

## Article

# Measuring the Primary and Secondary Bioreceptivity of Stone and Their Implications for Heritage Conservation

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**Abstract:** Bioreceptivity measures the ability of a surface to develop and maintain a permanent ecosystem of microorganisms. In the historic built environment, this is characterised visually by the greening of monuments and other outdoor heritage. Primary and secondary bioreceptivity refer to the potential for biological growth on fresh and weathered stone, respectively. Measuring bioreceptivity helps us understand biological growth rates and allows researchers to characterise the impact of stone conservation treatments on colonisation. Understanding the relative bioreceptivity of stones allows heritage professionals to make more informed selection of replacement stone types for repairs to existing structures. The primary bioreceptivity of stones from different geographical areas cannot currently be easily compared due to a lack of consistency in approaches to measurement. We propose a repeatable lab-based methodology for measuring colour and chlorophyll a levels from a standard algal culture grown on the stone surface as a standardised testing protocol for primary and secondary bioreceptivity. This protocol controls for the effects of mineralogical colour change during testing, which is something that has not been addressed in other methodologies. This method was successfully applied to case studies measuring the bioreceptivity of English granite, sandstone and limestone, determining their position in a bioreceptivity index. Our results demonstrated that for the stones tested, primary bioreceptivity was categorised as very low or low. Secondary bioreceptivity was increased compared to primary bioreceptivity. This research is the first to fully categorise any stones for secondary bioreceptivity and provides the first primary and secondary bioreceptivity data for any UK stones. We encourage others to follow this standard protocol to add additional data and build an international bioreceptivity database accessible to heritage practitioners.



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**Keywords:** stone conservation; biodeterioration; bioreceptivity; heritage science; bioreceptivity index

## 1. Introduction

The bioreceptivity of a surface is a measure of the physical and chemical characteristics that define its ability to maintain a permanent ecosystem of microorganisms, as opposed to finding transient bacteria or fungi present. Methods used to measure bioreceptivity are variable, and as such, results are presently not comparable between different research groups. Guillitte & Dreesen [1] defined three types of bioreceptivity, which have since been further subdivided into four [2]. Primary bioreceptivity is the initial potential for the biological colonisation of sound stone, secondary bioreceptivity is the potential for the colonisation of weathered stone, tertiary bioreceptivity is the potential for stone to support new growth after conservation treatments that do not leave a deposit on the surface (e.g., laser cleaning or cleaning with deionised water) and quaternary bioreceptivity refers to the potential for colonisation after conservation treatments that result in the modification of the surface (e.g., the application of bioinhibitors or paints). As part of selecting an appropriate replacement stone, understanding and measuring bioreceptivity will help heritage professionals select stone types that not only match the physical characteristics of the original stone but will support biological growth in the same fashion, allowing the repair to blend in aesthetically over time. In addition, bioreceptivity allows them to

determine whether the biological growth they are finding on a surface is expected and thus determine whether levels of regrowth after cleaning are also normal.

The bioreceptivity of stone is driven by the stone's properties, which research suggests are primarily the water absorption by capillarity coefficient and surface roughness of the samples [3–5]. Capillarity is defined as the rate at which stone absorbs liquid water due to capillary force (the movement of liquid in a space without the influence of external forces such as gravity). Surface roughness measures the small changes in the stone surface due to its overall structure. A rougher surface will mean the stone has a larger exposed surface area. Both the amount of absorbed water and the surface area can influence colonisation by microorganisms [4]. The chemical composition of the stone will influence the component species present in the microbiome [6,7] but to date has not been shown to influence the bioreceptivity of the material.

