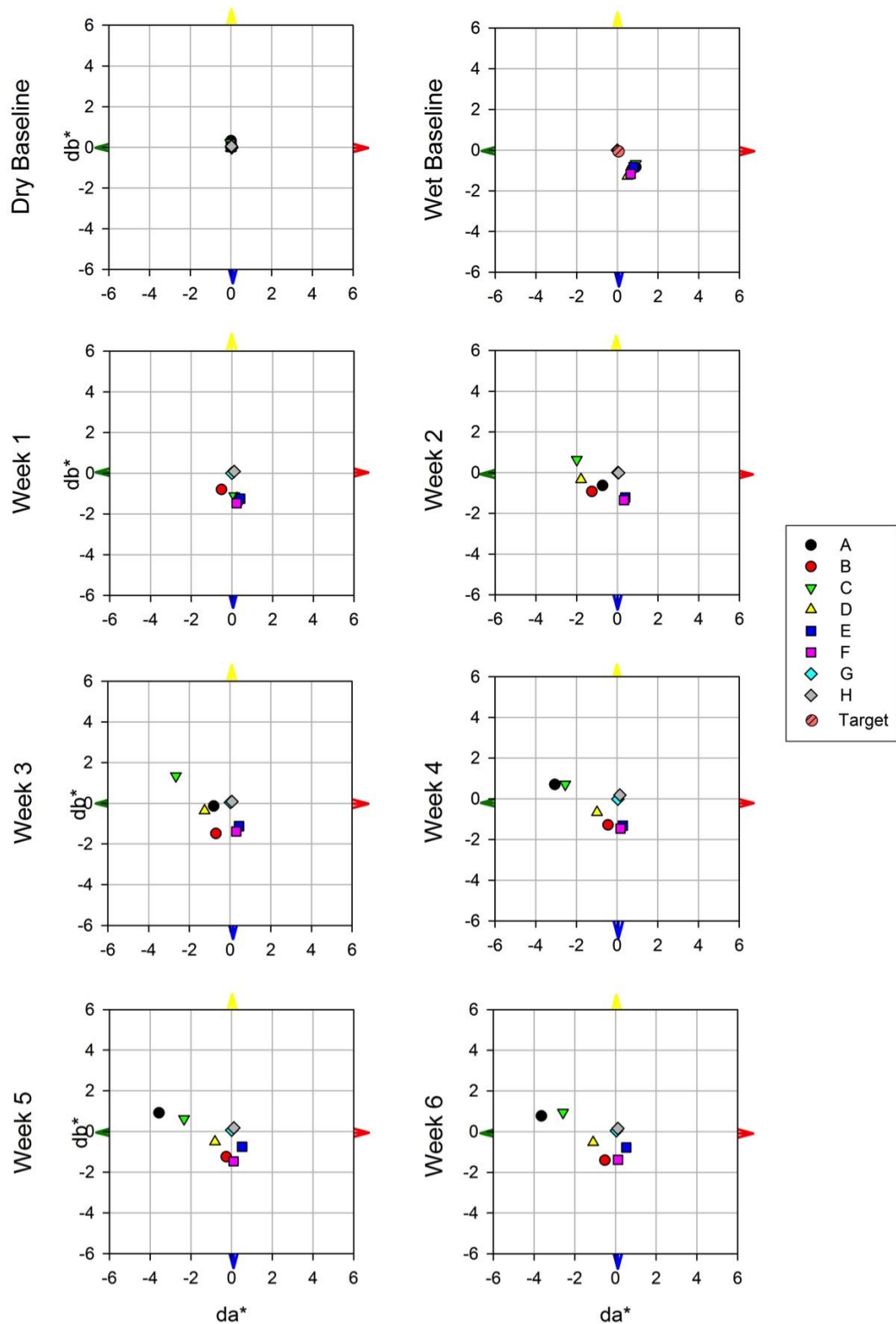
Data points represent the average (mean) colour change of each sample group (A-H).

Plots indicate directional colour change in reference to the averaged target colour (the central point of each plot) of all sample groups.

db\* represents movement in the yellow-blue axis and da\* represents movement in the red-green axis.

NB. Groups G and H were not subject to 'wetting' prior to the experiment.

Figure 79: Movement of St Bees samples through 2D CIE L\*a\*b\* colour space over time



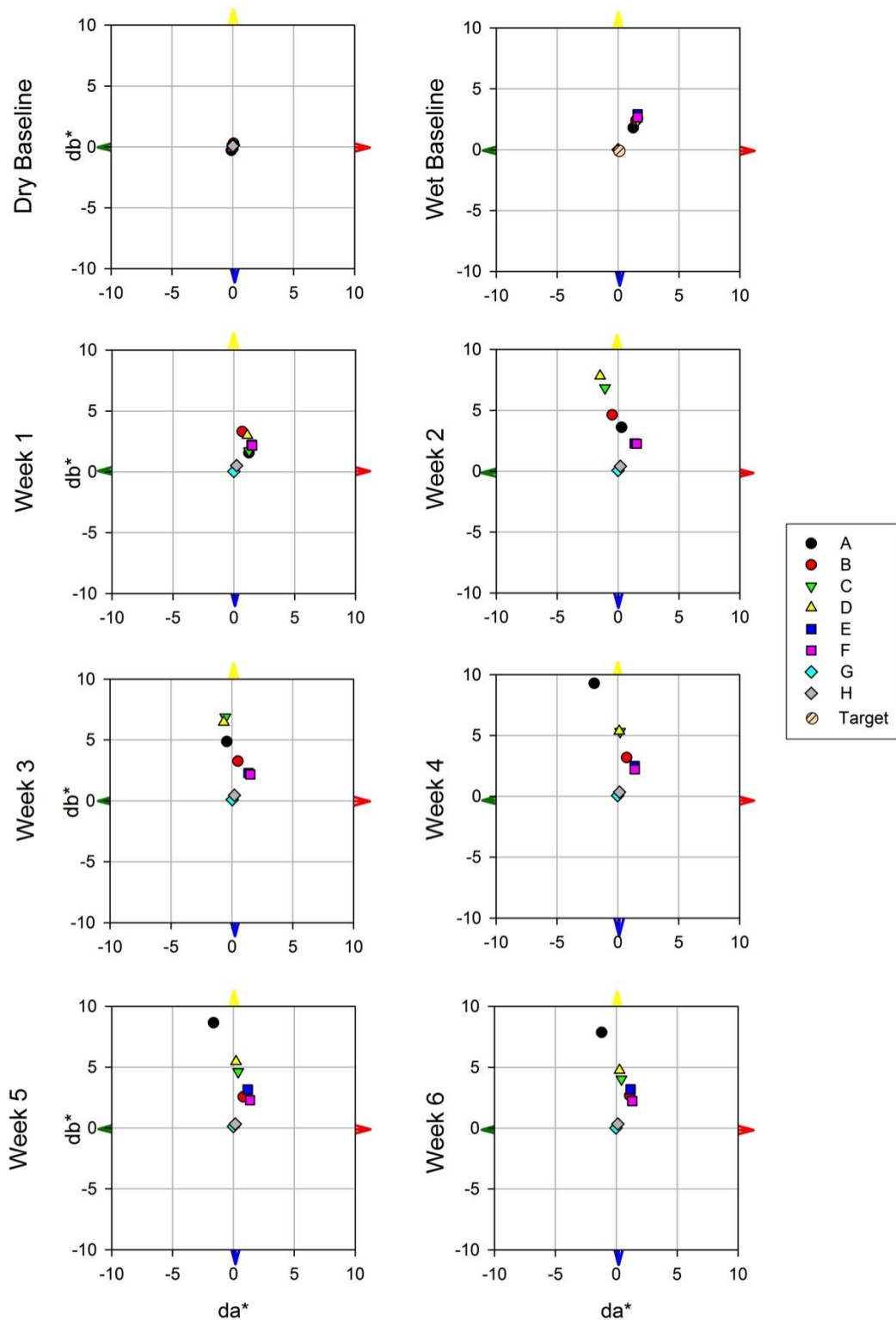
Data points represent the average (mean) colour change of each sample group (A-H).

Plots indicate directional colour change in reference to the averaged target colour (the central point of each plot) of all sample groups.

$db^*$  represents movement in the yellow-blue axis and  $da^*$  represents movement in the red-green axis.

NB. Groups G and H were not subject to 'wetting' prior to the experiment.

Figure 80: Movement of Peak Moor samples through 2D CIE L\*a\*b\* colour space over time



Data points represent the average (mean) colour change of each sample group (A-H).  
 Plots indicate directional colour change in reference to the averaged target colour  
 (the central point of each plot) of all sample groups.  
 $db^*$  represents movement in the yellow-blue axis and  $da^*$  represents movement in the red-green axis.  
 NB. Groups G and H were not subject to 'wetting' prior to the experiment.

When plotted in colour space (see Figure 78 and Figure 80), these samples made a clear movement from the wet baseline point to the yellow-green quadrant, with a strong bias towards yellowing. Beyond this peak point, samples made some movement back to the wet baseline point, with colour stabilising between weeks five and six. By the end of the experimental period, all inoculated samples had become more yellow-green than the target and wet baseline points.

For both buff coloured stones, group A (inoculated with *S. bacillaris*) samples demonstrated a similar trajectory of yellowing-greening to other inoculated groups (B, C and D). However, peak greening occurred at a later point at around week four (see Figure 77a and b). When plotted in colour space (see Figure 78 and Figure 80), the colour of these samples was more green and less yellow than other inoculated samples. Indicating perhaps, that stone samples treated with *S. bacillaris* may have gained greater total biomass or indeed, sustained healthier colonies.

St Bees samples displayed a noticeably different trajectory of colour change to the buff coloured stones, owing to the red base-colour of the substrate. Dry control samples (groups G and H) did not move noticeably from the target point whereas wet control samples (groups E and F) became more blue and red (see Figure 79) after wet baseline treatment. Wet controls did not move much beyond this position for the duration of the experiment.

All inoculated samples (groups A, B, C and D) moved towards the green-yellow quadrant of colour space (Figure 79) with the colour bias being towards greening and blueing. When plotted in colour space (see Figure 79, in particular weeks three to six) it is clear to

see that for St Bees, samples inoculated with *S. bacillaris* (group A) and *D. olivaceus* (group C) demonstrated the most greening (see also Figure 77b) and the most profound total change in colour from the wet baseline point.

## 6.5 Discussion

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This experiment was set up to compare the inherent bioreceptivity of three commonly used building stones to colonisation by single and mixed green algal cultures. Stone tablets were inoculated with algae and incubated. The bioreceptivity of these stones and the growth patterns of each of the treatments are discussed below.

### *Bioreceptivity of the three stone types*

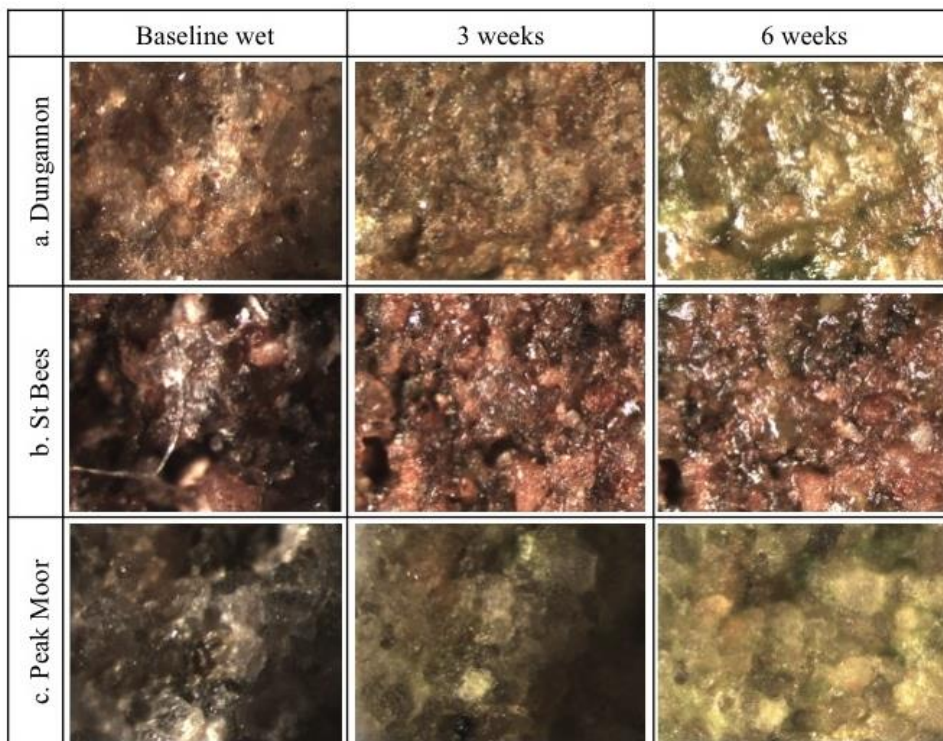
Treated and wet control Dungannon and St Bees samples gained significantly more weight by the end of the experiment than Peak Moor (see Table 23); a function of their finer texture and higher porosity. For both Dungannon and St Bees, each treatment group gained an average of around 3% weight after 42 days and the trajectory of weight gain was similar for both. Peak Moor samples however, gained the least weight in all instances of treatment (each treatment group gained an average of around 1.9% weight, after 42 days). Studies demonstrate that finer, higher porosity sandstones are better able to retain moisture and for longer periods, resulting in more prolonged periods of algal growth (Young and Urquhart, 1998) and better established colonies in the long term (Warscheid et al., 1991). Conversely, coarser grained sandstones encourage temporary microbial colonisation due to their greater permeability (Warscheid et al., 1991).

Results indicate that by the end of six weeks incubation, despite significant differences in

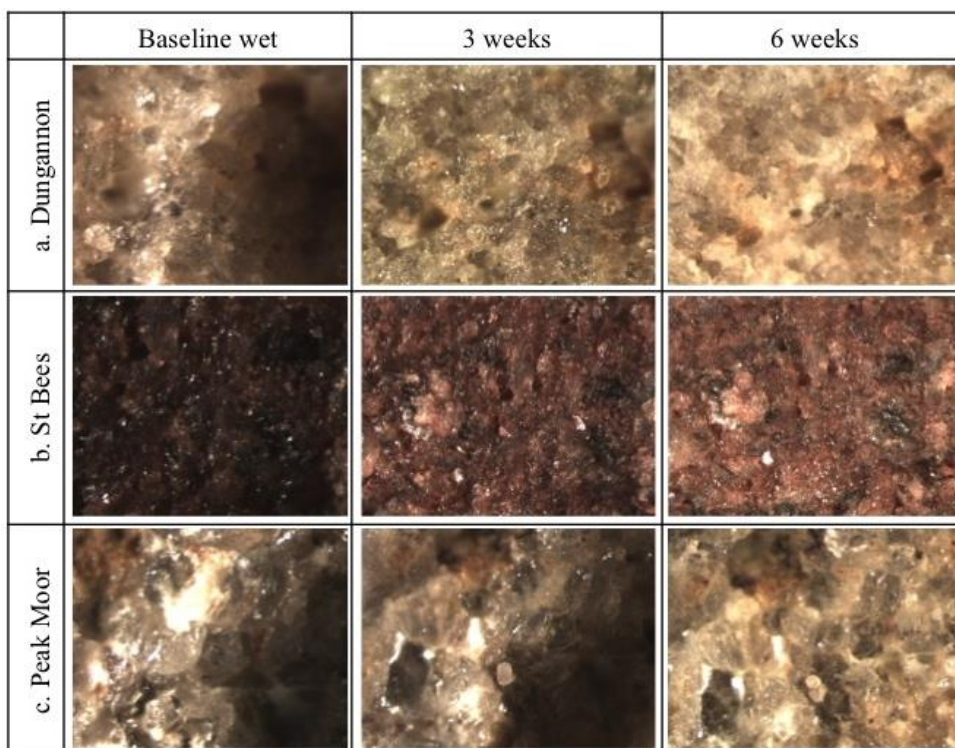
total weight change, there was no significant difference between biomass cover between stone types (see Table 24); demonstrating little influence of moisture uptake or porosity on total biomass observed. However, colonisation on the finer stone types appeared healthier and cells more embedded in EPS than on Peak Moor samples (see Figure 81 and Figure 83).

The bioreceptivity of a stone is not only measured by total biomass, but the spread of colonisation. In their study, Manso et al. (2014) observed that finer lithotypes result in more even colonisation and more porous (and therefore more permeable) stones allow moisture to enter the stone surface resulting in more uneven colonisation. However, we observed that algal cover was most variable and patchy on finer Dungannon samples, than St Bees or Peak Moor.

In this instance, patchy colonisation observed on Dungannon samples is less likely to be a function of texture and more likely due to mineral composition (see Figure 60). In particular, initial colonisation and clustered growth on Dungannon samples was often observed along clay laminations (see Figure 71a and Figure 75c), making these areas more bioreceptive. These clay laminations may have provided nutritional enrichment, greater surface area for cell adhesion and may demonstrate the direction of pore-space networks within the stone and therefore the direction and area around which moisture moves within the stone surface. For example in their study of Trani limestone, Tiano et al. (1995) noted concentrated microbial growth along higher porosity stylolite joints which also represented a preferential moisture inlet route at the stone surface.

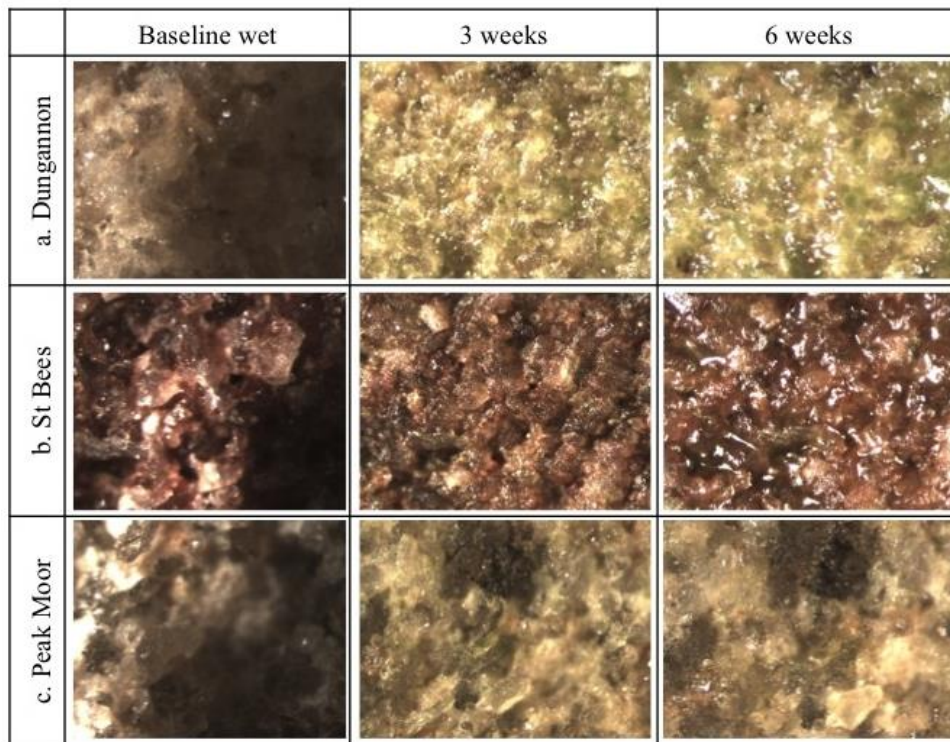
**Figure 81: Treatment A (*S. Bacillaris*) micrographs**

Micrographs at a magnification of 8 of samples A1, A9 and A17 (sample point **b**, see methodology section) at the wet baseline point and after three and six weeks of incubation

**Figure 82: Treatment B (*C. vulgaris*) micrographs**

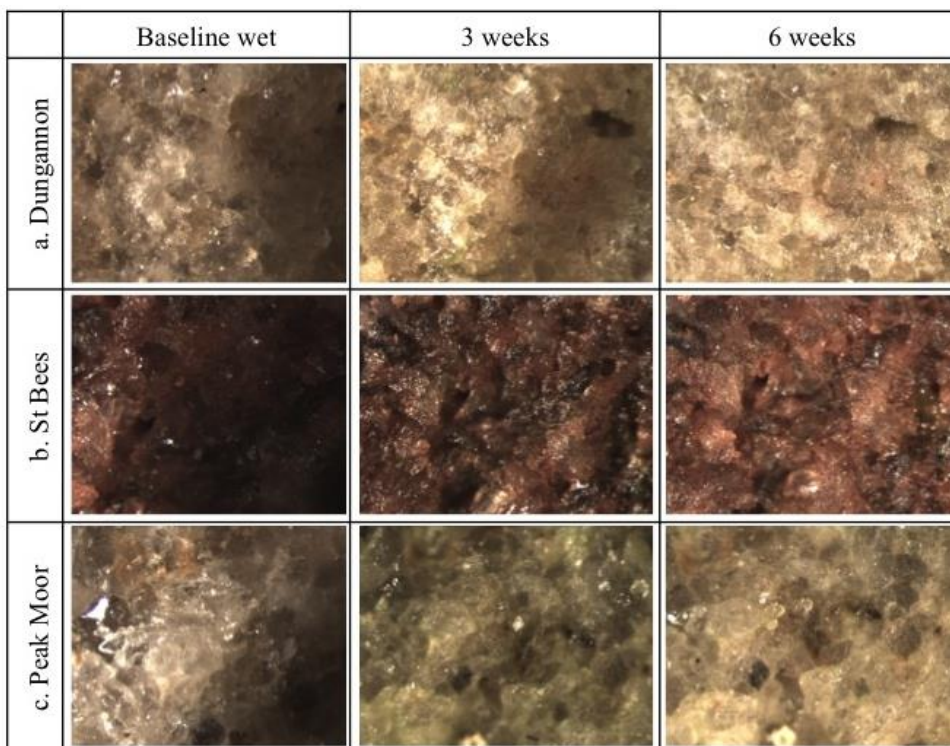
Micrographs at a magnification of 8 of samples B1, B9 and B17 (sample point **b**, see methodology section) at the wet baseline point and after three and six weeks of incubation

**Figure 83: Treatment C (*D. olivaceus*) micrographs**



Micrographs at a magnification of 8 of samples C1, C9 and C17 (sample point **b**, see methodology section) at the wet baseline point and after three and six weeks of incubation

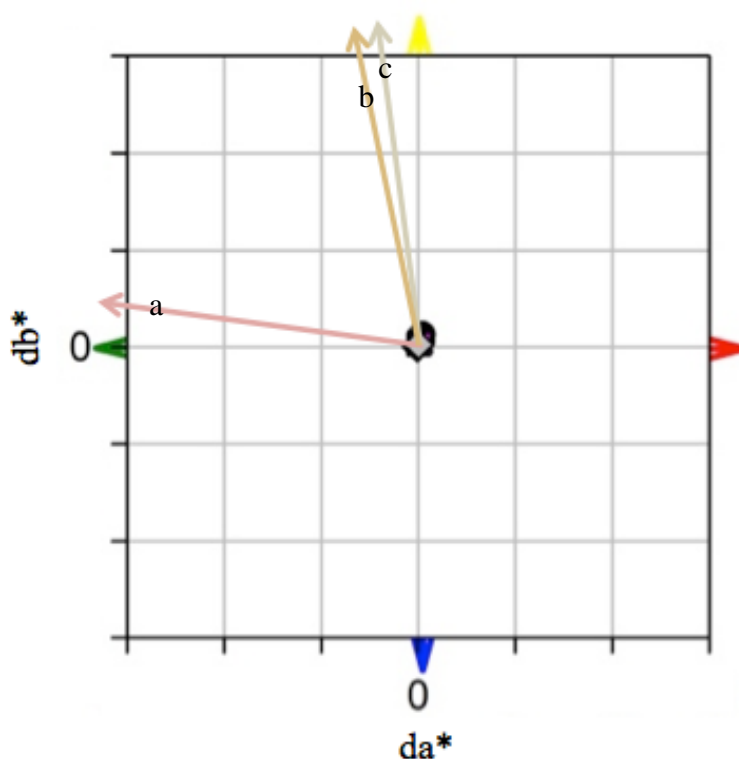
**Figure 84: Treatment D (mixed culture) micrographs**



Micrographs at a magnification of 8 of samples D1, D9 and D17 (sample point **b**, see methodology section) at the wet baseline point and after three and six weeks of incubation

Stone surface colour is also likely to have an impact on bioreceptivity and total amount of biomass observed (see Figure 60). After three and six weeks of incubation, total surface colour on the buff stones had changed significantly more than on St Bees (see Table 25). Whilst chromatic measurements indicate that most significant greening was observed on St Bees (see Table 26), as discussed, this is likely due to green pigments being more visible on the base red colour of the stone rather than being indicative of greater algal biomass more generally. Surface colour of inoculated St Bees samples moved towards the green-yellow quadrant of colour space, with chromatic bias being towards greater yellowing- greening with a loss in blue pigments (see Figure 85).

**Figure 85: Averaged trajectory of greening for each stone type, after 6 weeks of incubation**



*Schematic represents computed trajectory of greening based on the averaged position of inoculated samples (group A-D) in colour space at six weeks of incubation from the wet baseline point.*  
*a- St Bees, b- Peak Moor, c- Dungannon*

After 14 days of incubation, algal colonisation was more profoundly visible on the buff coloured lithotypes, particularly on samples treated with *S. bacillaris* (group A) (see Figure 71). This is perhaps due to the stone surface becoming more translucent as moisture was absorbed resulting in greater light penetration at depth in the buff coloured stones, than in the darker St Bees. In particular, after 42 days of incubation Peak Moor samples demonstrated significantly more greening than Dungannon samples (see Table 26), despite the trajectory of greening for the two buff stones appearing broadly similar under all treatment types (see Figure 77). However, the averaged trajectories of greening of the two buff coloured stones do highlight a small observable difference (see Figure 85). For both buff stones, colonisation resulted in surface colour moving distinctly to the yellow-green quadrant of colour space, as observed in previous laboratory studies (see Chapter 4, Figure 40). Unlike St Bees however, the chromatic bias was towards yellowing. The averaged trajectory does indicate that Peak Moor samples were more biased towards greening than Dungannon samples, indicative perhaps of healthier colonisation or greater total biomass on Peak Moor.

A microbial biofilm is likely to change the surface colour of a stone, which can affect its secondary bioreceptivity. The colour of a biofilm is dependent on its microbial constituents (Urzi and Realini, 1998) and the metabolites they produce. Chlorophyll (*a* and *b*), carotenoids and other associated compounds are often responsible for staining (McStay et al., 1996) and surface colour change. Whilst we focused on the primary bioreceptivity of the three stone types over 42 days and recorded notable greening of inoculated samples, a longer-term study would enable analysis of the impacts of surface colour change on secondary bioreceptivity. This might include impacts on albedo and light penetration at depth.

### *Treatment types*

Regardless of stone type, *S. bacillaris* (group A) replicates displayed the most logistic pattern of growth, whereby the initial, rapid phase of growth (2-4 weeks) levelled off after around 5 to 6 weeks of incubation (see Figure 72a, Figure 73a and Figure 74a). Peak growth for all group A replicates occurred at six weeks of incubation. Generally, *S. bacillaris* colonies had a darker and duller colour (see Figure 81) than the other species, which appeared a lot lighter, brighter and more translucent. In comparison to the other treatments, *S. bacillaris* cells appeared more embedded in grain spaces and better adhered to the surface; this was the case for all three stone types.

*S. bacillaris* growth was most widespread and homogenous on Dungannon samples (see Figure 72) followed by St Bees and Peak Moor (see Figure 73 and Figure 74 respectively), a likely function of the finer texture of Dungannon and St Bees. Growth appeared most attached and embedded in the smaller grain spaces of Dungannon and St Bees (see Figure 81a and b); a thick EPS layer was visible on these samples by six weeks of incubation. Growth of the species on Peak Moor was more patchy and there appeared to be less moisture and EPS accumulation above the surface (see Figure 81c), indicating perhaps that more moisture was absorbed into the stone subsurface owing to the more open texture of the stone (Manso et al., 2014).

We observed similar growth characteristics of *S. bacillaris* on Dungannon and St Bees samples in Chapter 5. In particular, in non-saline and low salinity conditions, cells appeared quite evenly spread on the surface and embedded in EPS (see Chapter 5, Figure 58d and e) as observed in this experiment. However, the biofilm layer appeared thicker and more luxuriant on larger 50 x 50 x 50 mm samples in Chapter 5 than on shallower 25

x 25 x 5 mm tablets as presented in this experiment. It is likely that the larger blocks of Dungannon and St Bees had a greater bioreceptivity due to their greater moisture retention capacity.

Of the four green algal inoculants, growth of *C. vulgaris* (group B) was most erratic. Whilst initial growth appeared healthy, algal cover and density peaked early at around 14 days (see Figure 72, Figure 73 and Figure 74). On all stone types, this was followed by colonies dying back with only limited growth visible in grain spaces on Dungannon (see Figure 82a, 3 weeks).

On all stone types, *D. olivaceus* (group C) and the mixed culture (group D) displayed a broadly similar pattern of growth (algal cover and density) over time (see Figure 72, Figure 73 and Figure 74). This may indicate that *D. olivaceus* thrived in the mixed culture environment, potentially out-competing other species. On both of the finer stone types (Dungannon and St Bees), at both three and six weeks of incubation visible clusters of cells were observed embedded in EPS (see Figure 83a and b), with noticeable accumulation of the sticky substance and surface moisture after three weeks. Biofilms on Peak moor samples seemed much less moist than on the finer stones (see Figure 81c and Figure 82c), with less accumulation of EPS. Cells appeared embedded deeper in pore spaces than on the finer stone types, likely due to the coarser texture of Peak Moor.

Biomass seems to be limited quite obviously, by nutrient availability. The blooms and rapid greening observed on groups B, C and D after 21 days and group A samples after 28 days can be explained by colonies taking advantage of the nutrients available and having adapted the stone to be a more hospitable surface (increased algal pathways and

EPS production). Consequently, maximum algal growth occurred at this point in time after which colonies began to die back or receded into the surrounding liquid in the petri dishes.

Beyond six weeks of incubation, the petri dishes themselves began to become colonised with green algae. In particular, cells were observed to adhere to the base and lower sides of the dishes. This indicates the strong influence of the inoculation protocol and enrichment (with growth medium) on the impermeable dishes, which have little to no nutritional property of their own. In their laboratory exposure study Guillitte and Dreesen (1995) noted a similar effect whereby polystyrene base stumps became colonised due to the exogenous influence of water treatment enriched with calcium carbonate. This suggests that with regards to the stone types analysed here, after a certain period of exposure time, the ability of inherent mineralogical properties of the stone to increase bioreceptivity may be reduced. However, mineralogical properties of the stone, which increase initial bioreceptivity (e.g. clay laminations in Dungannon, which increase the surface area) may go some way in catalysing early growth and promoting cell adhesion and health of more mature colonies.

## 6.6 Conclusions

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The first objective of this study was to compare the rate and morphology of growth of three axenic and one mixed green algal culture on three different building sandstones. Of the three stone types tested, the two finer stone types demonstrated greatest bioreceptivity. Dungannon and St Bees both showed evidence of the most widespread and healthy colonisation, with significant production of EPS by *S. bacillaris* and *D. olivaceus*.

Of the three stone types, the finer stones gained most weight (which can be used as a proxy indicator of moisture gain) by the end of the incubation period. Therefore, healthier colonisation observed on these stones may be explained by their greater porosity and capacity to absorb and store moisture. In addition, clay laminations in Dungannon were observed to catalyse growth in these areas, therefore increasing the stone's overall bioreceptivity. In the majority of instances however and given the nature of significance testing, it was not possible to determine absolutely which stone type was the most bioreceptive. This may indicate that algal treatment type or external influences (such as environmental conditions) may have more influence over colonisation rate and ecology than stone lithotype alone (Macedo et al., 2009).

Regardless of stone type, we found that growth of *S. bacillaris* was significantly more widespread than for other treatments. Approximations of algal density and measurements of colour also indicated denser growth and greening associated with this species. Patterns of growth and the trajectory of greening of *D. olivaceus* and the mixed culture were broadly similar indicating perhaps, dominant growth of this species in the mixed culture. In all instances, growth of *C. vulgaris* was least widespread and least healthy with colonisation peaking early at around one to two weeks of incubation, followed by a rapid decline in growth.

The second objective of this study was to investigate the nature of colonisation of each algal treatment on the stone types tested in terms of the components of colour. Previous experimental work (see Chapter 4) demonstrated that growth of *S. bacillaris* on Dungannon resulted in noticeable darkening and yellowing-greening of the stone surface.

We observed a similar trajectory of greening for all treatments on the buff coloured stones (Dungannon and Peak Moor) in this experiment. Greatest difference in the trajectories of greening was observed with St Bees. Whilst spectrophotometric measurements indicated that of the three stone types, St Bees exhibited greatest greening, this appears to be due to the base red colour of the stone. In our experiment, it is more likely that the lighter colour of the two buff stones may have increased their bioreceptivity due to the top few millimeters of the stone surface becoming translucent with moisture absorption. This may have resulted in light penetration at greater depth resulting in more widespread colonisation, particularly in the first three weeks of incubation.

It is apparent that a range of inherent factors including texture, porosity, mineral composition and surface colour affect the intrinsic bioreceptivity of a stone. The influence of these factors is not independent; rather, factors work together to determine the overall bioreceptivity of a stone to different green algal treatments.

# Chapter 7:

## The influence of location, aspect and surface geometry on algal greening

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### Chapter Summary

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Detailed observations of building façades show that algal biofilm colonisation is often patchy, with some areas much more prone to ‘greening’ than others. Preferential algal greening is hypothesised to relate to factors which improve the bioreceptivity of the surface. This includes factors inherent and external to the stone and those related to its location and geometry (architectural). Together these factors determine moisture regimes, which have a major impact on algal colonisation patterns. We designed a field experiment to evaluate the factors influencing algal biofilm growth patterns on real, complex architectural elements in order to provide a better model of patchy greening and identify which factors (shade, aspect and geometry) are most important. Therefore, this chapter directly addresses Objective 5 of the overall thesis (see section 1.9). We propose the novel use of hydrological terminology to identify rainfall stores and spillways, which are likely to increase the surface bioreceptivity of vulnerable sandstone structures.

Six weathered sandstone balusters, previously part of a building façade in northern England, were characterised and then exposed in a one year field experiment in central Oxford. Initial characterisation of the balusters involved surveys of surface moisture and colour. These measurements were repeated at the end of the experiment. During the field experiment surface moisture, temperature and relative humidity patterns were monitored regularly at six monthly or bimonthly intervals. The results indicate the complexity of

algal biofilm colonisation on real architectural elements in relation to any measurable explanatory factors and identify surface geometry as the most important factor controlling colonisation patterns.

## 7.1 Introduction

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Algal greening on real architectural surfaces is controlled by an array of factors, including the inherent characteristics of the building stone (such as porosity, mineralogy and surface roughness), the location (such as aspect, presence of shade) and the specific geometry of the architectural element. Together, these factors influence the interaction between the stone and its local microclimate, producing some zones which encourage moisture retention and others which shed water from the surface. Given that moisture contents are known to influence algal colonisation patterns, these factors are thus critical to explaining the patterns of algal colonisation observed.

### *Microclimatic influence*

Photosynthetic species must be able to tolerate the harsh conditions present at the lithosphere-atmosphere boundary (Gorbushina, 2007). This includes fluctuations in temperature, relative humidity, rainfall and light levels. Organisms present on a stone building must tolerate ambient changes in these conditions as well as changes at the micro-scale. Photosynthetic green algae are highly tolerant of environmental and in particular, osmotic stress (Gustavs et al., 2010) caused by the extreme dynamism of micro conditions (Cutler and Viles, 2010).

All buildings form a distinct relationship with the local climate that surrounds them. The traditional architectural features that characterise our built heritage be they turrets, buttresses, balustrades or the walls themselves, intercept and alter wind, moisture and solar conditions at the boundary layer. A number of microclimates can form around a stone construction within the range of a few hundred metres. For example, significant temperature differences can occur between one side of a building and another (Pope et al., 2002). These variations can go some way in explaining the complex patterns of algal greening observed on sandstone buildings across the northern parts of the United Kingdom.

The influence of *aspect* on patterns of biological colonisation has been studied quite extensively on building monuments in the UK (Jones et al., 1999; Robinson and Williams, 1999; Mottershead et al., 2003) as well as more recently in exposure trials in Northern Ireland (Adamson et al., 2013a). The orientation of a building will influence the levels of wind-driven rain and solar incidence walls receive and consequently their time-of-wetness (Smith et al., 2008; McCabe et al., 2013). The time-of-wetness (surface and interior) of stone walls greatly affects their bioreceptivity and ability to be colonised by different microbial organisms (Guillitte, 1995; Di Giuseppe, 2013). In the United Kingdom therefore, it is the south-west faces which receive the most direct rainfall and wind ventilation whilst northern faces remain relatively shaded.

The example of St. Mary's Church, Lisbellow in north-western Ireland (see Figure 86) demonstrates that differences in microclimatic conditions related to aspect can have significant impacts on the nature of biological soiling. Here, the northern façade exhibits pronounced growth of reddish-brown patinas of *Gloeocapsa* (low light intensity and

moisture-loving species). Conversely, there is green growth on the southern façade owing probably to a greater level of solar incidence and ventilation. In both cases however, growth is concentrated around areas of the facade where there is likely to be moisture accumulation. These dense patches of colonisation commonly occur around architectural detailing such as stone buttresses and string courses as well as near the ground due to capillary rise from ground water and splashback from the ground.

**Figure 86: St Mary's Church, Lisbellaw**



*Left) Northern façade, Right) Southern façade. Photos courtesy: Adamson et al. (2009)*

### *Architectural features*

Architectural features add to the aesthetic appeal of historic buildings. However, these features often have a more fundamental purpose. Buttresses for example, are paramount in providing structural support for immense, solid stonewalls. Other features have a more modest purpose; windowsills, string-courses and gargoyles for example are strategically placed to shed excess rainwater. However, it is often these features that lead to the stagnation of moisture and dust leading to microbial colonisation (Khan and Kulathuran, 2010). The bioreceptivity of a stone feature, which refers to the material properties of the substrate which contribute to its colonisation (Guillitte and Dreesen, 1995) is therefore,

greatly influenced by its intended purpose within the building matrix as well as by its mineralogy, porosity and surface roughness.

Observations of historic sandstone buildings in the north west of the UK and elsewhere demonstrate a complex relationship between the location of architectural features and algal greening (see Figure 87). Mapping colonisation patterns (see Figure 88) reveals a concentration of greening around joints, windowsills, string-courses and buttresses. This indicates that certain areas of a façade are naturally more receptive to colonisation. This is primarily due to the frequency they are wetted by rainfall and the moisture retention capacity of the stonework (which is influenced by ambient wind and temperature conditions).

**Figure 87: Patterns of biological colonisation on sandstone balusters in contrasting locations**



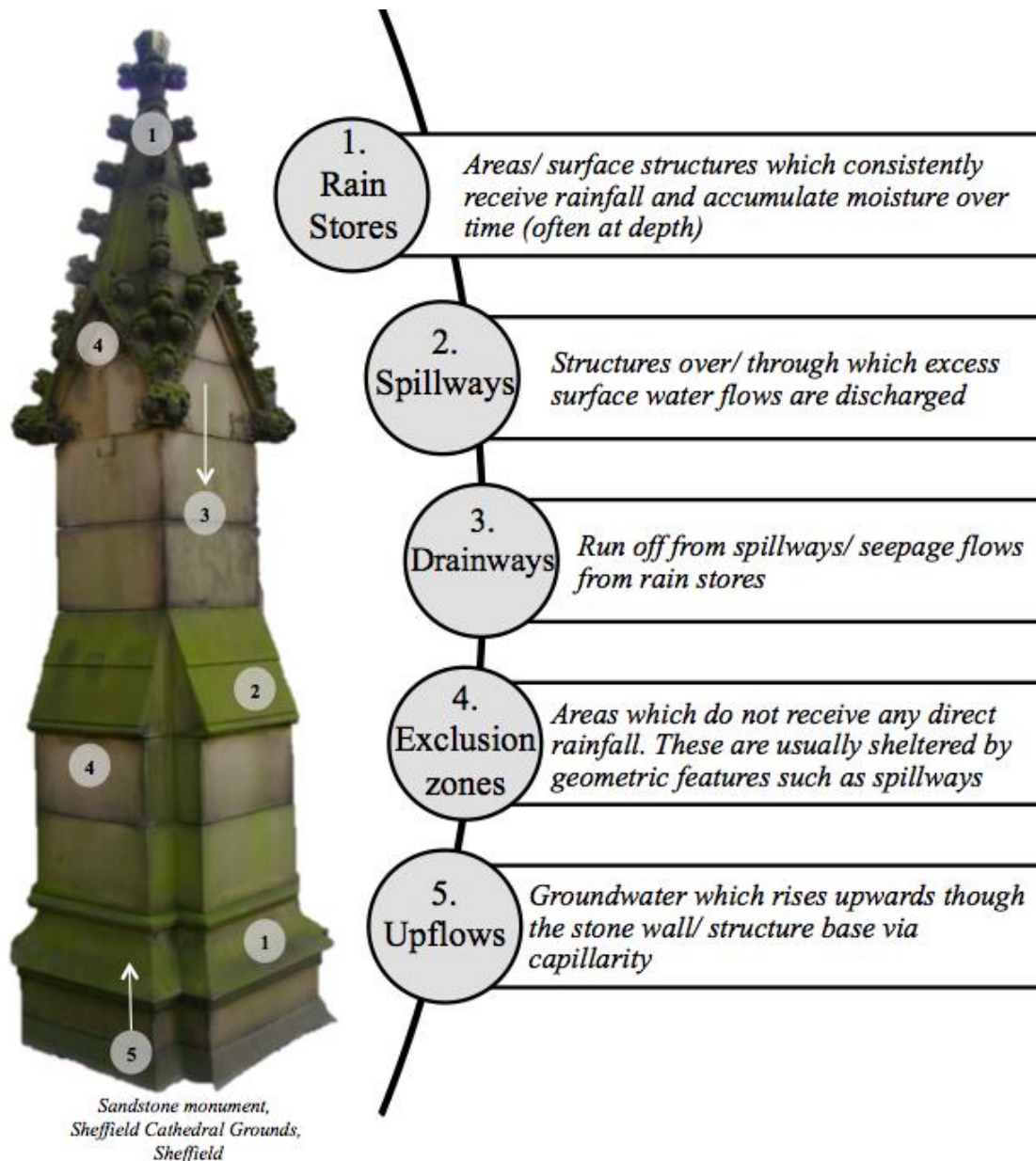
**Left:** Biological decay leading to replacement of sandstone baluster (2 Waterloo Place, Edinburgh),  
**Right:** Colonisation of cornice and plinth above and below recently cleaned brownstone balusters (Cedar Hill Bridge, Central Park, New York)

**Figure 88: Patchy algal greening, eastern facade, Sheffield Cathedral**



Green algal colonisation on sandstone facades is often very patchy in nature. However, the inherent bioreceptivity of the underlying stone is significantly increased by prolonged time-of-wetness (Smith et al., 2004; Jain et al., 2009). Hydrological terminology can be usefully applied to sandstone structures to identify regions of moisture accumulation and loss (see Figure 89). Zones of moisture accumulation will be more bioreceptive in comparison to drier areas. It is the rainfall stores, spillways and active drainways which are most likely to be colonised first. In many cases, moisture catalyses the rate and extent of colonisation across a façade. The presence of moderated shade and wind shelter exacerbates this effect. Smaller features in the stone such as indentations and concave detailing can form ideal niches for dust and moisture accumulation and therefore, algal colonisation over time (Ortega-Calvo et al., 1995; Miller et al., 2010). In all cases, the effect of moisture is moderated by the influence of aspect and angles of both rainfall and incident solar radiation.

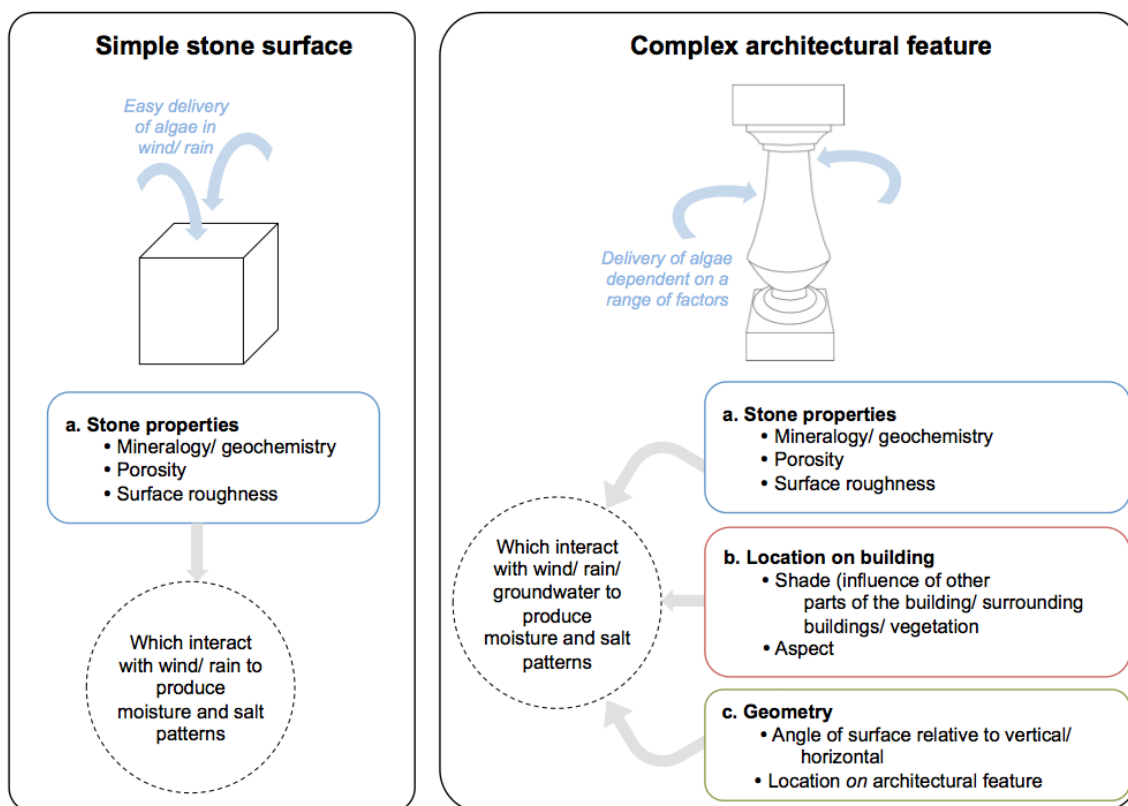
Figure 89: Hydrological features applied to sandstone structures



### *Baluster field experiment*

Previous experiments on green algal colonisation have looked at simple stone surfaces under controlled laboratory experiments. Here we have designed an experiment to better evaluate the range of controls on algal colonisation on more complex surfaces. As Figure 90 summarises, these controls relate to the stone feature's properties (inherent bioreceptivity), its location on the building and its geometry or morphology.

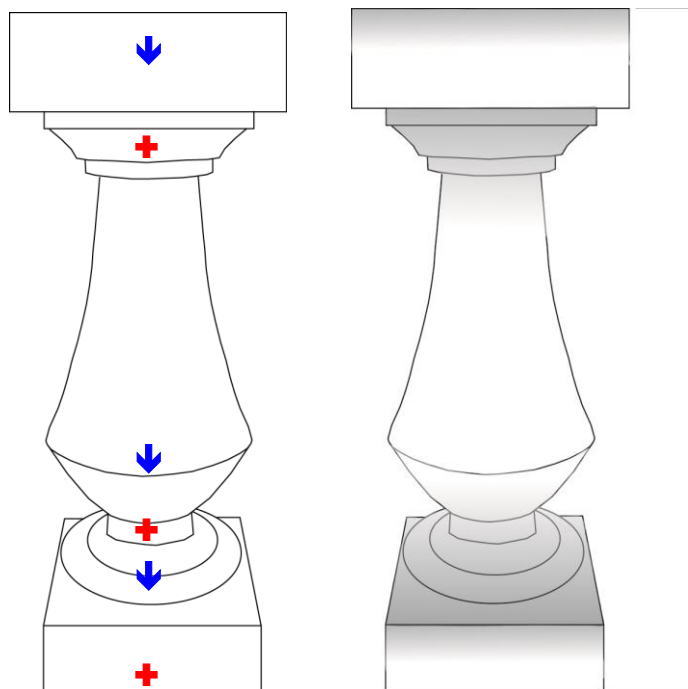
Figure 90: What controls algal colonisation patterns on complex architectural features



Stone balusters were chosen to represent the variety of both ornamental and architectural features commonly found in historic buildings.

A typical baluster consists of a protecting course (upper stone cap), moisture stores and spillways (see Figure 91). As a result of its geometry a baluster will form a dynamic relationship with its surrounding microclimate. For example, a single baluster might typically be exposed to differential patterns of incident solar radiation and shading, as well as intercepting and therefore modifying the wind regime around it. However, the impact of these microclimatic variables is moderated by the aspect and height at which the baluster is situated as well as ambient and macro-scale climate (including changes in seasons).