Previous research to measure bioreceptivity has attempted to characterise stones from predominantly southern Europe, for example [3,4,8–19]. However, the lack of consistency between methods of both creating and measuring surface growth in a laboratory environment means that work between different research groups cannot be cross-compared. Some research groups inoculate the stone surface with microbiome samples from the environment. Although this approach is more representative of the natural environment, it lacks repeatability due to local variations in the microbiome [12,20]. Growing a complex mixed culture of microorganisms in liquid culture to produce the initial inoculum for the experiment results in the loss of species from the culture roughly 30% of the time [21]; as algae give a higher chlorophyll fluorescence yield than cyanobacteria [22], changes in the applied culture will impact the reproducibility of this study. This introduces the need for regular monitoring of the species components of the culture to ensure that the inoculum remains consistent within and between experiments, increasing the overall time and expense in carrying out the methodology. The substrate to which the inoculum is applied also has a profound influence on the species composition of the biofilms that develop on it [23], and therefore an environmental mixed culture that has adapted to one type of stone may be inappropriate for others.

Studies that have measured bioreceptivity using fungi and lichen growth used single-species samples that were clearly identified in their published research papers [15,24–26]. In many cases, the fungal culture methodology involved the continuous application of growth media to the stone surfaces throughout the whole growth cycle [15,25,26]. The bioreceptivity would have been artificially enhanced by the additional nutrients, and therefore this approach does not demonstrate the ability of the stone surface itself to support growth.

The use of known algal or cyanobacterial cultures is the final commonly used approach to investigate bioreceptivity. Studies generally use between three and six defined species that are representative of those commonly found in the environment [4,10,16,27–29]. In some cases the algae used are isolated from the local environment and characterised before use; this can lead to issues with reproducibility as strain variants of the same microorganisms isolated from different locations can have significantly different levels of biofilm production [30] and growth rates [31]. This can be addressed by using known isolates from culture collections [32]. In our methodology, we therefore propose the use of a known, commercially available algal species to ensure the reproducibility of results.

Issues of reproducibility and cross-comparability in bioreceptivity studies also arise from different growth conditions with light/dark cycles ranging from 'natural cycles' [3,5], in which the light/dark period is not reported, to using a climate-controlled chamber in which the light/dark period, temperature and relative humidity are all reported [4,33–35]. It is important to consider the biological growth conditions used for this study. A climate chamber using controlled growth conditions for light/dark periods, relative humidity and temperature provides repeatable conditions that can be used between different research groups, whereas natural light cycles are dependent on season and location.

There have been a wide range of different measurements used for calculating bioreceptivity, including visual observations of microbial growth [4,24], microscopic cell counts [20,24] and chlorophyll fluorescence or photography to generate a percentage area coverage [8,10]. The most common approach with visual observation is to rank the surfaces as having high, medium or low bioreceptivity based on how they look in comparison with each other. Although this is a rapid and inexpensive method, it is highly subjective [36]. Direct microscopic cell counts can provide an accurate measurement of the level of colonisation at the time of sampling. There is an initial cost in the purchase of a microscope, but the consumables are inexpensive. However, this technique is time-consuming and requires trained and experienced microbiologists to carry out the counts; otherwise, it will be subjective and prone to error [37]. Analysing the bioreceptivity by percentage area coverage delivers a result that is, depending on the inoculation method, reproducible. A significant limitation is that it does not reflect the cell density, for example, 2000 cells or 100,000 cells per  $\text{mm}^2$  would both be counted as 1  $\text{mm}^2$  coverage; as such, this method is best carried out in conjunction with other methodologies [14].

L\*a\*b\* colourimetry and the spectrofluorometry and visible light spectrophotometry of chlorophyll all provide quantitative results that are less prone to operator bias [36]; however, all three approaches require more expensive equipment than those mentioned previously. Of the three methods, L\*a\*b\* colourimetry is the most prone to user bias; this can be overcome by having rigorous protocols in place to ensure that the operators measure the same location and are not asked to measure the area of the highest colour change [38]. Most colourimeters require direct contact with the sample, which introduces the possibility of contamination of the surface and, as such, is best used as a spot measurement with an appropriate microbiological aseptic technique. Chlorophyll fluorescence can be carried out using two approaches, either with a camera or spectrofluorometer. Both are highly time-dependent methods, as the fluorescence starts to drop as soon as UV exposure stops. Fluorescence is also problematic as it is a relative measurement [39], and readings cannot be compared to results from other instruments without an associated calibration curve being used to change the fluorescence reading into a quantified result, usually  $\mu\text{g}/\text{cm}^2$  [34]. Studies that have not published their fluorescence control results or converted to a non-relative measurement, such as  $\mu\text{g}/\text{cm}^2$ , cannot be compared with the rest of the literature. The visible light spectrophotometry of extracted chlorophyll gives a quantitative measure of the amount of chlorophyll extracted, which relates directly to the level of algal growth on the surface, which is again calculated in  $\mu\text{g}/\text{cm}^2$  [11,27,28].

In 2018, to address the need for a cross-comparable methodology, Vázquez-Nion et al. [34] proposed an index for calculating bioreceptivity based on results from seven granites, but this index has not been tested on other stone types. They suggest calculating the following three aspects: total bioreceptivity ( $BI$ ); bioreceptivity based on growth ( $BI_{\text{growth}}$ ); and bioreceptivity based on colour change ( $BI_{\text{colour}}$ ). All three measures are beneficial, as end users of the stone may have different requirements. For example, where the aesthetic impact is less important,  $BI$  or  $BI_{\text{growth}}$  would be most suitable. For the integration of a stone into a façade or into the landscape where aesthetic properties are key,  $BI_{\text{colour}}$  or total  $BI$  may be most suitable.

Vázquez-Nion [34] proposed that calculating  $\Delta E^*ab$  and pulse amplitude modulated fluorometry to give a measure of chlorophyll a ( $\mu\text{g}/\text{cm}^2$ ) would provide measurements associated with the key characteristics related to bioreceptivity and could therefore be used to calculate their bioreceptivity index. However, differing research methods used in previous studies to obtain the raw data for this calculation mean that the bioreceptivity index calculations cannot be applied to previous results from a range of research groups. In order for a bioreceptivity index to be broadly applicable for future studies, it is necessary to have a more standardised protocol. We have explored whether their proposed bioreceptivity index is applicable to a wider range of stone types through colourimetry measurements on an L\*a\*b\* colour scale and chlorophyll a measurements through spectrophotometry ( $\mu\text{g}/\text{cm}^2$ ) and have identified modifications to make the bioreceptivity index more widely applicable.

No UK stones have previously been fully characterised for bioreceptivity and associated properties to date. Therefore, we have tested our proposed methodology through the characterisation of three English stones, including granite, sandstone and limestone, demonstrating the effectiveness of the methodology on a range of stone types. Foggintor granite, Dartmoor, Devon, has been used in the main column of Nelson's Column [40], as well as many other notable London buildings. Howley Park sandstone, Leeds, West Yorkshire is found in historic buildings such as the Coliseum Theatre in Leeds [41] (p. 132) and Wentworth Woodhouse in South Yorkshire [42]. Jordans Basebed is a Portland limestone that originates from the Isle of Portland, Dorset. Portland limestones were widely used by Christopher Wren in the rebuilding of London following the Great Fire in 1666 and have been used in buildings such as St. Paul's Cathedral, Somerset House and the British Museum [41] (pp. 180–182). These stones are sufficiently different in geochemical and physical properties to ensure that the methodology developed is suitable for the majority of international building stones. This also adds new information regarding these specific types of UK stones, which will aid decision-making for those working with these stone types.

Our work focusses on the lab-based measurement of primary and secondary bioreceptivity. The characterisation of bioreceptivity properties has been carried out to confirm the success of the methodology, and utilising a lab-based environment means that experiments are carried out in a controlled and repeatable fashion. The same techniques can then be used as a model to test the impact of conservation treatments on re-colonisation (tertiary and quaternary bioreceptivity). Through a review of published papers, we have selected the most effective and reproducible methodologies, which will allow bioreceptivity testing to be carried out in a manner that facilitates the cross-comparison of results across different research groups. The use of our robust standardised methodology will enable conservators to compare bioreceptivity measurements from all future studies that use this set of techniques, and these methods can be applied internationally with equipment and materials that are readily available.