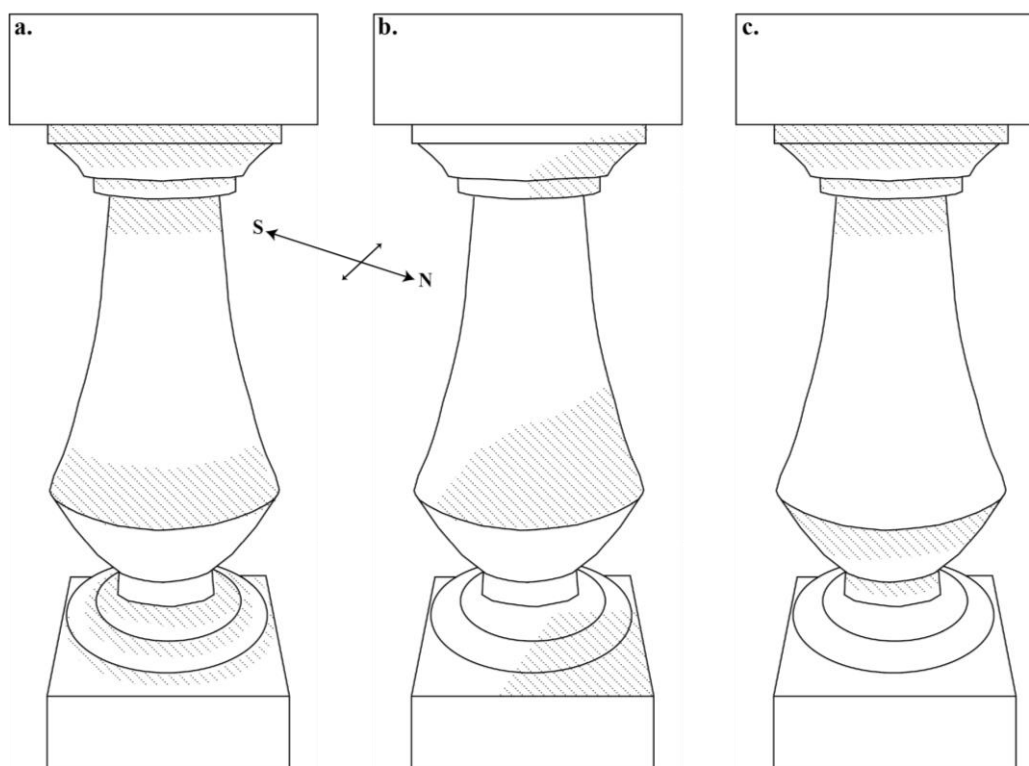
**Figure 91: Modelled interactions between architectural geometry with surface moisture and light**



*Left: Surface moisture model- Rain stores + and spillways ↓ on a typical baluster*  
*Right: Light model- Natural light shading on a typical baluster*

In order to produce a testable model of greening on a complex architectural object like a baluster, three experts on stone deterioration were asked to map onto a sketch of a baluster where they would predict greening to be most pronounced. Figure 92 illustrates the different predictions made by considering the morphology of a typical baluster and the ways in which it might be affected by micro and macro climate. Such differences in greening may also lead to patterning of associated stone decay (Papida et al., 2000).

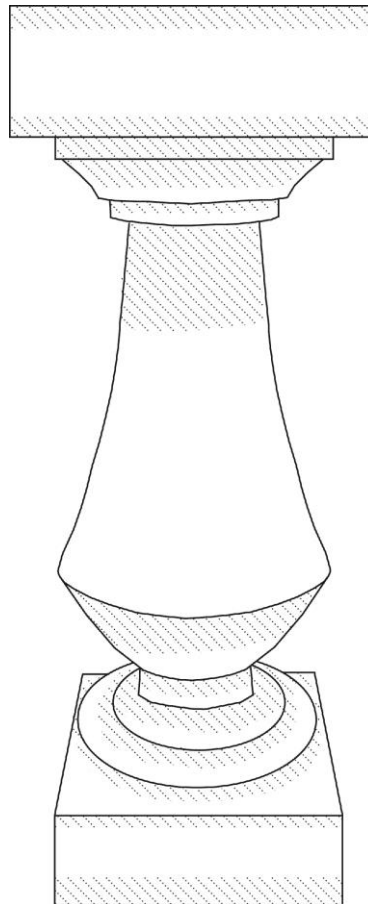
This investigation aimed to explore the interaction between microclimatic variables and the shape of a stone object and consequently, the role this plays in algal greening.

**Figure 92: Proposed models of greening**

*Models of greening shaded independently by: Professor Heather Viles, Dr Julie Eklund (SoGE, University of Oxford) and Dr Stephen McCabe (Queens University, Belfast)*

Our hypothesised model of greening, based on laboratory and field observations, the location of potential rain stores and spillways (see Figure 91) and informed by expert opinions (see Figure 92) is presented in Figure 93. A hierarchical approach was taken, in which we analysed the influence of location (shade vs exposed conditions), aspect and surface geometry in influencing greening patterns. In particular, we hypothesised that colonisation would be most pronounced in zones of moisture accumulation (moisture stores) whilst spillways and exclusion zones (see Figure 89) would remain comparatively free of colonisation. These hypotheses are based on the assumption that prolonged wetting and moisture accumulation result in more widespread and healthier biofilm development. Overall, the study evaluates whether the morphology of the object in question makes it more or less susceptible to greening.

**Figure 93: Hypothesised model of algal greening**



## 7.2 Aim and key questions

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**Aim:** To investigate the role or *architectural factors* in the development of algal greening by exposing sandstone balusters in a year- long field experiment

The research questions posed were:

- R1.** Does the degree of shade experienced by the baluster influence the nature and patterns of algal colonisation?
- R2.** Does aspect influence the nature and patterning of algal colonisation?
- R3.** Does position, in relation to the complex geometry of the baluster, influence the nature and patterning of algal colonisation?

## 7.3 Materials and Methods

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### *Stone preparation*

To test the validity of our hypothesised model of greening (see Figure 93), six reclaimed sandstone balusters were sourced from a building in Stirling, most likely constructed in the late 19<sup>th</sup> Century. The weathered balusters, Ionic in order, encompassed a range of angles as well as areas prone to shading and other, more open areas (see Figure 91). The stone, buff in colour, is likely to be a local sandstone from the Millstone series.

### *Field Sites*

Two exposure sites were chosen in a private garden in the grounds of Worcester College, in the centre of Oxford (see Figure 94). Both sites experienced the same ambient conditions yet offered contrasting microclimates. Site one was an ‘exposed’ location (see Figure 95 and Figure 96) providing some, moderated shade in the form of a roof overhang. Site two was ‘shaded’ throughout the year to varying degrees by overhanging trees and surrounding shrubbery. Three balusters were placed at site one and two at site two. At each site, the balusters were placed atop a base of gravel, bedded with sand to reduce water backsplash. One baluster was kept as a control under laboratory conditions. Balusters were monitored for 12 months from June 2012 to May 2013.

### *Sample monitoring*

Each sample baluster was extensively characterised prior to, during and after exposure. The monitoring plan (which includes a list of the parameters measured and their frequency) is summarised in Table 27. All measurements were non-destructive.

Figure 94: Location of field site in Worcester College grounds, Oxford



Field site marked by asterisk. Photo courtesy: Worcester College, 2013

Figure 95: Baluster experiment field site schematic

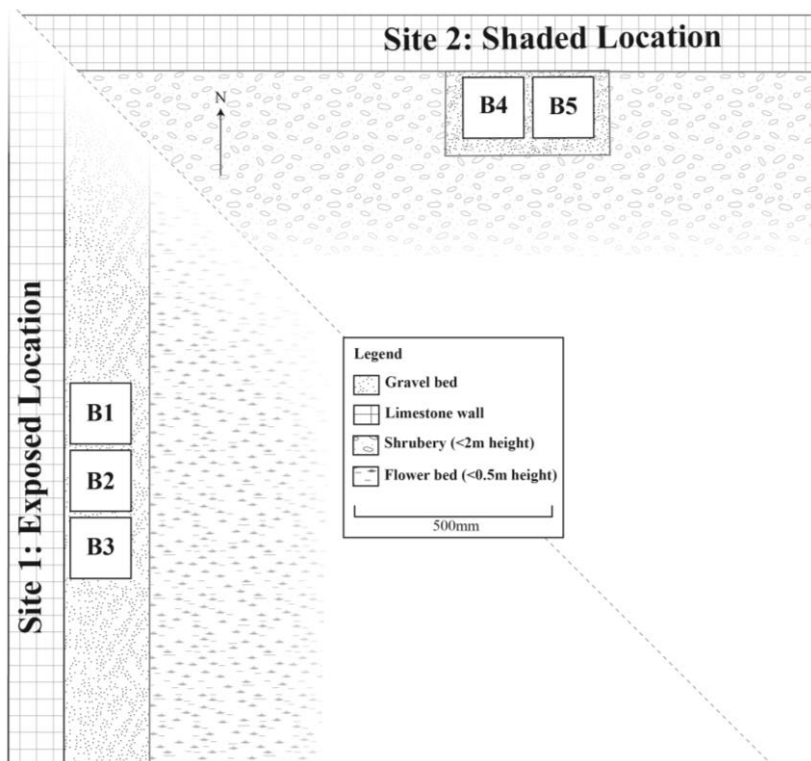


Figure 96: Field site photographs



Site 1: Exposed Location



Site 2: Shaded location

Table 27: Summary of monitoring plan

Parameter		Measurement device	Method for each baluster	Frequency
Moisture	Surface resistance	Protimeter	25 point transect x4 faces	Bi-monthly
	Near- surface capacitance	CEM	23 point transect x4 faces	Bi-monthly
Surface colour		Spectrophotometer	14 point transect x 4 faces	Six- monthly
Photography		Digital SLR with consistent field tripod set-up	8 photos (4x faces and 4x corners)	Six- monthly
Time-lapse videography		Digital TLC with semi-permanent field tripod set-up	NA	Ad hoc
Humidity		i-button	2 i-buttons per baluster	Hourly measurements, averaged bi-monthly
Temperature		i-button	2 i-buttons per baluster	Hourly measurements, averaged bi-monthly
Rainfall		RMETS maintained AWS Radcliffe Camera	NA	Daily accumulated rainfall

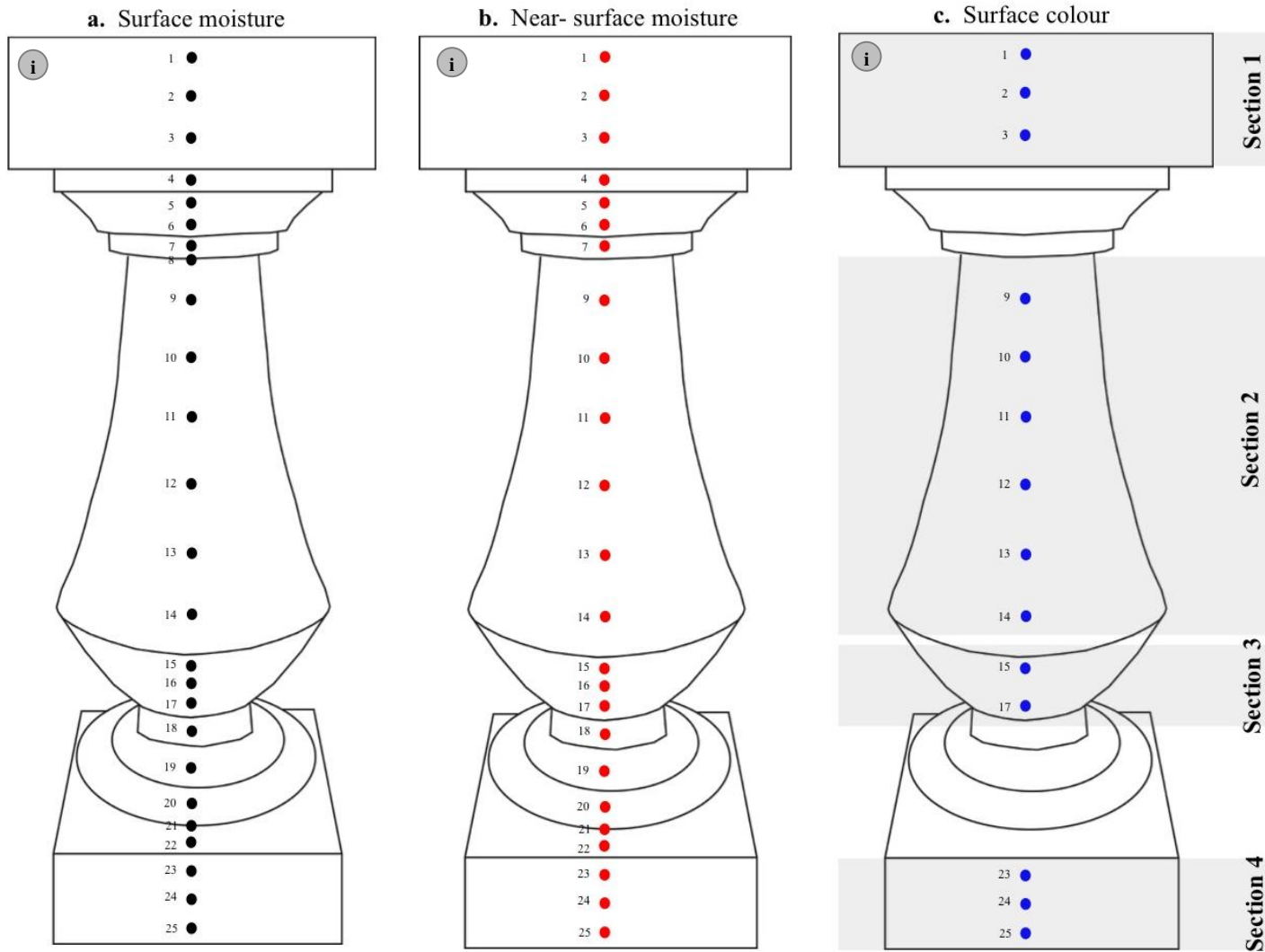
Vertical transects (containing a maximum of 25 points, see Figure 97) were used to enable study of different geometric features on the four cardinal aspects (N, E, S, W) of the six balusters. Transects were marked on each baluster using a non-toxic semi-permanent pen. Surface resistance and near-surface capacitance were measured in order to provide a measure of surface moisture contents. Resistance was gauged using a Protimeter Surveymaster moisture meter (GE; London) and capacitance using a DT-128 non-contact moisture meter (CEM; Shenzhen, China).

Colour, more specifically *greening*, was measured using a portable spectrophotometer device (CM-700d; Konica Minolta, Japan) using the CIELAB colour system. Greening was measured along the 25-point transect excluding points which were not possible to survey due to the morphology of the baluster and the size and shape of the measurement aperture (see Figure 97c). For the purpose of colour data analysis, the baluster transects were divided into four broad sections of morphology.

An average of 5 measurements were taken within 10 mm<sup>2</sup> of the transect point, using the 7mm Medium Area View (MAV) aperture, under standard illumination (D65: average daylight) conditions. Colour data was analysed using SpectraMagic™ NX colour data software (Konica Minolta, Japan). Only ‘Specular Component Excluded’ (SCE) data are presented here, as this measurement excludes gloss effects caused by surface water.

Measurements of moisture were taken bi-monthly and colour every six months. Baseline measurements of moisture and colour were taken 24 hours after initial exposure in the field to allow the stone time to settle in the external environment.

Figure 97: Transect point diagrams for moisture and colour measurement



*iButton's were attached with white-tac to the top left of the front and back of each baluster. Missing values occur in locations where it was not possible to collect data due to the curved morphology of the balusters and the aperture shape and size of measurement instruments (this affected colour measurements in particular)*

Detailed profiling photographs were taken every six months using a consistent camera, tripod and location set-up to allow for photographic comparisons. Ad hoc time-lapse videos (using a Brinno TLC100) were recorded of rain events at both sites. See Figure 100 for Time Lapse Camera (TLC) set-up.

**Figure 98: Time Lapse Camera (TLC) set-up at both field sites**



*The Time Lapse Camera (Brinno TLC 100) was positioned on a fixed tripod  
150cm away from the balusters*

Micro-scale relative humidity (RH%) and temperature data was logged by small iButton® Hygrochron devices (Maxim; California, USA). Ibuttons were attached to the top left corners of the front and back of each baluster (see Figure 97 and Table 28) using white-tac. Data was logged hourly. Synoptic conditions were monitored using rainfall data for central Oxford over the experimental period (obtained from the Radcliffe Meteorological Station).

Table 28: iButton coding key

iButton number	Site	Baluster Number	Location on baluster
1	Exposed site	1	Front left
2		1	Back left
3		2	Front left
4		2	Back left
5		3	Front left
6		3	Back left
7	Shaded site	4	Front left
8		4	Back left
9		5	Front left
10		5	Back left

### Statistical analysis

A range of statistical techniques was used to analyse data. This included simple descriptive statistics to summarise data as well as ANOVA tests with pairwise comparisons for multivariate analyses to examine the influence of location (shade/exposed), aspect and surface geometry on greening. The details of tests used for each dataset and corresponding results tables are presented in the following section.

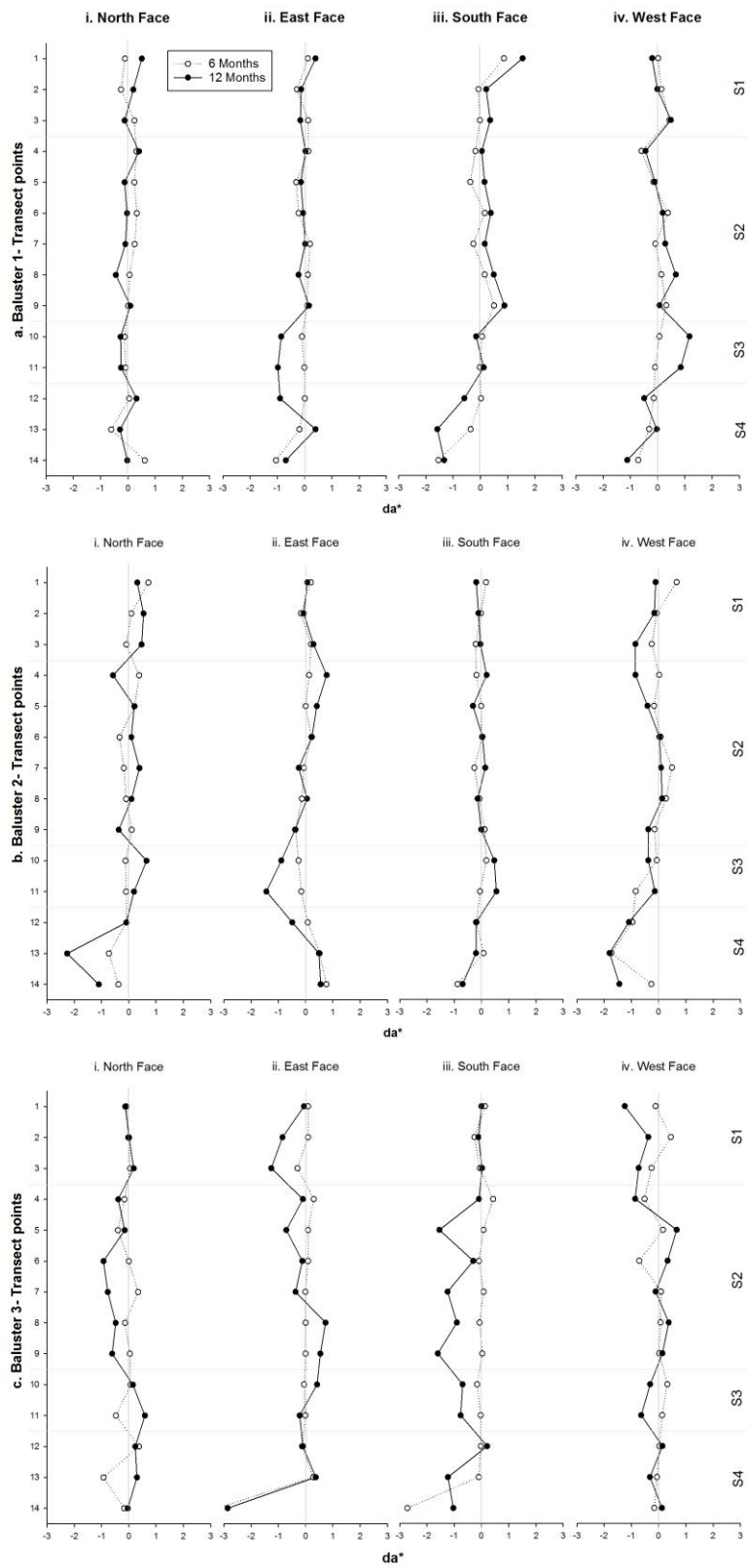
## 7.4 Results

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### Colour

Change in colour along the green-red colour axis ( $da^*$ ) was the primary component of colour measured here, where greening is taken to be indicative of the growth of photosynthetic microorganisms. Figure 99 and Figure 100 chart change along this axis at six and 12 months after the baseline point (one day after exposure- see section 7.3). Negative values indicate greening and positive values reddening. Values for the laboratory control are presented in Figure 101; the maximum value for colour change (in either direction) on the  $da^*$  axis was  $\pm 0.3$  CIELAB units.

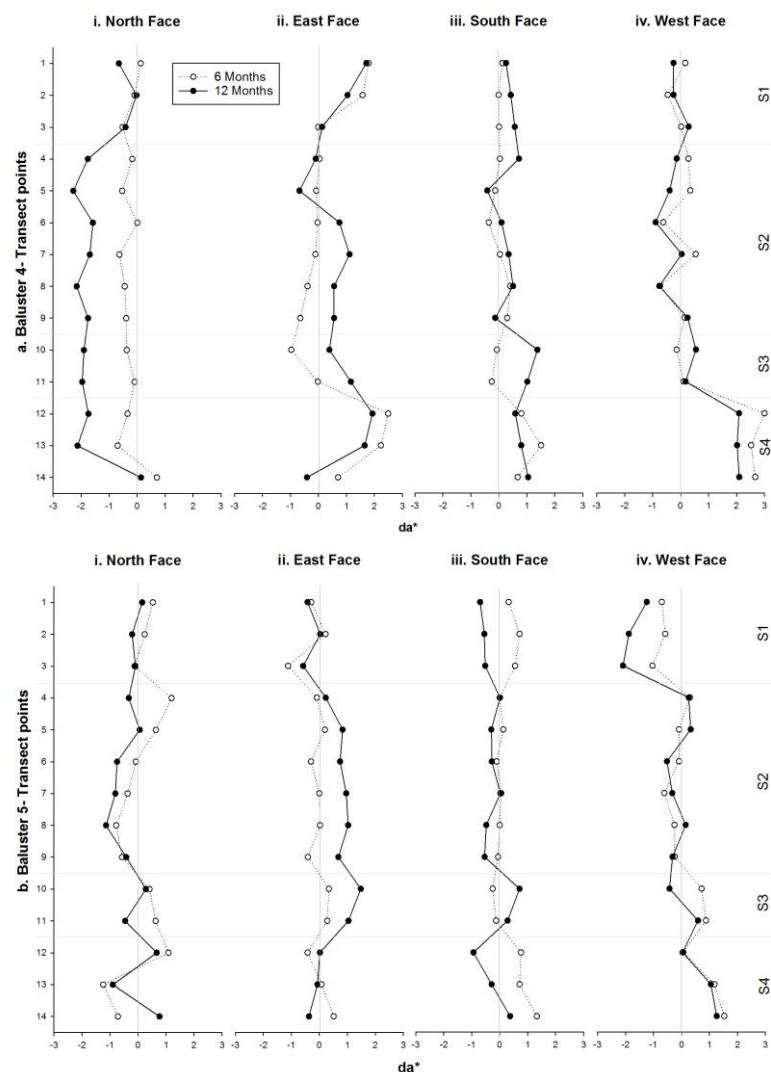
Figure 99:  $da^*$  colour transects for 'exposed' balusters 1-3



Colour change ( $da^*$ ) along the a-b colour axis is presented here. Change is calculated from the 'baseline' point at six and 12 months of exposure.

Each transect is divided into four broad sections by morphology.  
Negative values indicate greening and positive values reddening.