## 2. Materials and Methods

UK stones used were Portland limestone, Jordans Basebed, an oolitic limestone (supplied by Albion Stone, Portland, Dorset, UK), Howley Park Quarry 3 sandstone, a dolomitic sublithicwacke-stone (supplied by Hutton Stone, Berwick Upon Tweed, Northumberland), and Foggintor granite, a biotite-bearing syeno-granite pegmatite (supplied by Blackenstone Quarry, Devon, UK). The limestone and sandstone samples were 40 mm × 40 mm × 10 mm, and the granite samples were 50 mm<sup>3</sup> due to preparation constraints for stone cutting. All samples were produced with a sawn finish with no visible saw marks. These samples were used for both characterisation and inoculation for the bioreceptivity tests.

Stone samples were artificially weathered using a Genlab oven, heating for a minimum of 1 h at 105 °C, followed by 1 hour cooled in deionised water at 20 °C. This was repeated for 20 cycles. This protocol was developed based on BS EN 14066:2013 [43], with modifications based on other studies within the field [44–51]. To confirm this had caused ageing (in terms of morphological and mineralogical changes) to the stone, petrographic analyses of the sound (unweathered) and artificially weathered samples were carried out by Petrolab Ltd., C. Edwards Offices, Gweal Pawl, Redruth TR15 3AE, UK. Thin sections were prepared by impregnating the samples in epoxy resin with yellow dye to highlight voids and cracks. Thin sections were visualised using a Zeiss AxioImager M2m polarising microscope (Zeiss, Oberkochen, Germany). A modal analysis of composition was carried out using a Pelcon 64-channel electromechanical point counter (Pelcon, Ballerup, Denmark) with 1 mm stepping and traverse intervals and 500 step points counted per sample.

The water absorption coefficient by capillarity was measured on both the unweathered and artificially weathered samples to a minimum of  $n = 3$  for each sample type following BS EN 1925:1999 [52]. In brief, samples were dried for 24 h at 70 °C prior to testing and then cooled to room temperature in the oven. Each sample was weighed to an accuracy of 0.01 g, and the area of the base to be immersed was calculated to the nearest 0.1 mm<sup>2</sup>.

Samples were placed in a tank on thin supports and immersed in deionised water to a depth of 3 mm up from the base of the sample; this level was maintained throughout the whole testing cycle. Samples were weighed at 1, 3, 5, 10, 15, 30 and 60 min and then every 60 min from that point onwards if necessary. For longer periods, a cover was used to prevent evaporation. Measurements were stopped if the sample was visibly saturated or if the difference between the current and previous recorded weights was less than 1% of the mass of water absorbed by the specimen. Results were plotted on a graph with 'water mass in g/area of base' on the Y axis and 'square root of time in seconds' on the X axis. The correlation coefficient between the measured points of the linear section of the graph was found to be greater than 0.9 in all cases, and as such, the capillarity was calculated as the slope of the linear section of the graph.

Surface roughness measurements were performed with a Surtronic S-128 surface roughness tester (Taylor Hobson, Leicester, UK) per the manufacturer's guidelines. The scan length was set to 4 mm with a PK-02 5 µm pickup, giving a 50 nm resolution. Measurements were taken to a minimum of  $n = 6$  per sample.

The measurement of open porosity was based on protocols in BS EN 1936:2006 [53] and BS EN 13755:2008 [54] and taken to a minimum of  $n = 3$  per sample. Specimens were dried for 24 h at 70 °C and then cooled slowly to room temperature in the oven (Genlab, Widnes, UK). Each specimen was weighed to an accuracy of 0.01 g and then placed into an evacuation vessel. The specimens were covered with deionised water, which required approximately 1 litre of water in total. The pressure was then lowered with a vacuum pump for 10 min. The vessel was left for two hours under negative pressure to ensure that all the air in the specimens was replaced with water. The chamber was equilibrated to room pressure, and the samples were removed, wiped with a damp cloth and weighed to an accuracy of 0.01 g. Open porosity was calculated as follows:

$$\text{Open porosity (\%)} = (\text{wet weight} - \text{dry weight}) / \text{wet weight} \times 100$$

Algal species, CCAP211/11B, *Chlorella vulgaris* (type culture), CCAP379/1B, *Stichococcus bacillaris*, and CCAP219/5A, *Trebouxia decolourans*, were obtained from the Culture Collection of Algae and Protozoa (CCAP, Oban, UK) [55] and cultured at room temperature under natural light using the standard microbiological technique in 25 mL of BG-11 media from CCAP and took two weeks to grow to confluence. All three have been identified as growing on stone in previous studies [10,12,56]. *Trebouxia decolourans* was found to require a longer growth period, approximately one month to grow to confluence, and required a large surface area for oxygen exchange; this was achieved using 50 mL BG11 media in a 250 mL conical flask.