**Figure 100:  $da^*$  colour transects for 'shaded' balusters 4-5**

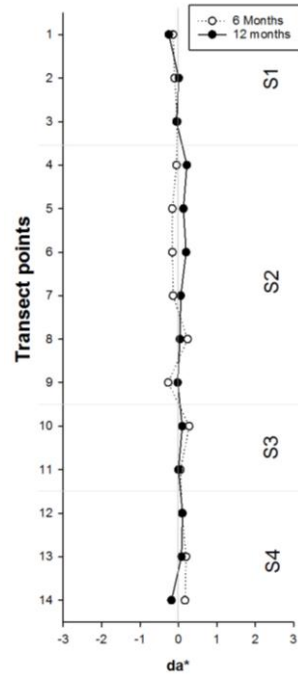


Colour change ( $da^*$ ) along the a-b colour axis is presented here.  
Change is calculated from the 'baseline' point at six and 12 months of exposure.  
Each transect is divided into four broad sections by morphology.  
Negative values indicate greening and positive values reddening.

Greening values in the range of 0 to -4 CIELAB units have been recorded along the  $da^*$  axis in previous controlled laboratory experiments (see Chapter 6, section 6.4). Here, the peak value recorded after a year of field exposure was of -2.86 units with the majority of mean values falling below -1 CIELAB units (see Table 29). This indicates that whilst greening was recorded, the levels are noticeably lower than observed in laboratory

experiments in which stone surfaces were inoculated with algae.

**Figure 101:  $Da^*$  colour transect for laboratory control baluster**



*A single representative transect on the laboratory control was selected for comparison with balusters exposed in the field environment.*

*Colour change ( $Da^*$ ) along the a-b colour axis is presented here.*

*Change is calculated from the 'baseline' point at six and 12 month intervals.*

*The transect is divided into four broad sections by morphology.*

*Negative values indicate greening and positive values reddening.*

Table 29: Averaged transect  $Da^*$  values after 12 months exposure

a. Exposed Site		b. Shaded Site	
Variable	Mean value ( $Da^*$ )	Variable	Mean value ( $Da^*$ )
All balusters	-0.18	All balusters	-0.02
B1 all transects	-0.02	B4 all transects	0.04
B2 all transects	-0.18	B5 all transects	-0.07
B3 all transects	-0.33		
All North transects	-0.09	All North transects	-0.82
All East transects	-0.20	All East transects	0.55
All South transects	-0.22	All South transects	0.06
All West transects	-0.21	All West transects	0.14
All Section 1's	-0.04	All Section 1's	-0.22
All Section 2's	-0.10	All Section 2's	-0.22
All Section 3's	-0.12	All Section 3's	0.27
All Section 4's	-0.52	All Section 4's	0.40

*Negative values highlighted in green denote greening along the  $a^*$  colour axis.*

*Positive values (highlighted in red) indicate reddening*

$Da^*$  values were highly variable across time and space. On average all 'exposed balusters' (1-3) became greener after 12 months (see Table 29a) with highest mean and point values recorded for baluster 3. Baluster 3 also displayed the most variability in colour change across transect sections (see Figure 99c). Contrary to what might be expected, the southern transects of the exposed balusters exhibited the most greening and northern facades the least. Whilst no clear trends were visible in terms of baluster aspect or exposure time, certain sections of the three balusters showed similarity. Indeed, the lowest section (section 4) of all the exposed balusters exhibited the greatest greening, with peak values (of up to -2.86 CIELAB units) recorded for transect point 14 in particular (see Figure 99). This is likely due to groundwater upflows and lower evaporation rates near the base of balusters (see Figure 89).

For balusters 2 and 3, aspect (north, east, south or west), section (section 1-4) or exposure time (6 or 12 months) did not have a significant impact upon levels of greening recorded (see Table 30b and c). Whilst not statistically significant, section did have a noticeable

*influence* on the greening of baluster 2 (see Table 30b). However, section did have a significant impact on baluster 1, where (as exemplified in Figure 99a) section 4 exhibited significantly lower (and therefore, greener)  $da^*$  readings than sections 1 and 2 in particular (see Table 30a).

Balusters situated at the 'shaded' site exhibited quite a different trajectory of colour change to the 'exposed balusters'. In particular, there was greater movement in the red-direction of the  $da^*$  colour axis (see Figure 100). When averaging transect points on all faces, baluster 4 became more red and baluster 5 greener (see Table 29b) after 12 months exposure. The north-facing transect was the only one to exhibit greening. Similarly, only the top two sections exhibited greening (see Table 29b).

Table 30: Exposed location ANOVA results-  
influence of aspect, section and time of exposure on colour change

<b>a. Baluster 1</b>	<b>Normality test</b>	Passed	P=0.055			
	<b>Equal Variance Test</b>	Passed	P=1.000			
	<b>Source of Variation</b>	<b>DF</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
	<i>Aspect</i>	3	0.354	0.118	0.914	0.449
	<i>Section</i>	3	1.756	0.585	4.538	<b>0.012</b>
	<i>Time</i>	1	0.000184	0.000184	0.00142	0.97
	<i>Residual</i>	24	3.096	0.129		
	<i>Total</i>	31	5.206	0.168		
	<b>Multiple comparisons:</b>	<b>Diff. of means</b>	<b>p</b>	<b>q</b>	<b>P</b>	<b>P&lt;0.05</b>
	<i>Section</i>					
	1 vs. 4	0.618	4	4.869	0.011	<b>Yes</b>
	1 vs. 3	0.223	4	1.752	0.609	No
	1 vs. 2	0.104	4	0.817	0.938	No
	2 vs. 4	0.515	4	4.052	0.04	<b>Yes</b>
2 vs. 3	0.119	4	0.935	0.911	No	
3 vs. 4	0.396	4	3.117	0.151	No	
<b>b. Baluster 2</b>	<b>Normality test</b>	Passed	P=0.160			
	<b>Equal Variance Test</b>	Passed	P=1.000			
	<b>Source of Variation</b>	<b>DF</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
	<i>Aspect</i>	3	0.898	0.299	1.627	0.209
	<i>Section</i>	3	1.487	0.496	2.695	0.069
	<i>Time</i>	1	0.1	0.1	0.545	0.467
	<i>Residual</i>	24	4.416	0.184		
	<i>Total</i>	31	6.902	0.223		
<b>c. Baluster 3</b>	<b>Normality test</b>	Passed	P=0.084			
	<b>Equal Variance Test</b>	Passed	P=1.000			
	<b>Source of Variation</b>	<b>DF</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
	<i>Aspect</i>	3	0.641	0.214	1.493	0.242
	<i>Section</i>	3	0.572	0.191	1.332	0.287
	<i>Time</i>	1	0.2	0.2	1.394	0.249
	<i>Residual</i>	24	3.435	0.143		
	<i>Total</i>	31	4.848	0.156		

*Shapiro-Wilk normality tests and Brown-Forsythe equal variance tests were used here (all passed), as part of the ANOVA analyses. Sources of variation: Aspect (North vs. East vs. South vs. West), Section (Section 1 vs.2 vs.3 vs.4) and Time (6 months vs. 12 months)*

Noticeable reddening of the other faces and lower sections was likely due to greater soiling (observed visually) and possibly the growth of other red hued moisture loving species (algae or cyanobacteria) as well as greater moisture accumulation over time.

The patterns discussed above indicate that section and aspect influenced the degree of colour change observed at the shaded location. Statistical testing demonstrates that for both balusters at the shaded site, section had a significant impact on colour change (see

Table 31a and b). Sections 3 and 4 displayed the most significant differences compared to the other sections. In contrast to the exposed location, these differences tended to be in the direction of red rather than the expected green (see Figure 100).

The influence of aspect on colour change was only significant for baluster 4, located at the shaded site. Here the northern transect became significantly greener (see Figure 100a) than the other aspects. This follows patterns of preferential greening observed on the northern facades of buildings (Adamson et al., 2013a).

In summary, for the majority of balusters, when looking at the influence of the variables: location (exposed/ shaded), aspect (N/ E/ S/ W) and surface geometry (section 1/2/3/4), *section* had the most significant influence on colour change. The influence of section and aspect on colour change (after 12 months exposure) was statistically compared (2-way ANOVA) between the two sites. Whilst the normality test for the datasets failed (see Table 32) and a significant ( $<0.05$ ) result was not detected, the results *indicate* that colour change ( $da^*$ ) does vary when both aspect and section are taken into consideration. That is to say, that the magnitude of the difference in  $da^*$  figures varied between the exposed and shaded locations.

Table 31: Shaded location ANOVA results-  
influence of aspect, section and time of exposure on colour change

<b>a. Baluster 4</b>	<b>Normality test</b>	Passed	P=0.769				
	<b>Equal Variance Test</b>	Passed	P=1.000				
	<b>Source of Variation</b>	<b>DF</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>	
	<i>Aspect</i>	3	11.138	3.713	7.821	<0.001	
	<i>Section</i>	3	7.599	2.533	5.335	0.006	
	<i>Time</i>	1	0.195	0.195	0.41	0.528	
	<i>Residual</i>	24	11.394	0.475			
	<i>Total</i>	31	30.325	0.978			
	<b>Multiple comparisons:</b>	<b>Diff. of means</b>	<b>p</b>	<b>Q</b>	<b>P</b>	<b>P&lt;0.05</b>	
	<i>Aspect</i>	E vs. N	1.456	4	5.975	0.002	Yes
		E vs. S	0.229	4	0.941	0.909	No
		E vs. W	0.0896	4	0.368	0.994	No
		W vs. N	1.366	4	5.608	0.003	Yes
		W vs. S	0.14	4	0.573	0.977	No
		S vs. N	1.226	4	5.035	0.008	Yes
	<i>Section</i>	4 vs. 2	1.284	4	5.269	0.006	Yes
4 vs. 3		1.077	4	4.42	0.022	Yes	
4 vs. 1		0.783	4	3.212	0.133	No	
1 vs. 2		0.501	4	2.057	0.48	No	
1 vs. 3		0.294	4	1.208	0.828	No	
3 vs. 2		0.207	4	0.849	0.931	No	
<b>b. Baluster 5</b>	<b>Normality test</b>	Passed	P=0.331				
	<b>Equal Variance Test</b>	Passed	P=1.000				
	<b>Source of Variation</b>	<b>DF</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>	
	<i>Aspect</i>	3	0.201	0.0669	0.216	0.884	
	<i>Section</i>	3	2.991	0.997	3.227	0.04	
	<i>Time</i>	1	0.273	0.273	0.884	0.356	
	<i>Residual</i>	24	7.416	0.309			
	<i>Total</i>	31	10.881	0.351			
	<b>Multiple comparisons:</b>	<b>Diff. of means</b>	<b>p</b>	<b>Q</b>	<b>P</b>	<b>P&lt;0.05</b>	
	<i>Section</i>	3 vs. 1	0.789	4	4.014	0.042	Yes
		3 vs. 2	0.449	4	2.283	0.39	No
		3 vs. 4	0.128	4	0.652	0.967	No
4 vs. 1		0.661	4	3.363	0.109	No	
4 vs. 2		0.321	4	1.631	0.661	No	
2 vs. 1		0.34	4	1.731	0.618	No	

Shapiro-Wilk normality tests and Brown-Forsythe equal variance tests were used here (all passed), as part of the ANOVA analyses. Sources of variation: Aspect (North vs. East vs. South vs. West), Section (Section 1 vs.2 vs.3 vs.4) and Time (6 months vs. 12 months)

**Table 32: Exposed vs. shaded location ANOVA results-  
influence of aspect and section after 12 months**

<b>Exposed vs. shaded sites</b>	<b>Normality test</b>	Failed	P<0.050			
	<b>Equal Variance Test</b>	Passed	P=0.132			
	<b>Source of Variation</b>	<b>DF</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
	<i>Aspect</i>	1	1.214	1.214	2.566	0.114
	<i>Section</i>	3	0.627	0.209	0.442	0.724
	<i>Aspect vs. Section</i>	3	3.869	1.29	2.727	<b>0.05</b>
	<i>Residual</i>	72	34.057	0.473		
	<i>Total</i>	79	39.612	0.501		

*Shapiro-Wilk normality tests and Brown-Forsythe equal variance tests were used here as part of the ANOVA analyses. Sources of variation: Aspect vs. Section*

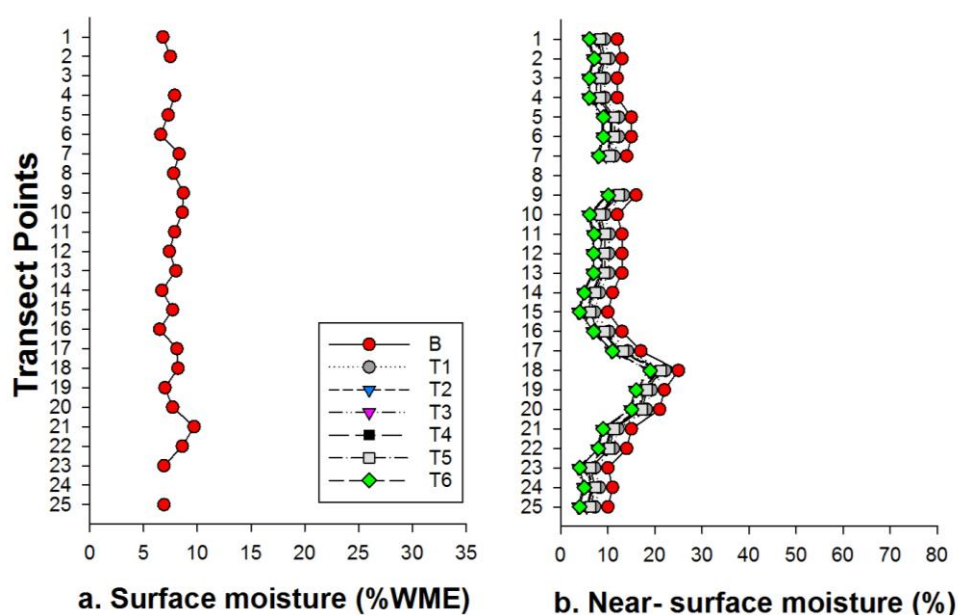
Colour profiles (see Figure 99 and Figure 100) highlight that for both sites the lower sections and for the shaded site, the northern transect displayed the most significant differences in colour in either direction on the  $da^*$  axis. However when considering *greening* exclusively, results demonstrate that the bottom sections of the exposed balusters and the top sections of the shaded balusters (see Table 29) were more receptive to green growth.

### *Surface Moisture*

Surface moisture was gauged using two devices measuring at different depths (see section 7.3). Figure 102 displays moisture transects for the control baluster, kept under consistent laboratory conditions. Surface moisture readings could not be taken beyond the baseline point as the control baluster became drier than the minimum reading capacity (<6% WME) of the Protimeter device (see Figure 102a).

Near- surface readings for the control baluster remained relatively low and consistent between the sampling intervals (T1- T6, see Figure 102b).

Figure 102: Laboratory control surface moisture profiles



- a. Surface moisture: as inferred by surface resistance measurements (Protimeter moisture meter)*  
*b. Near- surface moisture: as inferred by capacitance measurements (CEM moisture meter)*

Readings remained between an average range of 5-25% with a notable peak in transect points 17-20, corresponding to a modelled moisture store (see Figure 91). Mean moisture values for all balusters after 12 months of field exposure are expressed in Table 33.

Surface (SM) and Near-Surface (NSM) values were noticeably lower at the exposed site (see Table 33a) where greater drying out of the surface was due to higher levels of solar incidence and ventilation. Relating this to changes in surface colour, average colour values indicate that exposed balusters became greener than those at the shaded site after 12 months of exposure (see Table 29 'all balusters'). This would suggest preferential greening (and therefore, growth of photosynthetic green algae) on the more dynamic and drier exposed balusters.

**Table 33: Mean transect Surface (SM) and Near- Surface (NSM) moisture values after 12 months of exposure**

<b>a. Exposed Site</b>			<b>b. Shaded Site</b>			<b>c. Lab control</b>	
<b>Variable</b>	<b>SM</b>	<b>NSM</b>	<b>Variable</b>	<b>SM</b>	<b>NSM</b>	<b>NM</b>	<b>NSM</b>
All balusters	10.8	28.2	All balusters	12.7	33.45	1.1	10.2
B1 all transects	11.3	28.5	B4 all transects	12.7	33.0		
B2 all transects	10.7	29.5	B5 all transects	12.7	33.9		
B3 all transects	10.3	26.5					
All North transects	10.5	27.2	All North transects	12.4	32.6		
All East transects	10.5	28.4	All East transects	13.2	34.5		
All South transects	10.8	27.7	All South transects	12.4	32.9		
All West transects	11.3	29.3	All West transects	12.8	33.7		

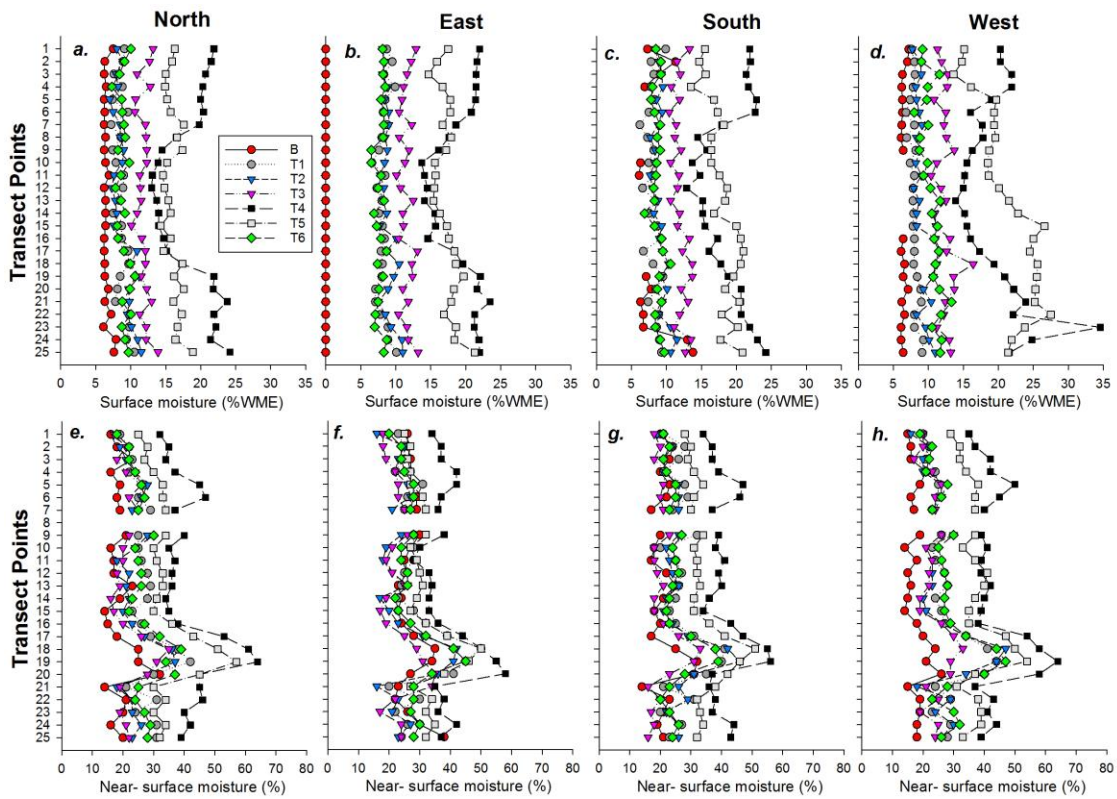
No clear trend was visible in terms of which aspect became most wet at both sites. At the exposed site both SM and NSM averages were highest for the western transects. At the shaded site, peak values for SM and NSM occurred on the eastern and northern transects respectively. This is most likely due to these transects facing towards stone walls (see Figure 95: Baluster experiment field site schematic) resulting in less drying out than other, more exposed transects.

To assess the influence of baluster geometry on surface moisture, comparative profiles of SM and NSM data at each of the 6 sampling intervals (T-T6) were plotted. Moisture profiles for balusters at the exposed site are presented in Figures 105-107 and those at the shaded site in Figures 108 and 109. In all cases, highest SM and NSM averages (see Table 34) occurred at sampling intervals T4 (Dec-Jan) and T5 (Feb- March) corresponding to lower temperatures, higher relative humidity readings and moderate to high rainfall during these periods (see ‘Climatic Conditions’ section).

Table 34: Mean SM and NSM values for each sampling period

	a. Exposed Site		b. Shaded Site		c. Lab Control	
	SM	NSM	SM	NSM	SM	NSM
<b>Baseline</b>	4.8	21.2	2.9	19.0	7.5	14.1
<b>T1: Jun- Jul</b>	7.8	27.6	9.5	31.3	0.0	11.3
<b>T2: Aug- Sept</b>	8.5	23.8	14.8	33.7	0.0	9.6
<b>T3: Oct- Nov</b>	11.1	23.3	13.4	30.9	0.0	7.8
<b>T4: Dec- Jan</b>	18.3	41.3	20.6	51.2	0.0	9.9
<b>T5: Feb- March</b>	16.5	34.1	17.4	37.6	0.0	10.4
<b>T6: April- May</b>	8.6	25.8	10.4	30.3	0.0	8.1

Figure 103: Baluster 1 SM and NSM profiles



Figures 105-109:

*a-d: Surface moisture: as inferred by surface resistance measurements (Protimeter moisture meter)*

*e-h: Near-surface moisture: as inferred by capacitance measurements (CEM moisture meter)*