Stone blocks were sterilised using an autoclave in borosilicate glass petri dishes (Fisher, Loughborough, UK). Each stone sample was inoculated with 61.6 µL/cm<sup>2</sup> of mixed algal culture with an OD<sub>750</sub> nm of 0.2 harvested while the algae was at exponential growth phase. This provided an inoculum equivalent to 25 µg/cm<sup>2</sup> of dry weight cells. The inoculated culture was spread evenly across the surface of the stone sample using a sterile spreader. Incubation was carried out at 23 °C, 80% RH [33], with a diurnal cycle of 16 h light (16,500 lux, Luxline Plus 4000 K colour temperature strip lights, which are suitable for algal growth) and 6 h dark [13,35,57] for 56 days in a Climacell 111 Ecoline climate chamber (MMM-Medcenter, Planegg, Germany). Samples were moved weekly in a random order to ensure that no sample was shaded by the others for too long per Vázquez-Nion et al. [16]. In addition to the inoculated samples, a non-inoculated sample of each stone type was incubated under identical conditions to control for chemical changes that could alter the base colour of the stone.

Sampling was carried out weekly for the first four weeks, which was then changed to fortnightly for the remaining four weeks. Sampling consisted of colourimetry and the removal of algal growth for chlorophyll extraction, as detailed below.

Colourimetry was carried out using a Konica Minolta CM-2600d spectrophotometer (Konica Minolta, Tokyo, Japan) with a medium aperture per the manufacturer's guidelines.

Measurements were taken to a minimum of  $n = 3$  per sample. Wet stone samples with algal growth were handled aseptically, with the colourimeter being wiped down with 70% ethanol pre- and post-reading and allowed to air dry in a sterile environment.  $L^*a^*b^*$  readings were taken prior to harvesting the cells for chlorophyll extraction. In order to avoid operator bias in selecting the area measured, each stone was measured at the centre of the sample area.  $\Delta E^*_{ab}$  was calculated using the equation from CIE [58], as shown below. Delta E represents the change in colour, where L represents a range between white and black, a is a range between red and green, and b is the range between yellow and blue.

$$\Delta E^* = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$$

Sampling of the growth on the stone in order to measure chlorophyll levels was carried out using an aseptic technique at all times. A 2 cm<sup>2</sup> area of the stone surface was sampled initially using one surface of a sterile cotton swab that was wetted with sterile deionised water, going over the surface twice in a crosshatch pattern to ensure consistent sampling. Once the surface algae had been removed, the same surface was scraped twice in a crosshatch pattern with a scalpel to remove the surface layer of softer stones and capture the subsurface algae. The scalpel sample was then applied to the surface of the wetted swab that contained the surface algae, and the swab was cut to remove the sample. The swab tip was then placed into a 1.5 mL microtube ready for chlorophyll extraction.

Chlorophyll extraction was carried out by adding 200 µL of dimethylsulphoxide (DMSO) (Fisher Scientific, UK, 10103483) to the sample. The sample was vortexed for 30 s then frozen for four hours at −20 °C. The sample was then defrosted, and 200 µL pure acetone (Fisher Scientific, UK, 10225900) was added. The sample was vortexed for 30 s to ensure that the DMSO and acetone were completely mixed and then centrifuged for five minutes at 15,100 rcf (15,000 rpm) to reduce debris in the supernatant. The supernatant was measured using a Biodrop Touch spectrophotometer (Biochrom, Cambridge, UK) with a 10 mm path length cuvette at 630, 647, 664 and 750 nm. The levels of chlorophyll a (chl a) extracted were calculated per UNESCO's determination of photosynthetic pigments in seawater, with modifications to the peak maxima in the equation due to the use of DMSO and revised extinction coefficients for chlorophylls a, b, c<sub>1</sub> and c<sub>2</sub> [59–61].

$$\text{chl a } (\mu\text{g}/\text{cm}^2) = ((11.85 \times (A_{664} - A_{750})) - (1.5 \times (A_{647} - A_{750})) - (0.08 \times (A_{630} - A_{750}))) / \text{area sampled } (\text{cm}^2)$$

Non-paired Student's t-tests were carried out using the embedded function in Excel. Testing for correlation between datasets was carried out using Pearson's correlation coefficient.