Figure 104: Baluster 2 SM and NSM profiles

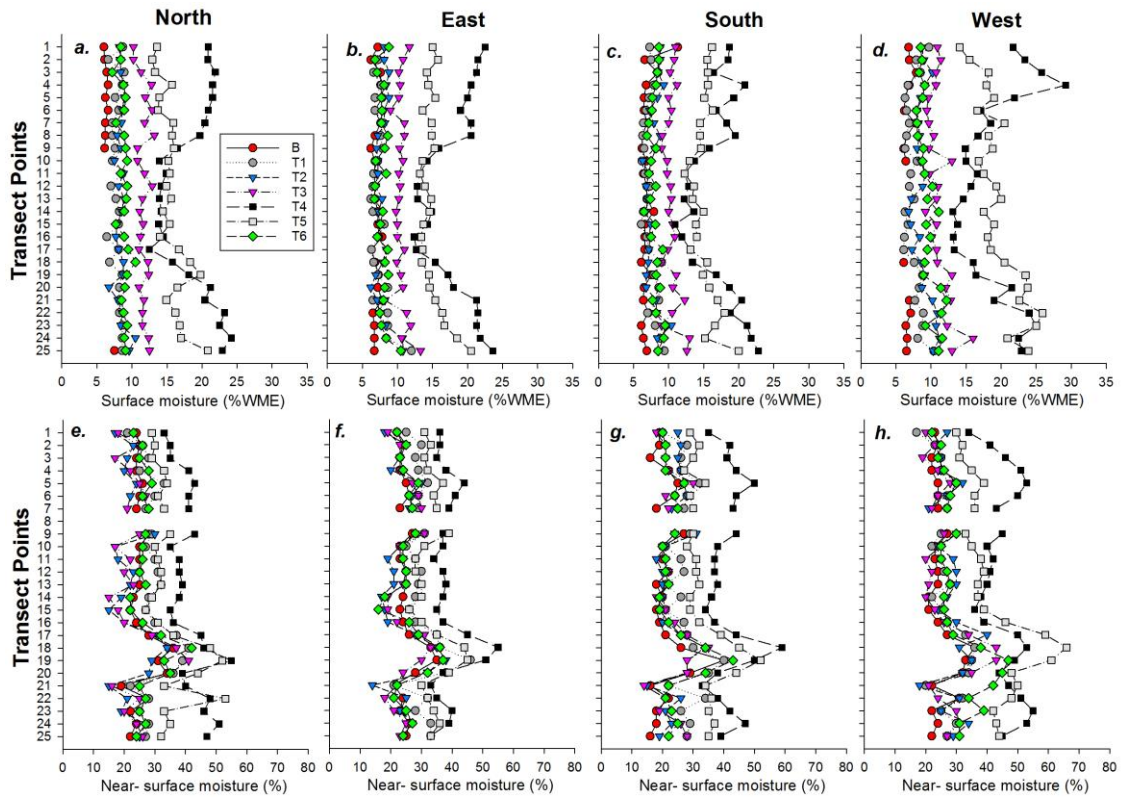


Figure 105: Baluster 3 SM and NSM profiles

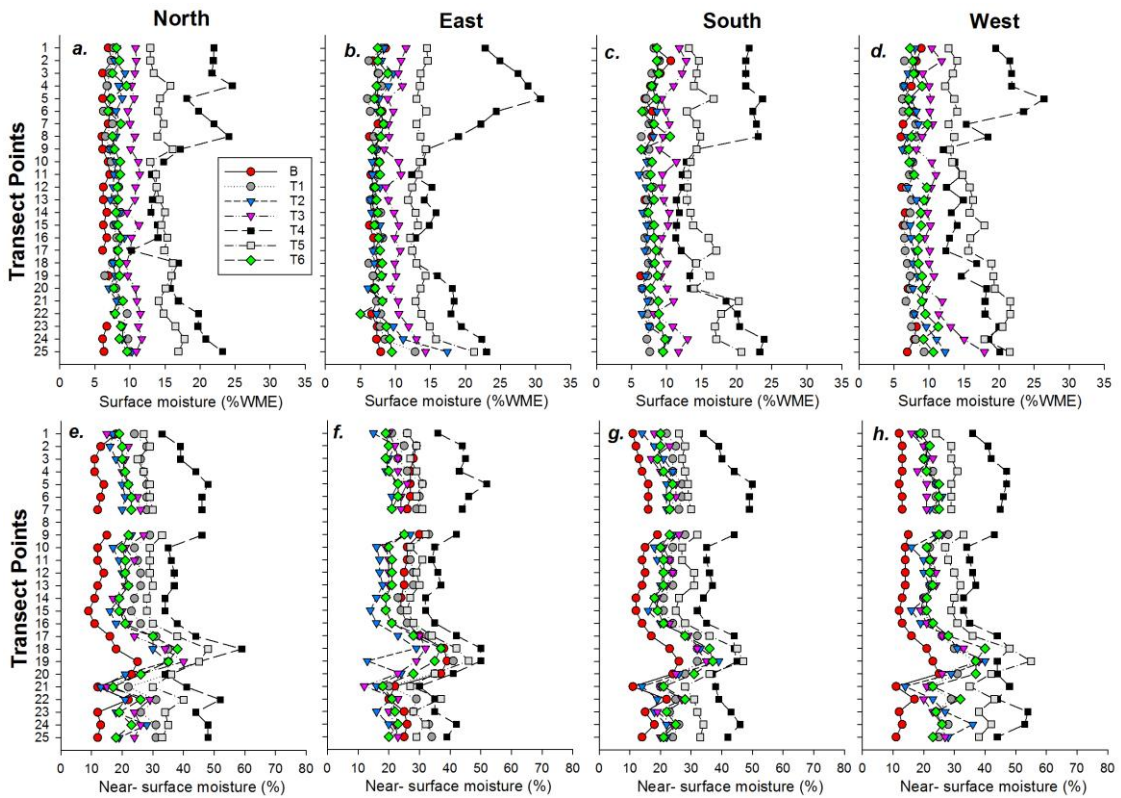


Figure 106: Baluster 4 SM and NSM profiles

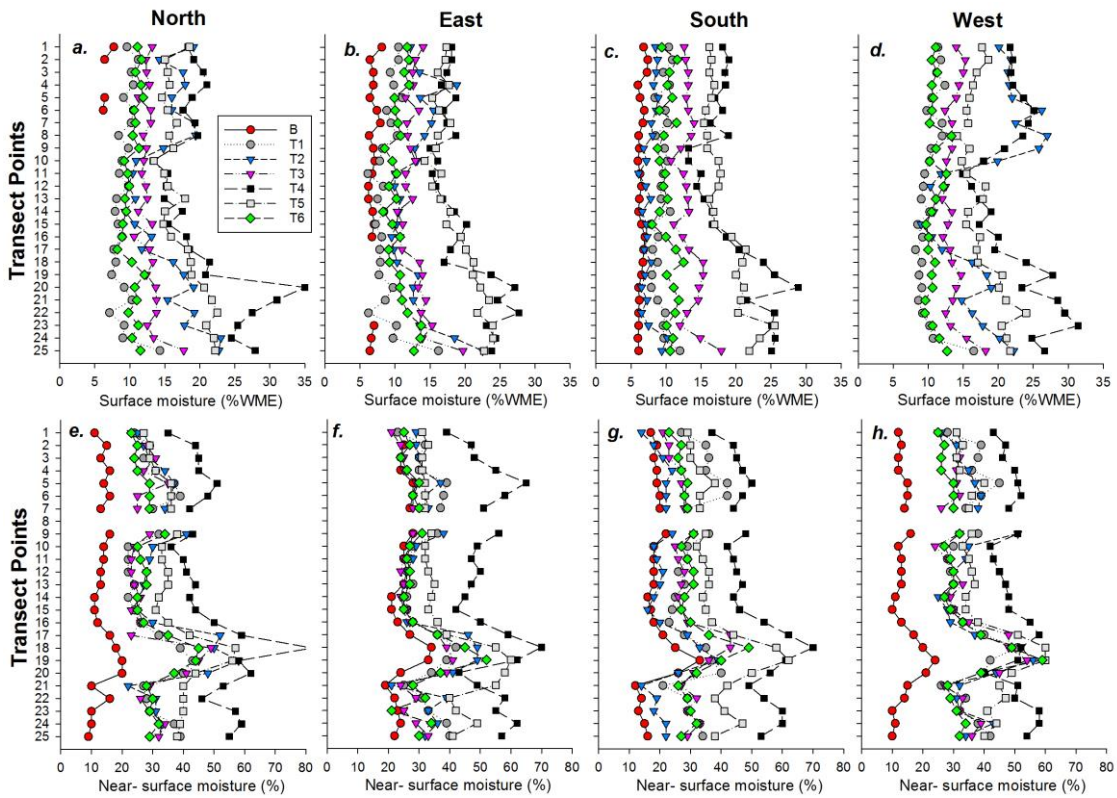
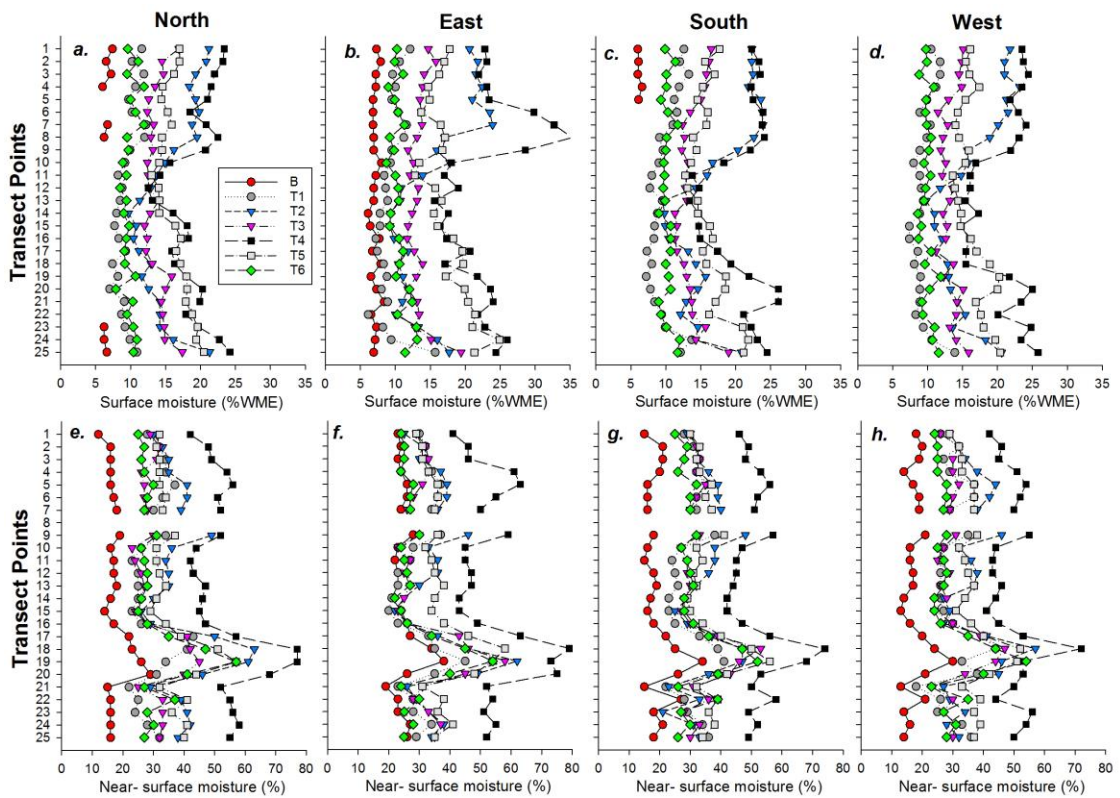
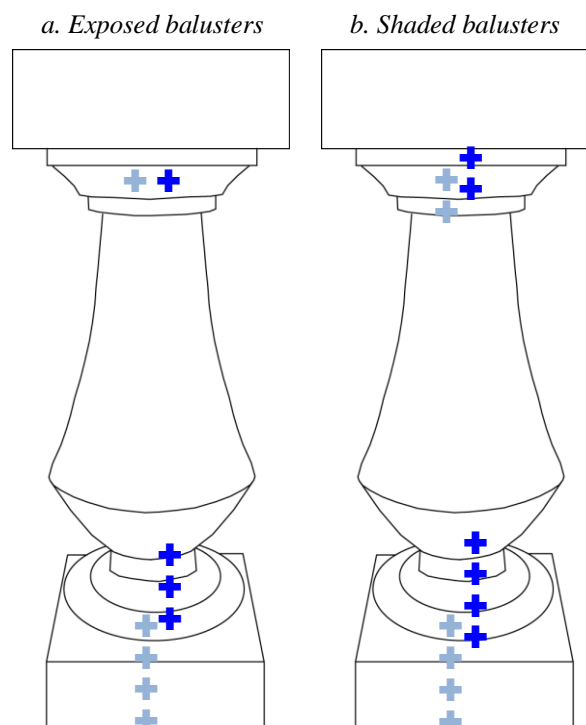


Figure 107: Baluster 5 SM and NSM profiles



Balusters at the exposed site (B1, B2 and B3) behaved similarly to one another in that peaks in both SM and NSM at each sampling interval (T1-T6) occurred around the same transect points (see Figure 103, Figure 104 and Figure 105). Peak SM values occurred between transect points 5-6 and 20-25 corresponding to areas of distinct geometry (see Figure 97), which have been previously modelled as rain stores (see Figure 91). For all profiles (T-T6) maximum SM values occurred at or around the very bottom of each exposed baluster. This could be the result of surface moisture accumulation at the baluster base or due to capillary rise of groundwater (upflow- see Figure 89). Peak NSM values for exposed balusters tended to occur around transect points 5-6 and 17-20. Whilst the top peak relates well to peaks in SM at the same location, the lower peak occurs higher on the baluster at a point of geometric narrowing (see Figure 108a).

**Figure 108: Observed surface moisture maxima**



Rain stores **+** as observed on exposed and shaded balusters  
 + =Surface Moisture, + =Near- Surface Moisture

Surface moisture profiles for balusters at the shaded site were much more variable than at the exposed site. In particular, there was greater difference between profiles for each of the sampling intervals than at the exposed site. There was also less overlap of peak SM and NSM values and these peaks occurred in less definitive locations on the baluster transects themselves. In general peak SM values occurred between transect points 5-8 and 20-25. Peak NSM values occurred between transect points 4-6 and 16-21 (see Figure 108b).

Simple regressions were run to assess the strength of the relationship between the two moisture variables (S and NSM) for balusters at each location. Figure 109 plots the positive relationship between surface and near-surface moisture for all balusters, irrespective of aspect. Whilst overall  $R^2$  are low, the  $R^2$  correlation coefficient for the exposed site (Figure 109a) is higher than the shaded site (Figure 109b) indicating that a stronger relationship occurs between the two variables of moisture at the exposed site.

ANOVA results (see Table 35) highlight the reliability of these results and demonstrate that for the exposed balusters, surface moisture is a significant predictor of near-surface moisture (and vice versa). The weaker relationship between the two variables of moisture at the shaded site may be due to damper microclimatic conditions at this location whereby less efficient evaporation and drying lead to more deep-seated moisture.

Figure 109: Relationship between Surface and Near- Surface moisture for exposed and shaded balusters

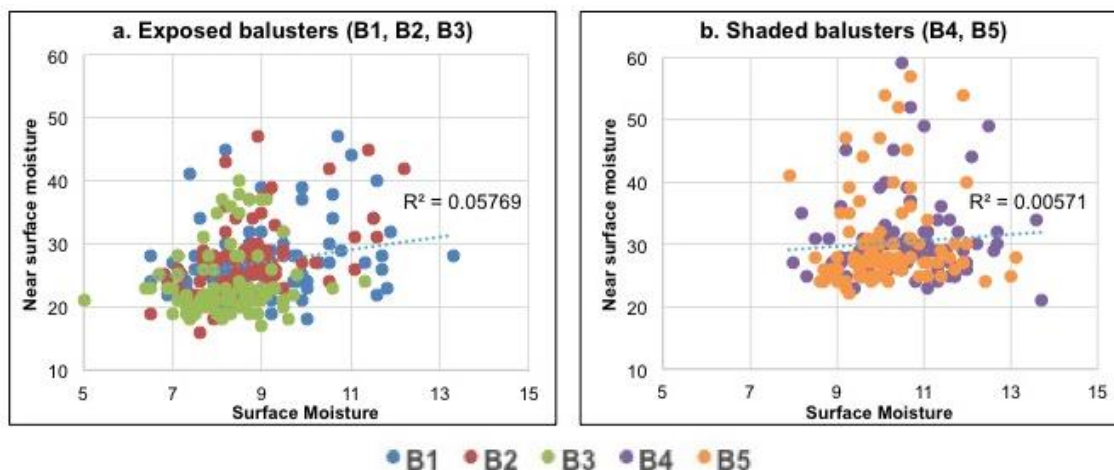
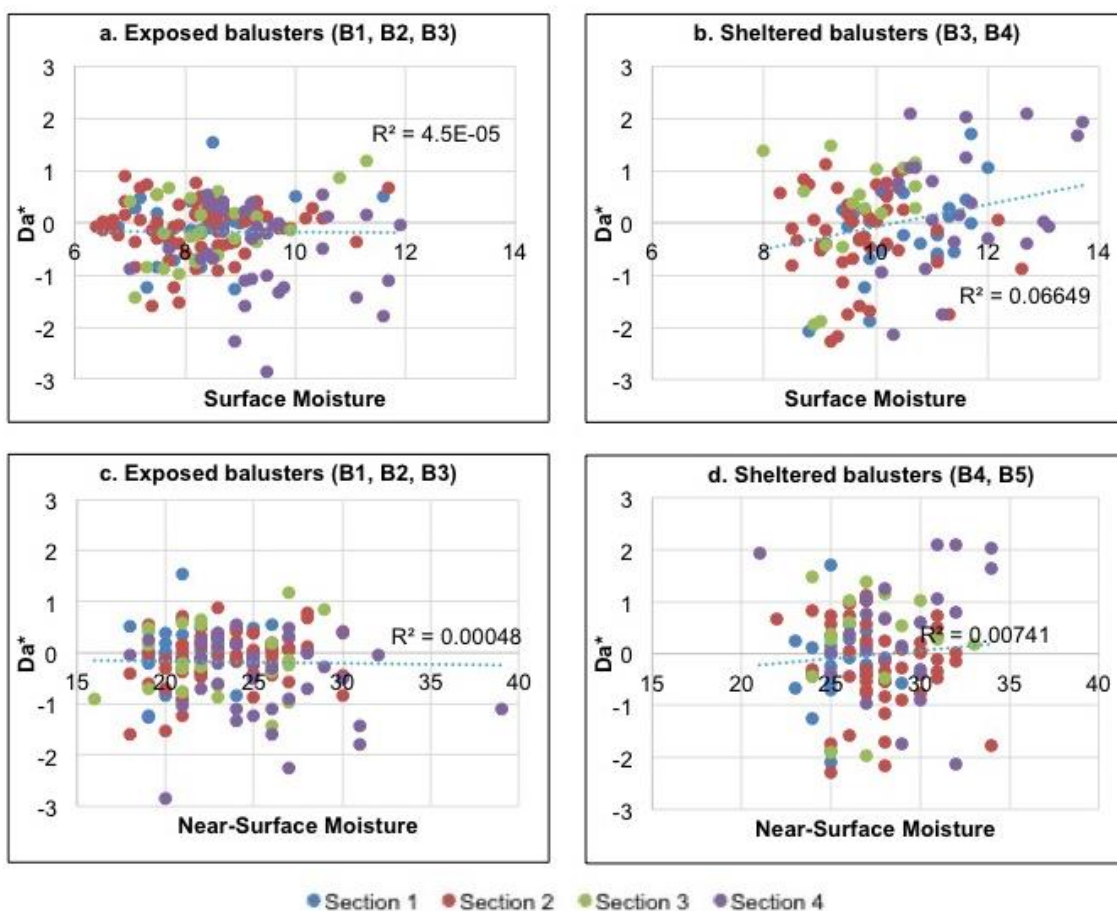


Table 35: Moisture regression statistics for Exposed and Shaded balusters

a. ANOVA for Exposed balusters (B1, B2, B3)					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
<b>Regression</b>	1	45.61941531	45.61941531	36.90322819	0.000000
<b>Residual</b>	286	353.5504458	1.23619037		
<b>Total</b>	287	399.1698611			
b. ANOVA for Sheltered balusters (B3, B4)					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
<b>Regression</b>	1	1.275688577	1.275688577	1.090368449	0.297716
<b>Residual</b>	190	222.2925927	1.169961014		
<b>Total</b>	191	223.5682813			

To compare the relationship between surface colour change ( $da^*$ ) and surface moisture, simple correlation plots were drawn (see Figure 110). On the exposed balusters, moisture and greening seems concentrated in the lower left zones of correlation plots (see Figure 110a and c). This indicates that these balusters were relatively dryer and greener (negative  $da^*$  values) than shaded balusters. For both sites, the relationship between greening and surface moisture (see Figure 110a and b) seems stronger than greening and near-surface moisture.

Figure 110: Correlations between Surface and Near-Surface moisture and greening ( $Da^*$ )



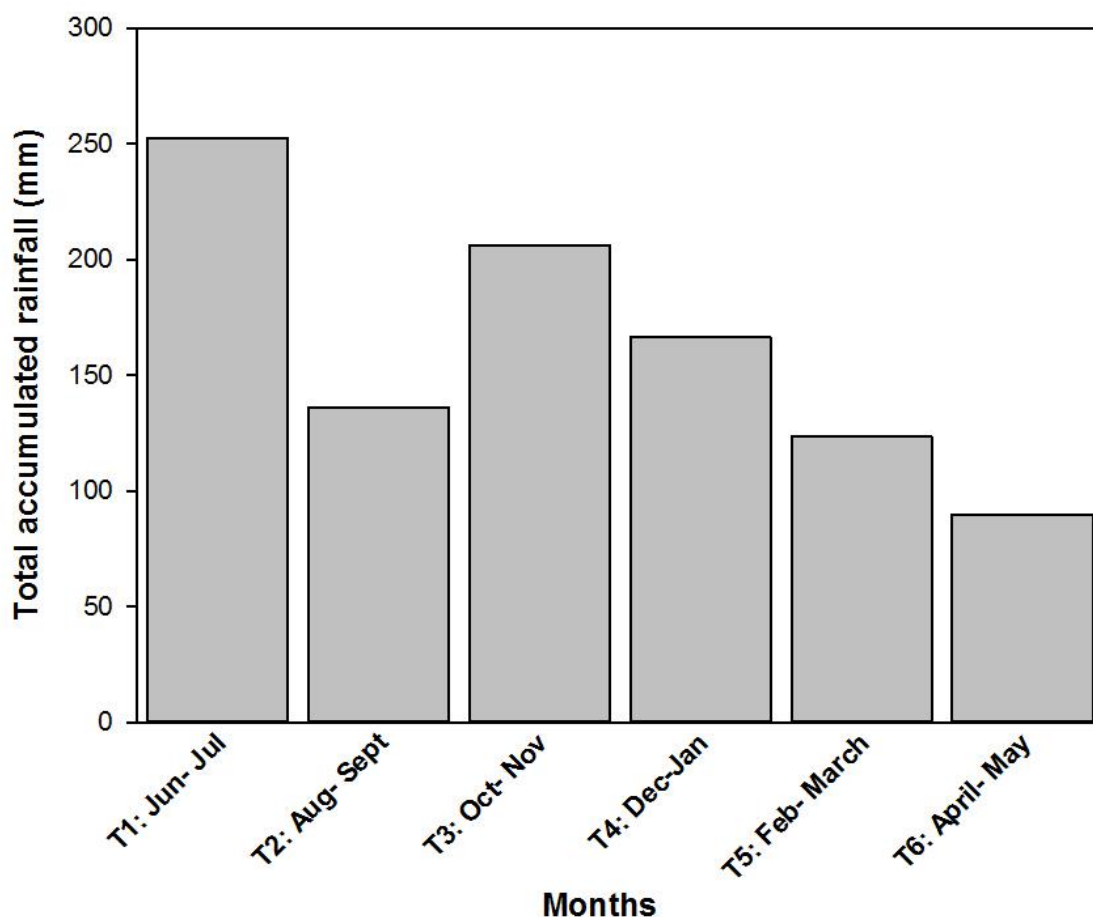
The relative shift of surface and near-surface moisture readings of shaded balusters towards the top right (see Figure 110b) and right generally (see Figure 110d) indicates that these balusters retained more moisture (and at depth) than exposed balusters. A noticeable relationship occurs between these higher moisture readings (particularly deep-seated moisture) and a bias towards the red end of the  $da^*$  colour axis. Indeed, the strength of the relationship between moisture and  $da^*$  (in either direction) is much more pronounced for the shaded balusters.

### *Climatic conditions*

#### ***Synoptic Conditions:***

High rainfall values were recorded for the experimental period with peak values for the summer months including June and July 2012 (see Figure 111, T1). Aside from the unusually wet preceding summer, October to January were the wettest months as would be expected. Rainfall was highest at sampling intervals T1 (June-July), T3 (October to November) and T4 (December- January). This corresponds to peak surface moisture profiles at T1 and T4 (see Figure 103- 109).

**Figure 111: Total bimonthly accumulated rainfall in central Oxford (June 2012- May 2013)**



*Rainfall data for the period June 2012- May 2013 was obtained from the Radcliffe Meteorological Station, Central Oxford*

#### ***Microclimatic Conditions:***

iButtons were placed on the front and back of each baluster (see Table 28) to measure micro-scale differences in temperature and Relative Humidity (RH%) between balusters and between exposure sites. Humidity data was corrected for saturation drift according to device manufacturer guidelines (see Appendix 4 for formula). Despite applying the correction, RH% values for the peak months of October to February exceeded 100% in some cases. In these instances, values were capped at 100%. Mean bi-monthly figures of temperature and relative humidity for all ten iButtons are presented in Table 36 and Table 37.