Bioreceptivity indices were calculated once the sampling demonstrated that the culture had reached equilibrium following equations from Vázquez-Nion et al. [34].  $\Delta E^*_{ab}$  was calculated using the equation from CIE as above [57], comparing the inoculated sample to the non-inoculated control.

$$BI_{\text{growth}} = 10 \times (\text{chl a } (\mu\text{g cm}^{-2}) / 4.14)$$

$$BI_{\text{colour}} = 10 \times \Delta E^*_{ab} / 24.25$$

$$BI = (2 \times BI_{\text{growth}} + BI_{\text{colour}}) / 3$$

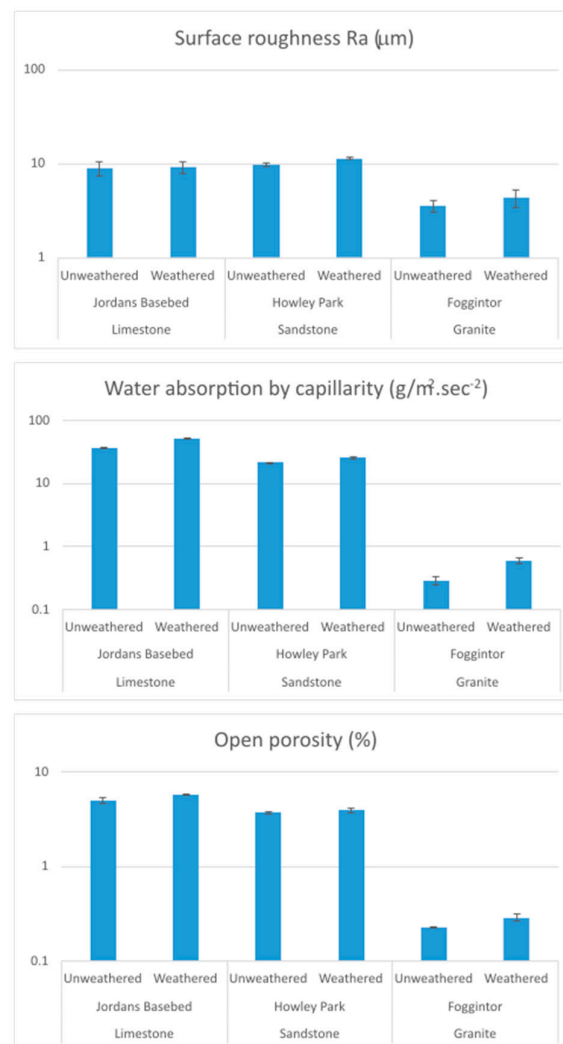
### 3. Results

#### 3.1. Physical Characterisation of Stone Properties

Primary bioreceptivity is measured using new, sound stone, and secondary bioreceptivity measurements are obtained using artificially weathered stone. Physical characteristics for the three stone types were measured pre- and post-artificial weathering in order to examine their relationship to factors influencing biodeterioration and characterise changes in properties after artificial weathering. Water absorption by capillarity and open porosity for unweathered Portland Jordans Basebed limestone fell within the expected range provided

by the technical data sheets [62]. No prior technical testing data were available for Howley Park sandstone or Foggintor granite.