**Table 36: Mean bimonthly temperature (°C) figures for each iButton**

iButton #	a. Exposed Site						b. Shaded Site			
	1	2	3	4	5	6	7	8	9	10
iButton location	B1 Front	B1 Back	B2 Front	B2 Back	B3 Front	B3 Back	B4 Front	B4 Back	B5 Front	B5 Back
<i>T1: June- July</i>	16.4	16.4	16.4	15.8	16.1	16.4	15.2	14.8	15.1	10.3
<i>T2: Aug- Sept</i>	14.3	14.4	14.3	13.9	14.1	14.5	13.6	13.5	13.7	13.7
<i>T3: Oct- Nov</i>	2.6	2.7	2.7	2.5	2.6	0.0	2.9	NA	2.9	2.8
<i>T4: Dec- Jan</i>	4.3	4.5	4.4	3.9	4.3	1.1	4.9	0.6	4.8	4.8
<i>T5: Feb- Mar</i>	2.9	3.1	3.0	2.5	2.8	3.0	3.4	2.9	3.4	3.3
<i>T6: Apr- May</i>	11.8	11.8	11.8	6.6	11.5	11.6	11.2	10.6	11.2	11.1

*B=Baluster. October data for ibutton 8 missing due to equipment failure*

**Table 37: Mean bimonthly relative humidity (%) figures for each ibutton**

iButton #	a. Exposed Site						b. Shaded Site			
	1	2	3	4	5	6	7	8	9	10
iButton location	B1 Front	B1 Back	B2 Front	B2 Back	B3 Front	B3 Back	B4 Front	B4 Back	B5 Front	B5 Back
<i>T1: June- July</i>	79.6	82.7	78.6	86.0	80.3	89.2	89.2	85.0	85.2	85.8
<i>T2: Aug- Sept</i>	66.9	69.6	67.8	72.4	67.1	79.5	79.5	70.1	73.4	69.8
<i>T3: Oct- Nov</i>	98.4	99.1	98.6	99.7	98.6	99.9	99.9	NA	98.8	98.9
<i>T4: Dec- Jan</i>	98.7	99.3	98.9	99.7	98.8	99.9	99.9	27.7	98.9	99.1
<i>T5: Feb- Mar</i>	92.3	93.9	93.1	95.7	92.6	95.8	95.8	94.0	92.5	92.8
<i>T6: Apr- May</i>	76.9	80.3	79.2	86.6	78.4	83.0	83.0	94.5	79.5	79.6

*B= Baluster. October data for ibutton 8 missing due to equipment failure*

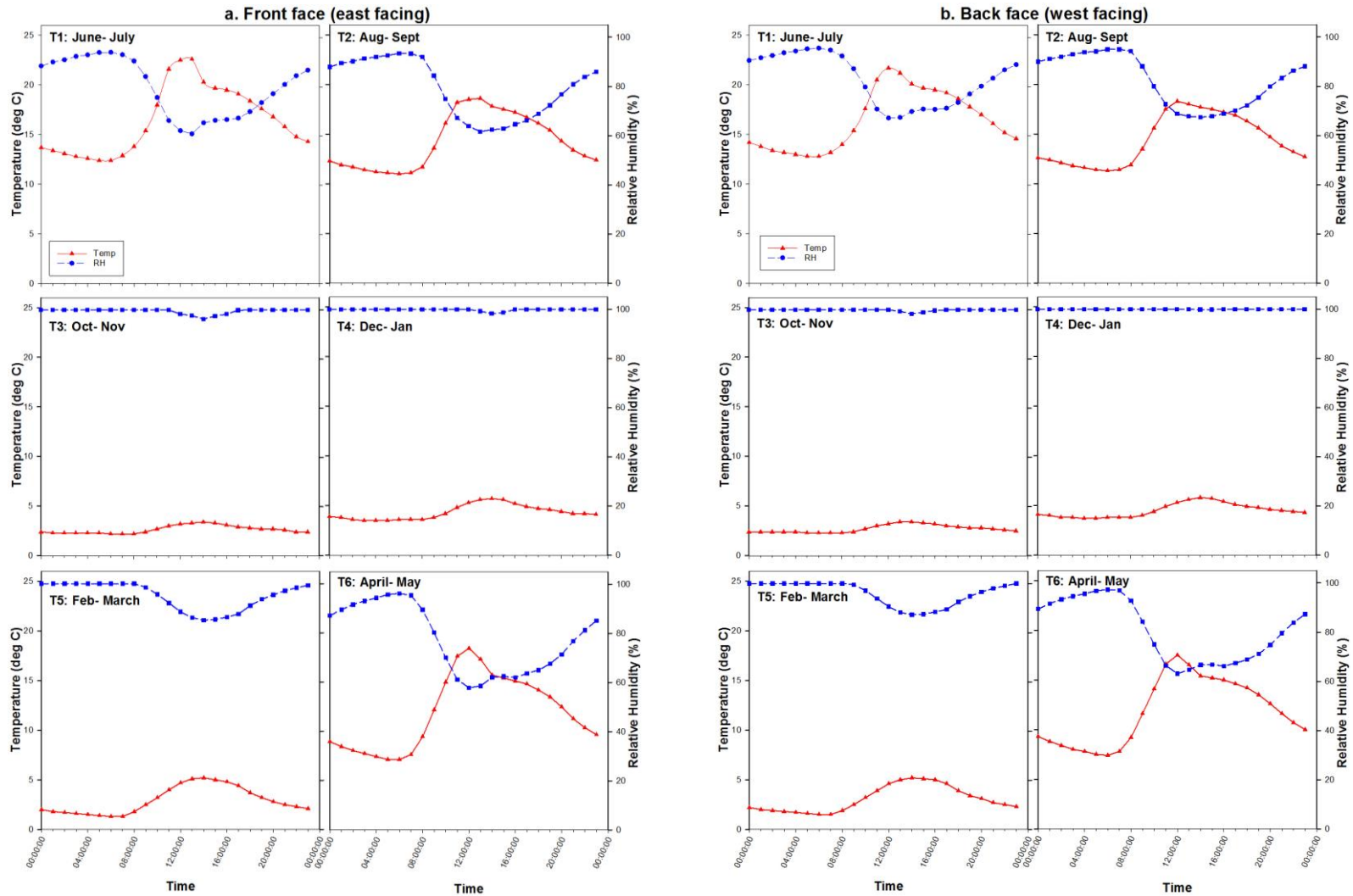
Hourly figures across two-month intervals were averaged in order to plot bi-monthly diurnal cycles of temperature and relative humidity for the exposed (Figure 112) and shaded sites (see Figure 113).

The greatest differences in diurnal temperature and relative humidity cycles between the two sites were observed in the summer- autumn months (T1, T2 and T6 see Figure 112 and Figure 113). In general, diurnal conditions at the shaded site were more moderated with less difference occurring between maximum and minimum temperature and relative humidity than the exposed site. This is due to the cover of overlying and surrounding vegetation at this site. Conversely, balusters at the exposed site were subject to direct solar incidence and rainfall, explaining the greater differences observed in daily extremes.

At the shaded site, a significant difference was not detected (paired two tailed T-Test,  $p < 0.05$ ) between ibuttons located on the front and back of each baluster. At the exposed site, a difference did not occur in temperature but relative humidity values were significantly higher (paired two tailed T-Test= 0.008,  $p < 0.05$ ) at the back of these balusters compared to the front. Therefore some micro-level differences did occur in the conditions balusters were exposed to.

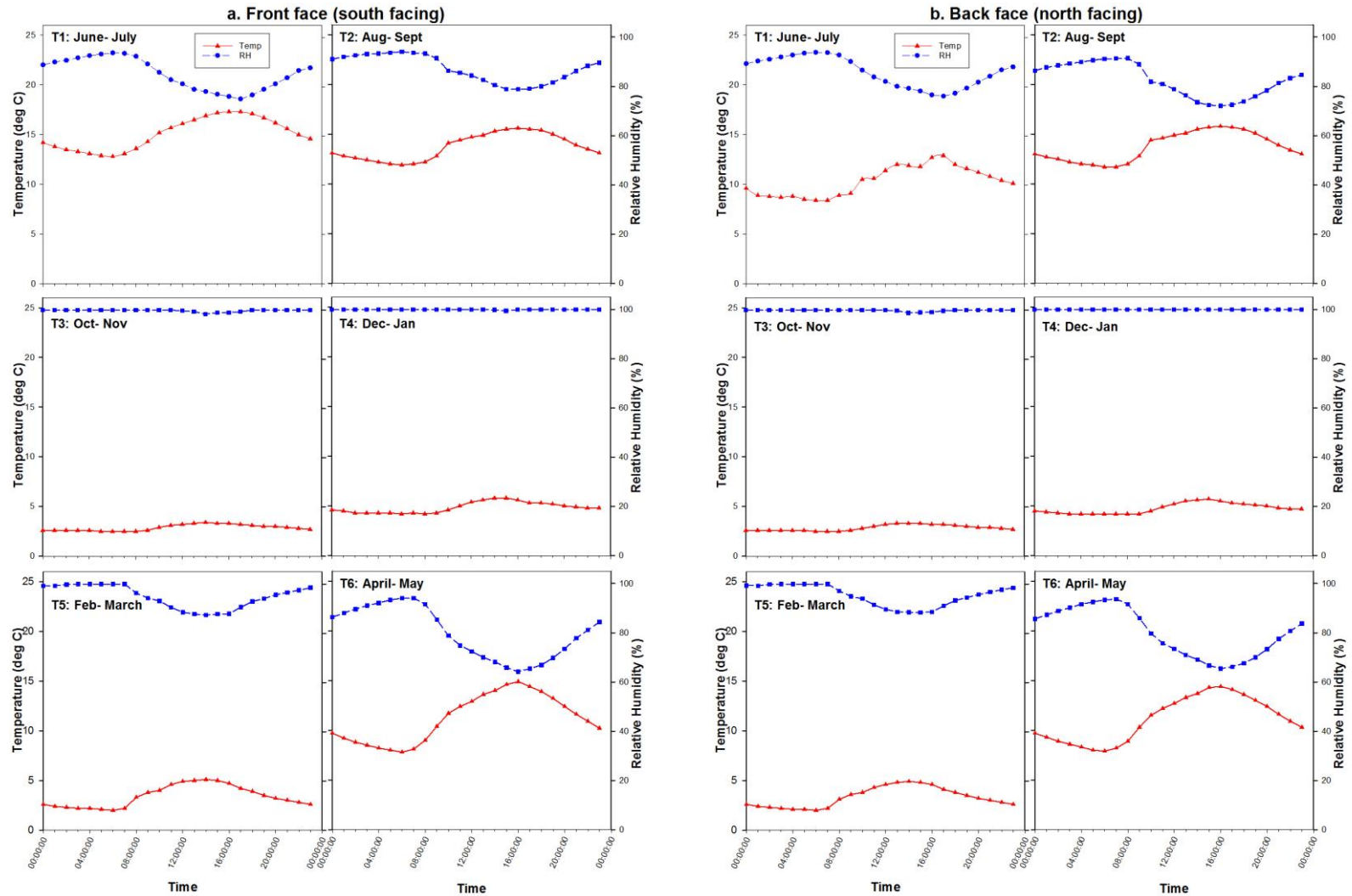
At the larger scale, when comparing all ibuttons (fronts and backs), a significant difference was not found in either temperature or relative humidity between the exposed and shaded sites.

Figure 112: Exposed site- Average diurnal cycles of temperature and relative humidity



*Hourly observations of temperature and relative humidity (obtained from ibuttons 1 & 2, positioned on baluster 1) were averaged (mean) across two-months to produce an average bimonthly diurnal cycle*

Figure 113: Shaded site- Average diurnal cycles of temperature and relative humidity



Hourly observations of temperature and relative humidity (obtained from ibuttons 9 & 10, positioned on baluster 5) were averaged (mean) across two-months to produce an average bimonthly diurnal cycle

### *Time lapse observations of rain events*

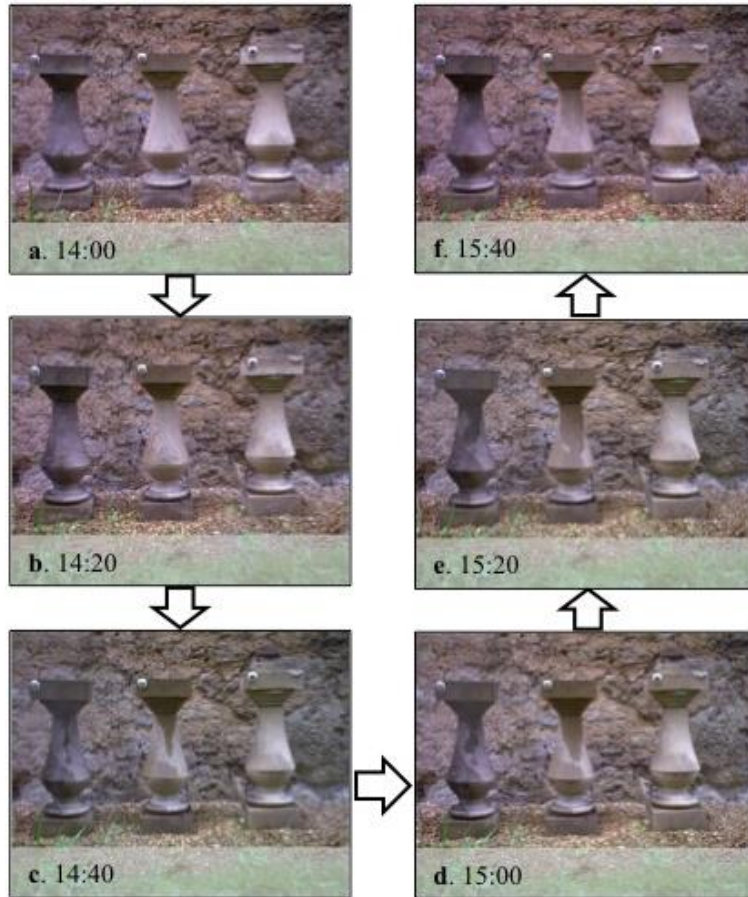
Time Lapse photography was used to document rain events at both the exposed and shaded locations. The main purpose of this was to compare wetting and drying patterns on each of the balusters. Sample rain events are presented in Figure 114 and Figure 115.

Generally, balusters at the exposed site were directly wetted on the top plinth and above the central band. Moisture elsewhere was in most cases the result of runoff and splash back from the ground. Only under very heavy downpours were other parts of the baluster directly wetted. In a typical rain event these two zones would receive the majority of direct rainfall and would shed this fairly quickly (see Figure 91). Moisture would then store in the narrower regions and in particular the bottom base of balusters (as evidenced by surface moisture measurements- see Figure 108: Observed surface moisture maxima). In the sample rain event presented (see Figure 114), balusters at the exposed site began to dry out only an hour after the initial rain shower. This is typical of balusters at this site due to greater exposure to sunlight and ventilation from prevailing wind.

Balusters at the shaded site followed a similar pattern of direct wetting to exposed balusters but in general remained moist for longer (in the presented example, balusters did not begin to dry out until almost three hours after initial wetting- see Figure 115) due to damper ambient conditions (see Figure 113). There was more variability between balusters at this site in that baluster 5 was generally wetted less frequently than baluster 4. This is likely due to the south-westerly prevailing wind and localised rain shading by overhanging trees.

Figure 114: Exposed site sample rain event, 23/09/2012

*a. Time lapse photo frames*



*b. Diurnal microclimate data*

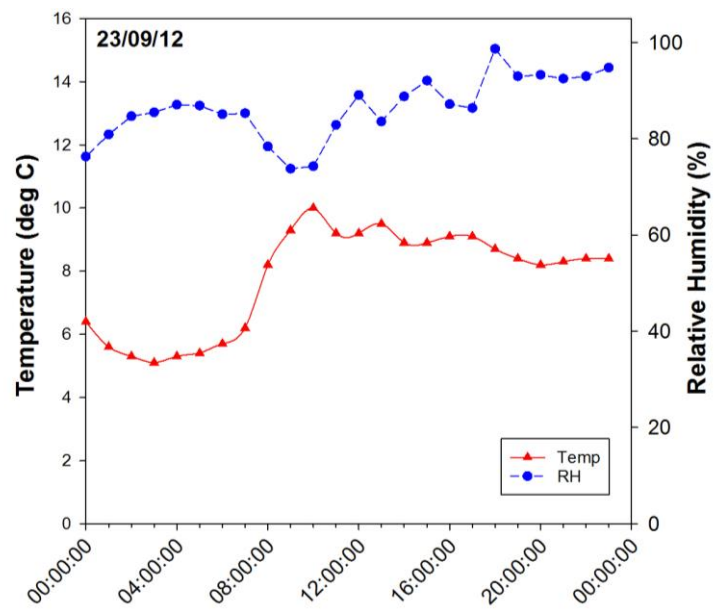
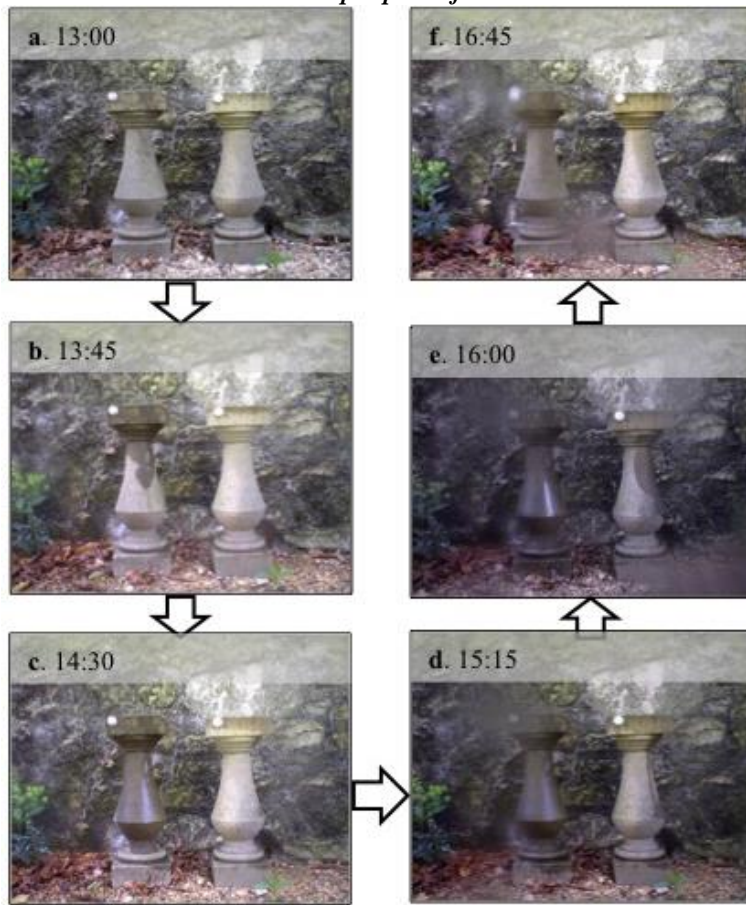
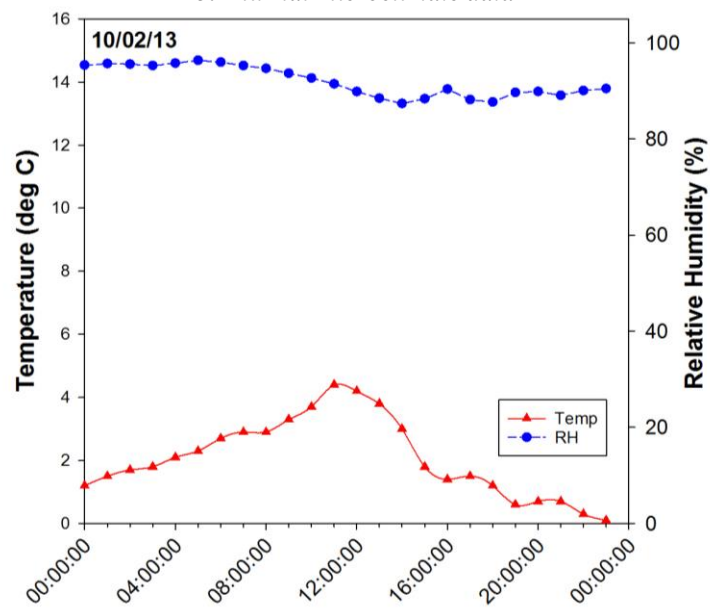


Figure 115: Shaded site sample rain event, 10/02/2013

*a. Time lapse photo frames*



*b. Diurnal microclimate data*



## 7.5 Discussion

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### *Location*

Greater levels of greening (negative  $da^*$  values) were recorded on balusters at the exposed site and greater levels of reddening (positive  $da^*$  values) at the shaded site. Surface and near-surface moisture did not correlate as well at the shaded site as at the exposed site (see Figure 109). This demonstrates a weaker connection between the variables, which is likely due to differences in microclimatic conditions between the sites. Conditions at the shaded site were much more moderated with less difference occurring between diurnal maximum and minimum temperatures and relative humidity (Figure 113). This led to generally moister conditions for greater periods of time than at the exposed site. Comparison of the time-lapse rain events highlights this effect whereby the shaded balusters remained moist for longer than exposed balusters (see Figure 114 and Figure 115), which had a tendency to dry out faster after rain events. There is a long established link between algal greening and prolonged time-of-wetness, particularly in winter months (Smith et al., 2009).

The relationship between surface colour change ( $da^*$ ) and surface moisture (SM) seems to be stronger than that for near-surface (NSM) moisture. Results indicate that lower values of surface and near-surface moisture (as observed at the exposed site) led to greater greening of the surface (see Figure 110a and c), attributed to growth of light-loving green algae (see Figure 116). Conversely, higher values (as observed at the shaded site) led to greater reddening of the surface (see Figure 110b and d), attributed to growth of red hued microorganisms and greater organic surface soiling (see Figure 117- West face in particular). Therefore, very moist/ wet conditions are more likely to favour growth of red-hued damp loving species rather than green algae (see Figure 86).

Figure 116: Baluster 3 (exposed site) 12 month photographic record

















































	North	N Corner	East	E Corner	South	S Corner	West	W Corner
a. Baseline								
b. 6 Months								
c. 12 Months								

Figure 117: Baluster 5 (shaded location) 12 month photographic record

	North	N Corner	East	E Corner	South	S Corner	West	W Corner
a. Baseline								
b. 6 Months								
c. 12 Months								

The location of balusters in relation to each other (intra baluster impacts) did not appear to have a noticeable impact on levels of greening observed. Whilst balusters were situated adjacent to each other, no obvious trend in greening or moisture patterns could be found due to the shading caused by adjacent balusters, this was likely due to the relatively low height (460mm) of the balusters minimising interactive effects. The proximity of balusters to stone walls (see Figure 95) however had a more noticeable impact. For example, the northern transects of shaded balusters had the lowest  $da^*$  readings (indicating greatest greening), these were the transects facing towards the stone wall (see *Aspect* discussion below).

### *Aspect*

For the majority of balusters, *aspect* did not have a significant influence on levels of colour change ( $da^*$ ) observed. Preferential greening only occurred on the northern transect of baluster 4 (see Table 31a and Figure 100a). This is likely to relate to higher surface moisture values (SM and NSM) occurring on the northern and eastern sides of the shaded balusters (see Table 33b) when compared to the southern and western sides. In their exposure trials in Northern Ireland, Adamson et al. (2013b) found a much more definitive relationship between greening and block orientation where northern-facing blocks exhibited greater greening and at a faster rate than southern-facing blocks.

### *Surface geometry*

Surface colour measurements reveal that peak colour change ( $da^*$ ) for all balusters occurs in the bottommost zone (see Table 29). Peak greening (negative  $da^*$  values) on exposed balusters occurred in the bottom two sections (see Figure 99) and on shaded balusters the top two sections (see Figure 100). Differences in microclimatic conditions and the

location of surface moisture stores at the two sites go some way in explaining the spatial variability in the bioreceptivity of balusters at either site.

Moisture (SM and NSM) profiles for all balusters (irrespective of location) reveal the upper and lower areas of narrowing and the base plinth of balusters as primary surface moisture stores (see Figure 108). A difference is apparent in the location of these moisture stores and the depth of moisture, where deeper-seated moisture (NSM) is more apparent in the narrow, more architecturally complex areas.

The location of moisture stores affects baluster bioreceptivity. In particular, peak colour change values for balusters at the exposed and shaded locations occurred in section 3 and 4 (corresponding approximately to moisture transect points 15-17 and 23-25 respectively). Surface moisture values (SM and NSM) were on average higher at the shaded location (see Table 33b) as were values of surface colour change along the  $a^*$  axis (red or green direction- see Table 29b).

Results indicate that microclimatic conditions can increase the bioreceptivity of vulnerable moisture stores where moderated light, temperature and moisture conditions (as seen at the shaded site) increase the bioreceptivity of these stores. It is likely that these stores experience reduced bioreceptivity in more dynamic locations (with greater diurnal variations in temperature and relative humidity, as seen at the exposed site).

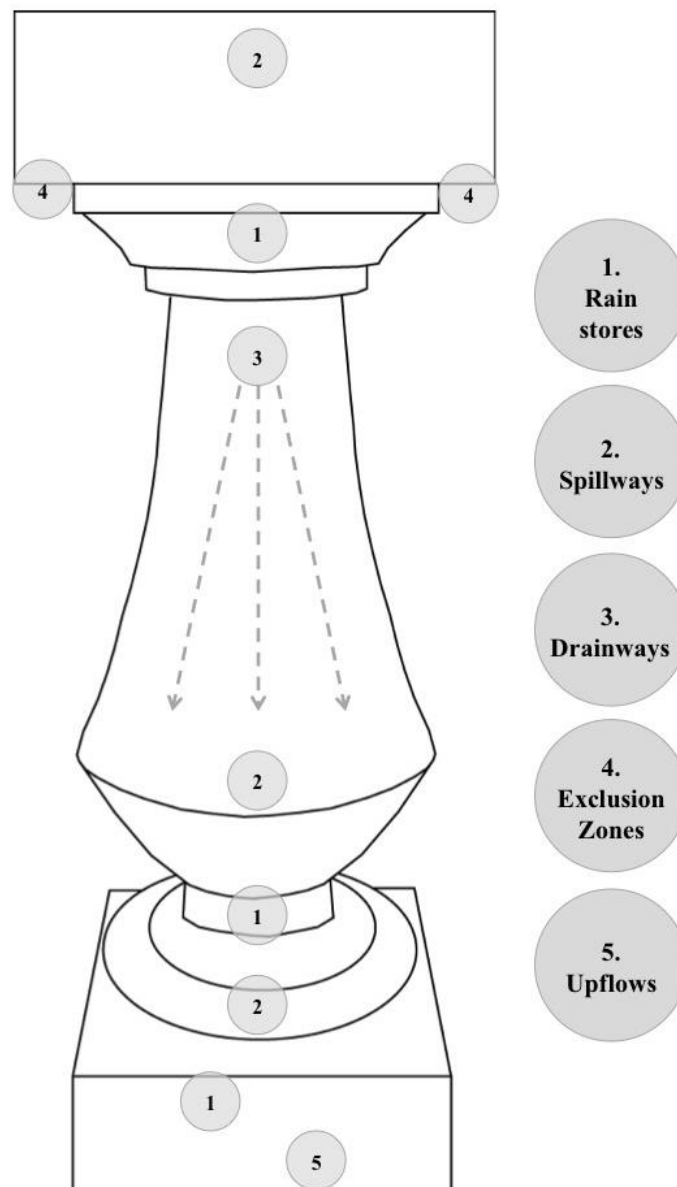
### *Models of greening*

Results from this exposure study demonstrate that a clear pattern of greening does not occur on complex sandstone structures such as balusters. Prior to the start of the study,

three experts were asked to ‘model’ where greening might occur on these structures (see Figure 92: Proposed models of greening). Whilst these models successfully highlighted likely moisture stores, in particular the upper and lower narrow regions (see Figure 108), no single model reflected the complexity of patchy colonisation observed in this experiment.