Jordans Basebed limestone showed increased open porosity and water absorption by capillarity after artificial weathering (Figure 1). Petrographically, there was evidence of fracturing, combined with a reduction in micritic and sparitic cement and evidence of an increase in microcrystalline calcite/micritic buildup on top of sparry crystals on the outer surfaces. There was also an increase in surface variability observed in the artificially weathered sample due to the movement of the intergranular micritic and sparry cement. The changes in surface variability were not large enough to cause a significant difference in the surface roughness measurement for unweathered and weathered samples, even though an actual change had occurred in the surface structure. The increase in fractures observed ties in with the measured changes in open porosity and water absorption by capillarity.



**Figure 1.** Comparison between physical characteristics of unweathered and weathered stones. All characteristics showed a significant change after artificial weathering ( $p$ -value  $< 0.05$ ), apart from surface roughness for the limestone and granite.

For the Howley Park sandstone, while the change in open porosity and water absorption by capillarity was the lowest numerical alteration after artificial weathering (Figure 1), the petrographic analysis demonstrated a shift towards a larger percentage volume of voids. The petrographic analysis also identified a change in the % volume of the iron oxide/hydroxide-containing minerals, as well as decreases in the carbonate and sulphate phases. The iron oxide-/hydroxide-containing minerals that showed an increase in percent-

age volume were those that contained manganese and sulphur, which will account for the decrease in the dolomite and calcium sulphate phases, respectively (Table 1). There was also a measurable increase in the surface roughness.

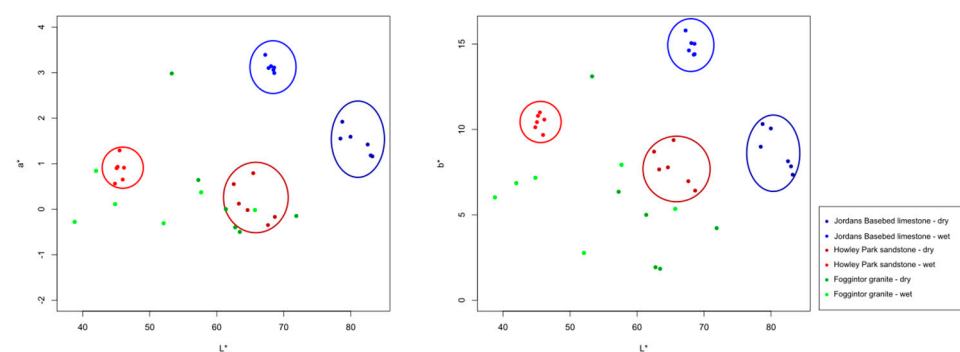
**Table 1.** Changes in the mineral composition of Howley Park sandstone pre- and post-artificial weathering.

| Mineral Composition    | Unweathered Volume (%) | Weathered Volume (%) |
|------------------------|------------------------|----------------------|
| Chlorite group         | 1.2                    | 1.6                  |
| Magnetite and hematite | 0.4                    | 1.2                  |
| Glauconite             | 0.4                    | 0.0                  |
| Iron oxides/hydroxides | 1.8                    | 0.7                  |
| Calcium sulphate       | 3.0                    | 2.0                  |
| Dolomite               | 14.4                   | 11.5                 |
| Ferroan calcite        | 1.3                    | 0.5                  |

The petrographic comparison of unweathered and artificially weathered Foggintor granite confirmed the observed increase in open porosity and water absorption by capillarity (Figure 1), as an increase in fracture occurrence and size was recorded. Additional information about petrographic changes observed for all stones can be found in our Historic England technical report [63].

A Student's *t*-test was used to compare pre- and post-artificial weathering readings to determine whether the weathering protocol had resulted in a significant physical change. All three stone types showed statistically significant increases ( $p < 0.05$ ) in water absorption by capillarity and open porosity after artificial weathering. Howley Park sandstone was the only stone to show a significant change in surface roughness. Taken together, this demonstrates that the artificial weathering protocol has caused physical changes to the stone.