Our proposed model of greening however (see Figure 93), did encompass the main patches of greening observed. *Section* or baluster geometry was identified as the most influential factor on colour change where the bottommost section of all balusters exhibited greatest change (in either the red or green direction on the  $da^*$  colour axis). Greening was strongly linked with the location of rain stores, spillways and upflows (see Figure 89 for term definitions) from groundwater in particular. Stone inoculation via rising groundwater has been observed in studies across the Mediterranean (Macedo et al., 2009). The location of important hydrological stores and flows on sample balusters (based on observations from time lapse photography and surface moisture measurements) is presented in Figure 118.

Figure 118: Observed location of hydrological features on sample balusters



Our hypothesised model of greening identified patches in the lower sections, where we found surface moisture readings to be the highest. These high readings were due to an accumulation of rainfall falling directly on the base, upflows of groundwater as well as drainways along the baluster shaft supplying a constant supply of rainfall run-off. Whilst total moisture content may be of primary importance, it is the *movement* of surface water via upflows and drainways, which supplies moisture stores with a constant supply of nutrient-rich moisture. We found greater surface greening at the exposed site where

balusters were subject to direct rainfall and more dynamic patterns of wetting and drying over time. This study highlights that a degree of dynamism in moisture movements may enhance a surface's bioreceptivity.

## 7.6 Conclusions

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Results indicate that the shape of a stone feature may contribute towards explaining the variable patterns of patchy colonisation observed on our built heritage. Some of the features traditionally designed as 'rain-shedding' elements have been very successful in achieving their purpose, as illustrated by the drier zones in the central part of the balusters in this study. However, this is often at the cost of adjacent features, which become water stores, such as the narrow and lower sections of the balusters.

The lack of a distinct pattern on all balusters is indicative of the patchy nature of colonisation as seen on building facades as well as in laboratory studies. The complex geometry of the balusters in addition to the differential degrees of weathering and any pre-existing microbial communities resulted in highly variable bioreceptivity across balusters. Section appeared to be the most influential factor in causing greening over aspect and time of exposure. The location of rain stores, spillways and upflows was closely linked to the location of patches of greening on the base of balusters. By identifying primary hydrological stores and flows on vulnerable building facades and monuments, it may be possible to highlight areas of enhanced bioreceptivity.

# Chapter 8:

## Discussion and Conclusions

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A range of both laboratory and field experiments has been conducted to investigate the factors which encourage colonisation of sandstone by green algae. This Chapter addresses the objectives set out in Chapter 1 by drawing together results and observations from the different experiments conducted.

### 8.1 Objective 1

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***O1: To develop improved laboratory experimental methods to control and monitor algal growth (Chapters 2, 3 and 4)***

Four laboratory and two field experiments were conducted within an overall integrated methodology. Laboratory experiments have consisted of inoculating stone samples with axenic and mixed cultures of green algae and incubating under optimised conditions in a growth cabinet. The laboratory experiments conducted are compared and summarised in Table 38. In each case, the inoculation procedure outlined in Chapter 2 (and detailed in relevant chapters) was successful in introducing algal cells to the surface of stone. Samples were not re-inoculated after the initial inoculation protocol (see Chapter 2, Figure 12) or treated with additional nutrients during incubation so as to better simulate a natural epilithic biofilm limited by available nutrition (Papida et al., 2000). The incubation regime tested and optimised in Chapter 3 was successful in catalysing algal growth, resulting in biofilm formation within a minimum of seven to fourteen days in most cases.

Table 38: Comparison of laboratory simulations conducted

Chapter	Variables tested	Sandstones used	Sample size and shape	Number of replicates/ controls	Algal treatment	Inoculation method	Incubation conditions	Duration	Methods for monitoring colonisation/ substrate change
3	Methods testing	Peak Moor	50 x 50 x 50 mm cubes	19 replicates, 3 controls of each type (25 total)	Axenic treatment w/ <i>C. vulgaris</i>	Treatment applied to surface of stone at start of experiment	Temperature 20±1 °C; relative humidity 85±5 %; photoperiod 16 hours light/ 8 hours dark in a growth chamber	35 days	Estimations of algal biomass (cover and density), Sample weight gain, Photography
4.I	Methods testing	Dungannon	25 x 25 x 5 mm tablets	6 replicates, 2 controls	Axenic treatment w/ <i>S. bacillaris</i>			35 days	Surface colour change, Sample weight gain, Photography
5	Influence of external salt treatment on bioreceptivity	Dungannon and St Bees	50 x 50 x 50 mm cubes	3 replicates and 1 control for each variable tested (32 total)	Axenic treatment w/ <i>S. bacillaris</i>			35 days	Estimations of algal biomass (cover and density), Sample weight gain, Photography, Optical microscopy, Dionex
6	Influence of inherent substrate factors on bioreceptivity	Dungannon, St Bees, Peak Moor	25 x 25 x 5 mm tablets	8 replicates/ controls for each variable tested (192 total)	4 treatments: 1- Axenic w/ <i>C. vulgaris</i> 2- Axenic w/ <i>S. bacillaris</i> 3. 1- Axenic w/ <i>D. olivaceus</i> 4. <i>Mixed Algal Population of above strains</i>			42 days	Estimations of algal biomass (cover and density), Sample weight gain, Photography, Surface colour change, Optical microscopy,

The literature reviewed in this thesis documents a range of sample shapes and sizes used for laboratory simulation of epilithic biofilms (see Chapter 1, Table 1), however, in this research we used two sample sizes depending on the objectives of the study (see Table 38). Where the objective was to monitor the influence of colonisation on moisture movements (and vice versa) 50 x 50 x 50 mm blocks of sandstone were used (see Chapters 3 and 5) rather than shallow tablets (see Tiano et al., 1995; Rogerio-Candelera et al., 2011). These were wrapped on four sides to minimise evaporation from side faces and to concentrate biofilm development on a single top face. Samples were configured in this way to encourage moisture retention within the stone, which is known to be conducive to biological colonisation (Gorbushina, 2007). This method was successful in initiating and sustaining surface algal growth.

Where the objective was to observe the influence of inherent stone properties on bioreceptivity (see Chapter 6) smaller, shallow tablets of 25 x 25 x 5 mm were used. By using smaller tablets we were able to maximise the number of control and replicate samples incubated in the limited space of the growth cabinet. This was particularly important when comparing the bioreceptivity of different sandstones to single and mixed algal treatments as there were multiple variables being compared.

Having critically reviewed other laboratory simulation studies, we found that in most cases only a limited number of replicates were used to test individual variables and very limited studies mentioned the use of control samples as a marker for cross-contamination (see Chapter 1, Table 1). In the case of our laboratory experiments, the maximum number of replicates and controls was limited by either the quantity of stone material available (we ensured that all replicates and controls were cut from single quarry blocks to avoid

the influence of inter- block variance in experiments) or the maximum storage capacity of the growth cabinet. The number of replicates was maximised in all experiments whilst allowing for a minimum number of representative and comparative controls (see Table 38). If it had been possible, the number of both replicates and controls would have been increased to enhance the resolution of results and the ability to draw significant conclusions from experimental findings. However, the results from Chapter 3 indicate that the number of replicates was adequate as very similar patterns of greening were observed on the majority of blocks.

The length of laboratory experiments was determined by the duration for which cabinet control samples (sterile samples treated with growth medium and incubated, not inoculated with green algae) remained free of colonisation. Whilst storage trays and the inside of the growth cabinet were sterilised frequently, due to the circling of humid air through the cabinet and therefore the free- movement of algal propagules, it was not possible to keep cabinet controls sterile indefinitely. Given this constraint, we found the maximum incubation length possible to be  $28 \pm 7$  days (see Chapter 3 and 6). However, where samples were kept partially covered (as in Chapter 6, where tablets were kept in petri dishes with lids slightly ajar) it was possible to extend this duration to 42 days (see Table 38 for a comparison of the experiment lengths). Of the studies surveyed, some authors were able to extend incubation periods by up to nine months (see Guillitte and Dreesen (1995)), in these cases samples were inoculated with mixed microbial populations and therefore, cross- contamination of samples may not have been an important consideration. However, our results indicate that great care should be taken when designing experiments involving green algal colonisation in order to reduce the threat of contamination.

Whilst our laboratory experiments have been successful in ‘simulating’ epilithic green algal biofilms, it must be noted that these were accelerated studies. That is, concentrated suspended solutions of green algae were artificially introduced onto fresh, unweathered stone samples, and incubated under optimal conditions of  $20 \pm 1^\circ \text{C}$ ,  $85 \pm 5\%$  relative humidity and a 12:8 hour light: dark regime. All of the studies surveyed used accelerated incubation conditions of around  $20 \pm 10^\circ \text{C}$  and  $85 \pm 20\%$  relative humidity to maximise biofilm growth (see Chapter 1, Table 1). We tried to keep conditions mild enough to represent the optimum growth season of spring- summer in the UK (Harding et al., 2015) and were able to obtain abundant algal growth within 28 days in most laboratory simulations, under these conditions. Therefore, we feel that our experiments have been effective in representing real world conditions.

In our studies, samples were kept in trays or petri dishes filled with water allowing constant supply of moisture to the surface via capillarity. Laboratory experiments were designed in this way to encourage maximum algal growth thereby ensuring enough biomass was produced to effectively assess the influence of factors such as stone porosity on bioreceptivity. Results must therefore be interpreted with a degree of care, as the rates of colonisation and volume of biomass observed may not represent biocolonisation as would be expected in the field environment. Furthermore, in the majority of experiments we used single axenic cultures to inoculate stone samples to simplify experimentation and to focus on the growth characteristics of individual species (see Table 38 for comparison of algal treatments). As Tiano (2002) stresses, studies of this type must be taken as indicative as in reality, epilithic communities are mixed and often diverse. However, isolating key variables and reducing other extraneous sources of variability is highly important to good experimentation and the experiments reported in this thesis have been

designed to simplify. The experimental design developed in this thesis could be expanded in future to provide more complex experiments.

However, whilst the laboratory set-up did not necessarily represent field conditions and the natural fluctuations and seasonality as experienced in the UK, the extent and variable nature of colonisation observed (such as the concentric patterns of colonisation observed in Chapter 3 and Chapter 5) is reminiscent of the kinds of patchy colonisation we observed on monuments in Belfast, Edinburgh and Sheffield (see Figure 1), thus giving additional support to the conclusion that these experiments are relevant to real world situations.

#### *Methods for monitoring moisture movements and surface colonisation*

We have used a range of simple methods to monitor moisture movements and surface colonisation, these methods have been used in both laboratory and field experiments. The fundamental role of moisture in the development of surface algal biofilms was highlighted in Chapter 1 and is discussed under Objective 2 below. Given the important role of moisture, we used weight change as a proxy for moisture ingress and egress from stone samples as well as measurements of surface moisture to assess the relationship between colonisation and moisture movements. Weight change is a highly versatile parameter, its main strength being that it is very simple and requires inexpensive and easily portable equipment. In particular, weight change results presented in this thesis have been useful in differentiating differences between colonised samples and uncolonised controls (see Chapter 3 and 5). Weight change does have its drawbacks however, as it is limited by the resolution of the balance used and can be affected by changing environmental conditions. We also found the patterns of weight change very variable between

experiments (see for example, Figure 24 compared with Figure 34) most likely due to the heterogeneity of the sandstones used for experimentation. Therefore, our results indicate that a large number of controls and replicates are required to successfully assess the influence of surface colonisation on weight change (and therefore, inferred moisture movements). Despite this, results highlight interesting differences between uncolonised controls and colonised replicates within individual experiments (see Chapter 3).

Capacitance and resistance (measured using CEM and Protimeter moisture meters, respectively) have been used as indicators of surface moisture change in both laboratory and field experiments. These measures are outlined in detail in Chapter 2 and have revealed interesting relationships between surface colonisation and moisture change. However, measures of conductivity (and capacitance to a lesser extent) are known to be affected by salinity (see Eklund et al. (2011)). Consequently, it is not possible to attribute high values of either measure to high moisture content alone. Salts are present naturally in building stones and can be deposited exogenously via rainwater or by metabolic activity of green algae themselves. For example in Chapters 3 and 5, peak conductivity values were recorded at the surface centre of blocks in addition to observations of mineral efflorescence in these regions. Despite this however, we have found the moisture meters used to provide a good *indication* of relative moisture content. Furthermore, they benefit from being highly portable, inexpensive and non- specialist devices to use.

The influence of surface colonisation on water penetration was assessed in Chapter 5 using vertical Karsten tubes. This is a very simple and once again, portable, non-specialised and inexpensive method. We found that it yielded reliable and repeatable results demonstrating that algal colonisation can reduce the water penetration capacity of

colonised surfaces. The primary disadvantage we found of using Karsten tubes was disruption or removal of surface colonisation when removing the tube and sealing putty. Therefore, these tests were only conducted after laboratory experiments had ended. This also meant we were unable to use the method in field experiments to prevent damage to vulnerable existing colonisation. However, in their field surveys of colonised sandstone buildings in Belfast, Cutler et al. (2013b) used the method with some success, demonstrating that it also has applicability in the field environment on less friable algal surfaces.

Two main methods were used to monitor surface colonisation in laboratory and field experiments. Spectrophotometry was used with a good deal of success in monitoring changes in the relative greening of samples from their original state (see Chapter 4 for an in-depth discussion of the method). However, use of portable spectrophotometers requires a degree of basic training and the use of specialised colour-assessment computer software. Given this and a general lack of non- specialist methods available to measure both the spatial variation and density of green algal colonisation, we developed a novel, simple and semi- quantitative ‘biomass mapping’ method to assess these parameters in combination (see Chapter 2, section 2.6 for further methodological details).

In brief, the semi- quantitative method assesses percentage algal cover and density of colonisation together, using photographs taken under controlled conditions. We found this method to be a very effective way of mapping spatial and temporal change in surface algal colonisation in laboratory studies. The primary drawback of the method is that it can be quite time-consuming and subjective depending on the observer. However, the results obtained from our laboratory experiments indicate that with some further

modification and testing (by incorporating automated quantification of spatial change, for instance using an imaging program such as ImageJ) it could have wider potential use in both laboratory and field applications.

## 8.2 Objective 2

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***O2: To investigate the fundamental role of moisture in the development of algal greening (all chapters)***

### *Moisture movements*

Whilst epilithic green algae are very tolerant and capable of growing in low moisture conditions, in laboratory experiments we found that maximum growth occurred within a narrow moisture range (see Chapter 3 and 5), as indicated by surface resistivity readings obtained from the Protimeter. In all cases, no growth occurred in the surface centre of blocks; these areas were characterised by peak resistivity readings from which we can infer either high moisture content or high salinity, or a combination thereof.

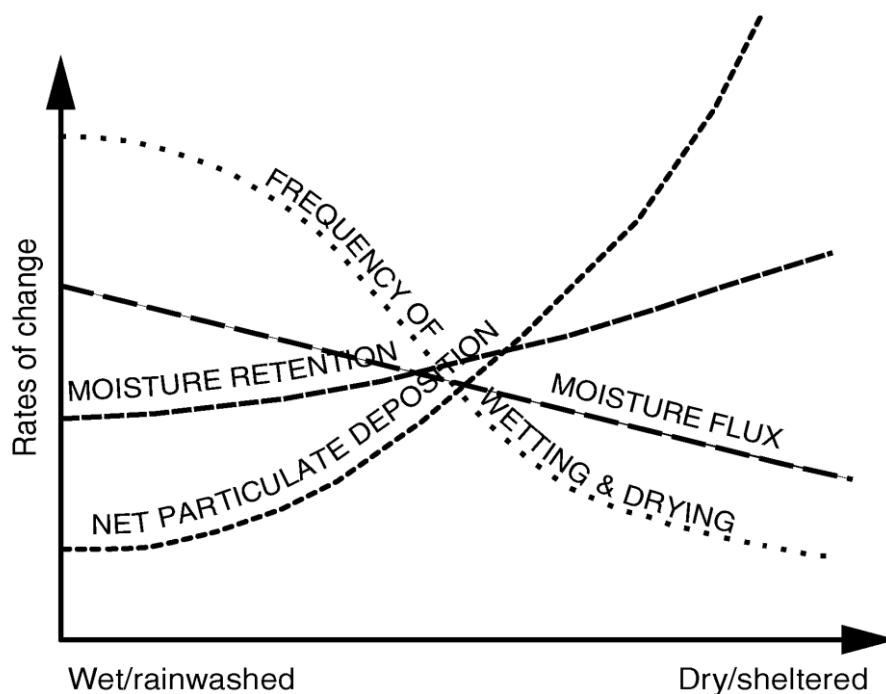
We hypothesise that the centre of blocks were saturated preventing movement of moisture and propagation of algal cells across the surface of blocks. It is likely that hygroscopic-saturation of the centre was due to the concentration of soluble salts, as evidenced by the precipitation of mineral efflorescence on the surface centre of cabinet control blocks (see Chapter 5, Figure 58a). The influence of soluble salts in inhibiting growth was explored in Chapter 6 and is discussed under Objective 3 below.

The distinct concentric patterns of algal growth observed in Chapter 3 and Chapter 5 may also have been a function of the preparation of the blocks themselves. These blocks were wrapped on four sides with impermeable tape and were placed in trays of water.

Therefore, colonisation (and importantly also, evaporation) was restricted to the top exposed face only. It is likely that the patterns of colonisation observed can in large part be explained by the restriction of the movement of moisture into, out and within these blocks (and the consequent mobilisation of salts), as a result of their wrapping. That is to say, sandstone blocks present in buildings are not likely to experience as restricted moisture movement as created in our experiments, and therefore such clear rings of growth are unlikely to occur on building stone in situ. However, some degree of moisture movement restriction does occur in building stone due to mortar joints and different building materials (of contrasting permeability) occurring adjacently. Indeed, patchy colonisation observed on buildings in the northern parts of the UK (see Figure 1) can most often be explained by the variability in moisture distribution (and therefore, dissolved salts and nutrients) across a façade.

Whilst moisture availability has been identified by many to be the most influential factor controlling the ecology of lithobiontic algae (Hoffmann, 1989; Crispim et al., 2003; Gorbushina, 2007), it appears from the results presented in this thesis that *movement* of surface moisture is an important requirement to promote surface colonisation by green algae. Using data and observations from their weathering study of sandstones in Belfast, Turkington et al. (2003) related exposure conditions with rates of change (see Figure 119). They found exposed rain-washed surfaces to have greater biological deposition than sheltered surfaces. Colonisation occurred on frequently wetted surfaces where moisture flux was higher whereas gypsum deposition was concentrated in sheltered areas where moisture retention was greater.

**Figure 119: Causes of differential decay on stone under a continuum of exposure conditions**



*From Turkington et al. (2003, p. 1214)*

Our observations from field studies in Northern Ireland (see Chapter 4. II) and Oxford (see Chapter 7) shed further light on this, where greater greening occurred on the more dynamic southern faces/ exposed sites and greater reddening was observed on moderated northern/ shaded sites. Reddening was concentrated in locations where there was likely to be greater moisture retention and prolonged drying out periods (as indicated by time lapse photographs of the shaded site in Chapter 7, for example). Adamson et al. (2009) observed similar preferential greening of the southern façade of St Mary's Church, Lisbellaw and reddening of the northern façade (see Figure 86). Whilst we were not able to identify the species responsible for the reddening we observed, red colonisation in temperate locations is most often attributed to growth of moisture- loving *Trentepohlia* (Rindi and Guiry, 2002), which acquires its distinct red- orange colour on exposure to light and in low- nutrient conditions (Wakefield et al., 1996a).

The movement of moisture across a stone façade also has key importance for algal propagation, as highlighted by preferential growth in areas of frequent moisture movement in our laboratory (see Chapter 3 and 5) and field studies (see Chapter 4.2 and 7). In particular, spatial observations of inoculated blocks in laboratory studies indicate that as early as two to three days after incubation, colonisation was quite clearly restricted to areas of moisture upwelling (see Figure 21) avoiding areas of moisture stagnation (centres of blocks). In the case of our experiments, the spatial distribution of colonisation (ring- like patterns) was established early due to propagation of cells (and nutrients) within a distinct region of moisture movement. Indeed, distinct streaking patterns of algal colonisation (along areas of frequent wetting such as windowsills, plinths, etc) are widespread on sandstone buildings (see Figure 1).

It appears that dynamic and frequently wetted surfaces are more receptive to green algal colonisation, which may be due to algal propagation and frequent supply of soluble nutrients. Little work has been conducted exploring the relationship between algal propagation, soluble nutrients and moisture movements on sandstone buildings and is an area that requires further study.

### *Moisture retention*

Results from the laboratory experiments in this thesis demonstrate that green algal biofilms may help to protect stone surfaces. Heavily colonised blocks had a lower absorption capacity than samples with less or no colonisation (see Chapter 5, Karsten Tube results). In their survey of sandstone monuments in Belfast, Cutler et al. (2013b) found some evidence to suggest that patches of green growth had a lower absorption

capacity than adjacent uncolonised areas. By reducing water penetration at the surface, surface biofilms may reduce moisture ingress into the wall, thereby reducing the likelihood of damage through moisture-induced dissolution. Conversely, this might also have the effect of ‘sealing in’ moisture and therefore soluble salts at depth, resulting in deep-seated decay (Smith et al., 2011). Further research is needed to investigate the influence that green algal biofilms have, through mediation of surface moisture regimes, on stone deterioration.

Algal colonisation had a variable influence on the moisture retention capacity of stone samples in laboratory experiments. Where weight change was used as an indicator of moisture ingress and egress from stone samples, we found that in some cases samples colonised with green algae gained significantly more weight (and therefore moisture) than uncolonised controls (see Chapter 3). However, in other laboratory experiments a significant difference was not found (see Chapters 5 and 6).

We expect that variations in the influence of colonisation on moisture retention were due to the inherent characteristics of the substrates tested, namely their texture and porosity and also due to the size of samples. For instance, colonisation of Peak Moor blocks (50 x 50 x 50 mm) in Chapter 3 resulted in greater moisture retention than uncolonised samples. However, in Chapter 5, a significant difference in weight change was not found between colonised blocks (50 x 50 x 50 mm) of Dungannon and St Bees compared with uncolonised controls. Furthermore, colonisation did not influence moisture retention on smaller tablets (25 x 25 x 5 mm) of Peak Moor in Chapter 6. Due to the smaller size, tablets would have had less moisture retention capacity than blocks and would have dried out faster due to the greater surface area/ shallower depth ratio.

Our studies have focused on the distribution and movement of moisture at the surface of sandstone blocks. However, Smith et al. (2011) emphasise the need to incorporate studies of deep wetness into models of sandstone bioweathering. They argue that it is often deep-seated moisture that can ‘feed’ biotic colonies, even when the stone surface may be dry. This deep wetting is likely to become more of a concern under projections of increased winter rainfall and enhanced seasonality in the north-west of the UK over the next 40 years (McAllister et al., 2011).

### 8.3 Objective 3

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***O3: To investigate the role of external factors in the development of algal greening (Chapters 5 and 7)***

External factors such as precipitation, temperature, pollution and the influence of marine salts can affect the bioreceptivity of a surface. Our study focused primarily on the influence of marine salts, as investigated in Chapter 5. These investigations were prompted by observations of patchy colonisation around mineral efflorescence (see Chapter 1, Figure 3) at Sheffield Cathedral and patterns of inhibited growth in areas of high resistivity recorded in earlier laboratory experiments (see Chapter 3).

We found that salinity (0.1% and 1% Artificial Sea Water treatment- ASW) delayed colonisation of blocks by at least 7 days and prevented growth all together on blocks treated with 10% ASW. Inhibition of growth was likely due to either desiccation of cells and or direct sodium toxicity. Green algal growth which occurred in saline conditions appeared visually desiccated with reduced deposition of surface EPS, colonisation also

appeared to cluster in and around grain-spaces which may have offered a protective environment (See Chapter 5, Figure 58).

We observed similar patterns of biofilm formation on salt- affected blocks as in our other laboratory experiments (see Chapter 3); rings of colonisation were observed on all blocks treated with 0, 0.1 and 1 % ASW. However, the outline of these 'rings' became more defined as the concentration of salt treatment was increased (see Figure 59). Defined lines of colonisation observed at the micro scale in this experiment were similar to patterns of patchy colonisation observed at Sheffield Cathedral (see Figure 47). This indicates that soluble salts may have a similar inhibiting effect on colonisation on heritage monuments.

As indicated by weight change, saline blocks accumulated more moisture over time than non-saline blocks, but had significantly less surface colonisation. Due to osmosis, salts dissolved in water are known to reduce the availability of that water to microorganisms (Madigan et al., 2009b). It is likely that saline blocks became supersaturated (hygroscopic) resulting in reduction of internal and surface moisture movements (and therefore mobilisation of essential nutrients and propagation of cells across the surface) resulting in inhibited growth.

A significant difference in biomass was not detected between untreated (0% salt) blocks of Dungannon and St Bees. However, differences between the two stone types were detected when blocks were treated with saline solution. That is, salinity reduced the bioreceptivity of St Bees to a greater degree than Dungannon. This cannot be attributed to stone structure alone as Dungannon has a higher porosity than St Bees, therefore one

would expect greater mobilisation and retention of growth- inhibiting soluble salts within its structure. Indeed, control samples of Dungannon gained more weight over time than St Bees. Furthermore, a significant difference in Chloride levels was not detected between the two stone types. Therefore, the reduced bioreceptivity of St Bees might be explained by other, possibly inherent factors (e.g. colour, surface texture, chemical composition).

## 8.4 Objective 4

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### *O4: To investigate the role of inherent factors in the development of algal greening (Chapter 6)*

The influence of inherent factors on the bioreceptivity of sandstones was primarily focused on in Chapter 6, which compared the bioreceptivity of Peak Moor, St Bees and Dungannon sandstones to green algal colonisation. In particular, the porosity, texture and mineralogy of these sandstones affected their receptivity to colonisation.

#### *Porosity/ permeability*

It is widely reported that substrates with a greater open porosity and associated high capillary absorption have the ability to retain more moisture and are therefore more receptive to biological colonisation (Guillitte and Dreesen, 1995; Prieto and Silva, 2005; Miller et al., 2006; Miller et al., 2010). For instance, in their study of Italian monuments, Tomaselli et al. (1999) noted that higher stone porosity and surface roughness had a greater influence in promoting microbial colonisation than other factors such as mineral composition.

In the experiment reported in Chapter 6, we found that treated and wet control Dungannon and St Bees samples gained significantly more weight by the end of the experiment than Peak Moor; a function of their finer texture and higher porosity. However, by the end of six weeks, a significant difference in levels of biomass did not occur between stone types. It must be noted that colonisation on the finer stone types appeared healthier and cells more embedded in EPS than on Peak Moor samples on which cells appeared more embedded in grain spaces. We observed similar embedding of colonisation and surface soiling in grain spaces on balusters exposed in Oxford (see Chapter 7). The lithology of Peak Moor and ‘baluster stone’ is very similar (see Chapter 2, Figure 7) and the nature of surface colonisation on these stone types is likely to be a product of their coarser texture when compared to St Bees and Dungannon sandstones.

Colonisation was most patchy on the finest of the stones, Dungannon. This finding is backed up by literature which suggests that larger- pored materials exhibit more even colonisation. For instance, in their accelerated algal fouling test, Manso et al. (2014) found larger- pored concretes to better absorb and retain water containing algal spores resulting in more more heterogenous growth of *C. vulgaris*. Furthermore in the case of our study, patchy colonisation on Dungannon is likely to be further pronounced due to preferential colonisation due to its mineral composition (see discussion below).

### *Mineralogy/ geochemistry*

As discussed above, a significant difference in biomass was not observed between St Bees, Dungannon and Peak Moor sandstones. However, greening was often concentrated around laminations on Dungannon samples, resulting in patchy surface growth. Indeed, on all Dungannon samples tested in Chapter 6, colonisation was observed to cluster

around laminations. Microscope observations and XRD analysis of sample chips suggest that these are fine clay laminations, predominated by kaolinite (see Table 4). Presence of clays and feldspar minerals is known to increase the bioreceptivity of stone surfaces by retaining moisture and providing microorganisms a greater surface area to adhere to (Warscheid et al., 1991). In the case of Stanton Moor sandstone, which is known to have a similar mineralogy to Dungannon stone, McKinley and Warke (2007) note that diagenetic clays infilling pore spaces in the mineral matrix provide points of weakness for the ingress of moisture. In Dungannon, clay laminations retain moisture resulting in preferential colonisation in these regions.

Cutler and Viles (2010) note that whilst a number of studies have been conducted to compare differences *between* stones, studies looking at the variability in porosity and mineral content *within* stones are limited. The intra-stone variability in bioreceptivity is an area that requires further study. This is particularly the case for inhomogeneous, but widely used British building sandstones such as Scrabo which is rich in feldspars (McGreevy et al., 2000; McKinley et al., 2001) and is known to be particularly vulnerable to patchy biological colonisation (McCabe et al., 2015).

### *Other inherent factors*

The colour of a surface biofilm is likely to influence temperature and moisture conditions at the stone surface. For instance, surface colonisation may impact “the rate at which thermal energy is gained and lost through albedo effect” (Coombes and Naylor, 2012, p. 100). Whereas, darker colonisation may enhance surface temperatures through increased absorption of solar radiation, which may foster further colonisation by warmth-loving microorganisms. However, the role of initial substrate colour on bioreceptivity is less well

understood.

We found total colour change was significantly greater on the buff coloured stones, but most significant *greening* occurred on St Bees. However, this result is likely to be an artefact of the measuring method. That is, due to the interrelationship between red and green hues on the CIELAB  $a^*$  chromatic scale, measurements of relative greening may be greater on red-coloured substrates, as is the case for St Bees. This does not necessarily mean greater biomass occurred on St Bees and highlights the risk of using spectrophotometry alone as an indicator of surface biomass.

Moreover, most greening was observed visually on the buff coloured stones (Peak Moor and Dungannon). This is likely due to photosynthetically usable light being able to penetrate at greater depth in the lighter sandstones (Manso et al., 2014; Hallmann et al., 2015). In terms of trajectories of greening, the buff coloured stones had a broadly similar trajectory but Peak Moor samples were more biased towards green than Dungannon, indicative of greater biomass and or healthier colonisation on the former.

#### *Differences between algal treatments*

Stone type did not appear to have a significant influence on levels of biomass (of any of the algal treatments). Of all the treatments, *S. bacillaris* exhibited the most widespread growth on all stone types. Despite healthy growth of *C. vulgaris* in previous laboratory experiments (see Chapter 3), in this experiment we found its growth to be highly variable with periods of healthy growth and dieback over the course of 6 weeks. This indicates that it might have been more sensitive to changes in ambient conditions/ nutrient changes than the other algal treatments. In all instances, biomass seemed to be closely related to

nutrient levels where most growth and colonisation occurred up to 28 days after which colonies often began to die back. We hypothesise this is because beyond this point, nutrients from the initial inoculation protocol began to be depleted. Growth patterns of the mixed culture were most similar to those of the axenic culture of *D. olivaceus*. This indicates that *D. olivaceus* might have out-competed the other two strains in the mixed culture.

## 8.5 Objective 5

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***05: To investigate the role of architectural factors in the development of algal greening (Chapters 3, 4.II and 7)***

Whilst the availability of moisture has been identified as a fundamental requirement for green algal growth, architectural factors such as the orientation of, or the angle at which, a surface is exposed influences the degree of moisture the stone receives and the duration for which it remains moist. Therefore, architectural factors can influence the bioreceptivity of a surface or building façade.

As discussed earlier, preferential greening was observed on the more dynamic frequently wetted south-west facing blocks in exposure trials in Northern Ireland (see Chapter 4.II) and on exposed compared with shaded balusters in Oxford (see Chapter 7). Results from our exposure trials are somewhat contrary to those conducted by Adamson et al. (2013a), who found northern-facing stone samples were the first to become colonised by green algae. In their experiment, they suggest that enhanced greening on the northern faces was due to increased time-of-wetness resulting from slower drying times. In the case of our experiments however, we propose that greater levels of greening on more dynamically

wetted or exposed faces occurred due to a number of contributory factors. Frequent wetting of these surfaces may have provided a ready supply of algal propagules as well as dissolved nutrients and salts required for growth. Furthermore, these faces receiving higher levels of solar incidence which may also have contributed to the preferential greening observed.

Conversely, in our studies greater reddening was observed on north-west facing blocks in Northern Ireland and at the shaded site in Oxford. This is most likely due to prolonged time-of-wetness and longer drying-out cycles (as demonstrated by time lapse photography, for instance) favouring growth of moisture and damp-loving red-hued microorganisms (likely to be Trentepohlia).

There is growing evidence to suggest that greater biocolonisation occurs on surfaces which remain moist for longer and or are supplied with deep-seated moisture (see McCabe et al. (2013), Smith et al. (2011)). Furthermore, Cameron et al. (1997) highlight that the period for which a stone remains moist is more important in encouraging colonisation than frequency of wetting. Our results suggest that whilst moisture may have an important influence on total biomass, the diversity of colonisation is dependent on environmental conditions, which are often moderated by architectural factors. Namely, more tolerant green-hued chlorophyta seem to thrive in dynamic zones of moisture movement and higher levels of solar incidence whereas locations characterised by longer drying-out periods and darker conditions support growth of red-hued organisms.

One of the primary goals of exposing balusters in the field environment (see Chapter 7) was to ascertain the influence of angles of exposure and architectural form on

bioreceptivity. In our study, aspect and time of exposure (six or twelve months) did not have a significant influence on levels of greening observed. However, section (surface geometry) did, in that the bottom most section of balusters at both the exposed and shaded locations became significantly greener than other sections. This correlated well with higher surface and near-surface resistance and capacitance readings, indicating greater moisture retention than other sections.

We suggest that by labelling building facades and monuments with potential hydrological stores and drainways (spillways and flows) it is possible to identify areas of higher and reduced bioreceptivity (see Chapter 7, Figure 118). In particular, our observations of building facades and monuments in the northern regions of the UK (see Chapter 1, Figure 1) indicate that drainways encourage biological colonisation. That is, dynamic zones of frequent moisture run-off are likely to provide algal cells with a constant supply of moisture and nutrients required for metabolic growth and propagation. Indeed, in addition to the relative moisture content of stonework, it the *movement* of moisture that enhances bioreceptivity and encourages colonisation.

The following section summarises the key findings from the linked laboratory and field experiments presented in this thesis followed by a summary of areas, which we have highlighted as requiring further study in the future.

## 8.6 Key Findings

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- Greater levels of greening were found to occur in dynamic moisture movement zones in both laboratory (central, ring zones on incubated blocks) and field experiments (south-western faces/ exposed locations). Reddening was more pronounced in areas of moisture accumulation and stagnation in field studies.

- A range of external, internal and architectural factors influence the degree and distribution of algal greening observed in accelerated laboratory simulations and naturalistic field experiments.
  - External factors- Due to the influence salinity has on the moisture retention capacity of sandstone; we found treatment with moderate to high concentrations of sea salt to significantly inhibit algal colonisation.
  - Internal factors- laboratory experiments demonstrated that porosity, mineralogy and surface colour influenced the degree and distribution of algal colonisation. Dungannon was the most bioreceptive of the three stone types tested (St Bees, Peak Moor and Dungannon) due to its comparative ability to retain moisture.
  - Architectural factors- surface geometry had a notable influence on the distribution of algal colonisation observed in field experiments. Zones of frequent wetting and moisture accumulation (spillways and stores) better retain moisture and support algal greening.

## 8.7 Recommendations for further study

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- Further development and testing of ‘biomass mapping’, the novel method developed to measure algal biomass (such as testing its applicability in the field environment).
- Assessment of the nutritive effect of rainwater in enhancing the bioreceptivity of sandstone surfaces. In particular does the frequency of wetting or the duration of moisture retention improve surface bioreceptivity?

- Investigations of the influence of substrate colour on bioreceptivity.
- Studies to test the influence of intra-stone variations on the bioreceptivity of inhomogeneous or clay-rich sandstones.
- As only primary bioreceptivity was assessed here, we recommend future studies explore the secondary and possibly, tertiary bioreceptivity of British building sandstones.

## 8.7 Conclusions

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### *Overall thesis aim*

**To investigate the factors which encourage colonisation of sandstone by green algae, through laboratory and field- based experimentation**

Our results demonstrate that it is possible to ‘simulate’ epilithic green algal biofilms under optimised laboratory conditions. These studies allowed for the monitoring of biomass change over time and the influence of inherent and external factors on bioreceptivity, such as intrinsic substrate characteristics and exogenous treatment with marine salts. Field experiments allowed study of complicated architectural factors (such as geometry, aspect and shading) on surface moisture movements and colonisation patterns. In combination, laboratory and field experiments aimed to investigate the factors which encourage colonisation of British building sandstones by green algal colonisation.

Our experimental results show that different algal species are likely to have different effects on different types of sandstone; it is therefore difficult to make generalisations. Furthermore, the results demonstrate that experimental design can have an impact on the

results obtained so care needs to be taken in comparing the results from different studies. Therefore we recommend stakeholders take a degree of caution when generalising results from laboratory experiments to specific buildings, particularly where contrasting stone types are used. Furthermore, the variability in results we obtained from different experiments highlights the importance of having a large number of replicate and control samples for comparison and to better enable generalisation of results. By using smaller stone tablets (25 x 25 x 5 mm) an effective compromise seems to be reached between maximising the number of samples incubated whilst retaining a suitable sample size to ensure sufficient surface area for study of spatial colonisation patterns.

The bioreceptivity of sandstones is highly complex and is influenced by a combination of inherent, external and architectural factors. Short-term laboratory experiments in combination with field experiments, as presented in this thesis, are an effective way in which to test the influence of these factors on green algal colonisation patterns. Laboratory experiments benefit from controlled conditions and the ability to monitor growth characteristics of individual strains on a variety of sandstone substrates. However, they are limited in duration due to the difficulty in maintaining sterile conditions. Field experiments benefit from being realistic as they rely on natural microbial propagation of samples. However, in these experiments it is difficult to establish cause- effect relationships between individual microbial genera and patterns of surface change. To improve the study of British sandstone bioreceptivity in the future, we suggest that the results from an increased number of short- term laboratory tests (to assess the bioreceptivity of a wider range of sandstones to a greater diversity of axenic and mixed green algal cultures as well as naturally isolated mixed microbial populations) are assessed in combination with results from longer- term field experiments. Successful

application of this combined approach will lead to more diverse set of results from different building stones and microbial populations, better enabling stakeholders to interpret and apply results to specific heritage buildings.

Simple semi-quantitative and qualitative methods as used in this thesis (such as biomass mapping and identifying hydrological stores and flows) can be a useful means by which to differentiate areas of greater/ reduced bioreceptivity and highlight the potentially vulnerable areas on a monument/ façade to green algal colonisation. The variety of non-specialist, inexpensive and portable methods used in this thesis demonstrate that it is not always necessary to use highly specialised or complex methods in bioreceptivity studies. Greater engagement of the architects, surveyors, builders and custodians managing our built heritage is required to address the limited knowledge of the bioreceptivity of British building sandstones to green algal colonisation. We hope that the accessibility of the methods proposed in this thesis, encourages a wide range of stakeholders to survey vulnerable buildings and build on gaps in knowledge.

Moisture plays a fundamental role in the development of green algal biofilms. Results from integrated laboratory and field experiments highlighted that in addition to the relative moisture content of stonework, it is the *movement* of moisture that enhances bioreceptivity and encourages colonisation. That is, green algae were found to thrive in dynamic moisture movement zones on blocks in laboratory studies and in areas of rainwater flow in field experiments. These dynamic zones are likely to facilitate colonisation by enabling greater movement of algal propagules and the movement of soluble nutrients required for growth. Therefore, by identifying these ‘dynamic zones’ (through mapping of rainwater flows across a façade or conducting simple moisture

surveys, for example), it is possible for stakeholders to pinpoint which areas of a building are most vulnerable to biological colonisation.

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# Appendices

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## Appendix 1: Recipe for 3N-BBM+V-growth medium

**Name:** 3N-BBM+V (Bold-Basal Medium with 3-fold Nitrogen and Vitamins; modified)

**Description:** Freshwater algae

### Stocks per litre

(1) NaNO<sub>3</sub> - 25.0 g

(2) MgSO<sub>4</sub>·7H<sub>2</sub>O - 7.5 g

(3) NaCl - 0.5 g

(4) K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O - 7.5 g

(5) KH<sub>2</sub>PO<sub>4</sub> - 17.5 g

(6) CaCl<sub>2</sub>·2H<sub>2</sub>O - 2.5 g

(7) *Trace elements solution*

Add to 1 litre of distilled water 0.75 g Na<sub>2</sub>EDTA and the minerals below in exactly the following sequence:

FeCl<sub>3</sub>·6H<sub>2</sub>O - 97.0 mg

MnCl<sub>2</sub>·4H<sub>2</sub>O - 41.0 mg

ZnCl<sub>2</sub>·6H<sub>2</sub>O - 5.0 mg

CoCl<sub>2</sub>·6H<sub>2</sub>O - 2.0 mg

Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O - 4.0 mg

(8) *Vitamin B1*

0.12 g Thiaminhydrochloride in 100 ml distilled water. Filter sterile.

(9) *Vitamin B12*

0.1 g Cyanocobalamin in 100 mg distilled water, take 1 ml of this solution and add 99 ml distilled water. Filter sterile.

### Medium

Stock solution **1** - 30.0 ml

Stock solutions **2 - 6**, 10.0 ml each

Stock solution **7** - 6.0 ml

Stock solutions **8 - 9**, 1.0 ml each

### Method

Make up to 1 litre with deionized water. Autoclave at 15 psi for 15 minutes.

*Available from: CCAP; Argyll, UK*

## Appendix 2: CIE 2000 Colour difference formula

This is the newest color difference formula intended to correct the differences between the measurement result and visual evaluation, which was the weak point in the  $L^*a^*b^*$  color space. The calculation is based on the lightness difference  $\Delta L^*$ , saturation difference  $\Delta C^*$ , and hue difference  $\Delta H^*$ , with correction using weighing coefficients (SL, SC, and SH) and constants called parametric coefficients (kL, kC and kH), as shown below.

$$\Delta E_{00} = \sqrt{\left(\frac{\Delta L^*}{k_L \cdot S_L}\right)^2 + \left(\frac{\Delta C^*}{k_C \cdot S_C}\right)^2 + \left(\frac{\Delta H^*}{k_H \cdot S_H}\right)^2 + \left(R_T \left(\frac{\Delta C^*}{k_C \cdot S_C}\right) \left(\frac{\Delta H^*}{k_H \cdot S_H}\right)\right)}$$

where

$$L' = L^* \quad a' = a^*(1+G) \quad G = 0.5 \left( 1 - \sqrt{\frac{\bar{C}_{ab}^*}{\bar{C}_{ab}^* + 257}} \right)$$

$$b' = b^* \quad C' = \sqrt{(a')^2 + (b')^2} \quad h' = \tan^{-1} \left( \frac{b'}{a'} \right)$$

The weighting coefficients SL, SC, and SH are defined by the following equations

$$S_L = 1 + \frac{0.015 (\bar{L}' - 50)^2}{\sqrt{20 + (\bar{L}' - 50)^2}} \quad S_C = 1 + 0.045 \bar{C}' \quad S_H = 1 + 0.015 \bar{C}' T$$

$$T = 1 - 0.17 \cos(\bar{h}' - 30) + 0.24 \cos(2\bar{h}') + 0.32 \cos(3\bar{h}' + 6) - 0.20 \cos(4\bar{h}' - 63)$$

The rotation factor RT is determined as shown below. (The hue angle and  $\theta$  are in degrees.)

$$R_T = -\sin(2\Delta\theta) R_C \quad \Delta\theta = 30 \exp \left( - \left( \frac{\bar{h}' - 275}{25} \right)^2 \right)$$

$$R_C = 2 \sqrt{\frac{\bar{C}'^7}{\bar{C}'^7 + 257}}$$

Note 1: The bar over the values indicates the average value for the two colors.

Note 2: The parametric coefficients kL, kC, and kH may vary according to the actual measurement conditions, but all three are set to 1 under the standard conditions specified for CIE 2000.

*From Konica Minolta (2007a)*

### Appendix 3: Seawater Corrosion Test Mixture kit contents

- Product no. 331533P, VWR International Ltd., Poole
- Synthetic seawater approximating to that from the North Sea of about 3.5% by weight total dissolved salts
- Artificial sea water contents per litre:
  - Sodium chloride 26.5g,
  - Magnesium chloride 2.4g,
  - Magnesium sulphate 7H<sub>2</sub>O 3.3g,
  - Calcium chloride 2H<sub>2</sub>O 1.1g,
  - Potassium chloride 0.73g,
  - Sodium bicarbonate 0.2g,
  - Sodium bromide 0.28g.

## Appendix 4: iButton Hygrochron Temperature/ Humidity Logger (DS1923) Saturation Drift Compensation method

H<sub>corr</sub> is the humidity reading with the software correction algorithm for humidity already applied, as explained in the previous section. The function and values of the other parameters are explained in the table below.

### Software Saturation Drift Compensation

Capacitive humidity sensors read higher humidity values than the actual humidity level when they are exposed to a high-humidity environment for an extended time period. The DS1923's humidity sensor produces readings that are higher than the actual humidity when exposed to humidity levels of about 70%RH and higher. This shift continues to increase while the device remains at 70%RH and above. This effect is called saturation drift, or hysteresis. This drift is reversible. Readings return to their regular level when the DS1923 is removed from a high-humidity environment.

It is possible to compensate for most of the error introduced by the saturation drift by post-processing tem-

perature and humidity logs using the equation below, which is based on laboratory tests and curve-fitting techniques.

$$H_{Scorr} = HT_{corr} - \sum_{k=1}^N \frac{0.0156 \times ARH_k \times 2.54^{-0.3502 \times k}}{1 + (T_k - 25)/100}$$

ARH<sub>k</sub> The average software-corrected and temperature-compensated humidity reading of the k<sup>th</sup> hour that the device is continuously exposed to 70%RH or higher.

T<sub>k</sub> The average software-corrected temperature reading of the k<sup>th</sup> hour that the device is continuously exposed to 70%RH or higher.

N The number of hours that the device is continuously exposed to 70%RH or higher.

HT<sub>corr</sub> The humidity reading **at the end** of the N<sup>th</sup> hour with the software correction algorithm for humidity and temperature compensation already applied. See the *Software Correction*

### Numerical Saturation Drift Compensation Example

SAMPLE INPUT DATA (N = 8)			APPLICATION OF CORRECTION ALGORITHM
k (HOUR)	T <sub>k</sub> (°C)	ARH <sub>k</sub> (%RH)	PARTIAL CORRECTIONS (INDIVIDUAL ADDENDS)
1	25.1	91.1	1.024321
2	25.0	92.5	0.751140
3	24.9	92.9	0.544824
4	25.0	93.1	0.393535
5	25.1	93.2	0.283950
6	25.1	93.3	0.205086
7	25.0	93.6	0.148591
8	24.9	93.7	0.107428
HT <sub>corr</sub> = 93.70207%RH			Sum of partial corrections: 3.458875

*Algorithm for Humidity and RH Temperature Compensation sections for details.*

The numbers in the equation are derived from curve fitting. They apply to a time scale in hours.

$$H_{Scorr} = HT_{corr} - \text{Sum of partial corrections}$$

$$= 93.70207\%RH - 3.458875\%RH$$

$$H_{Scorr} = 90.24319\%RH$$

The data in this example was taken from devices that were exposed for several hours to 90%RH at 25°C in a test chamber. The drift per hour decreases the longer the device is exposed to high humidity. The correction algorithm compensates for the drift reasonably well. For some applications, compensation may not be necessary since the error introduced by the saturation is relatively small.

*From Maxim Integrated Products Inc (2015)*