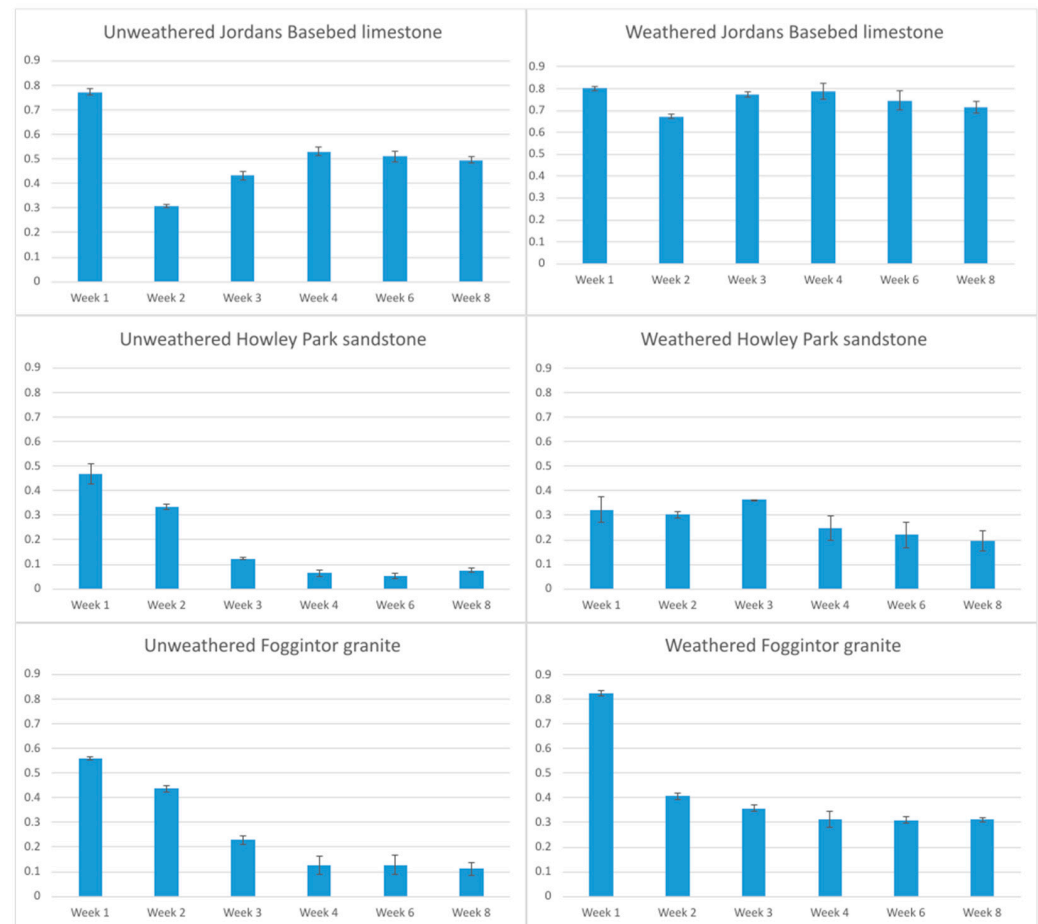
As the most immediate public impact of bioreceptivity is visual, characterising the colour of the stone surface is important to provide a baseline for bioreceptivity observations. The three stone types showed a noticeable difference in colour when comparing  $L^*a^*b^*$  colourimetry readings between wet and dry stones. Perhaps unsurprisingly, given the nature of granite, Foggintor granite demonstrated the highest level of variability in colour but had the greatest overlap of colour readings between wet and dry measurements (Figure 2). Howley Park sandstone and Jordans Basebed limestone both demonstrated a consistent shift in colour when saturated. As the bioreceptivity tests are carried out on wet stone in a humid environment in order to produce suitable growth conditions for the algae, it is important that a wet control is used to exclude colour changes due to water absorption of the stone.



**Figure 2.** Comparison between  $L^*a^*b^*$  colour readings for wet and dry stones prior to artificial weathering. Limestone (red) and sandstone (blue) show separate clusters (circled) of the wet (lighter red and blue) and dry (darker red and blue) readings together, whereas granite (green) demonstrates overlapping colour measurements.

### 3.2. Characterisation of Bioreceptivity

Unweathered and weathered stone blocks were inoculated with algae, and chlorophyll levels were measured over an eight-week growth period in order to provide data for calculating primary and secondary bioreceptivity. Algal growth levels were monitored every 7 days up until week 4 and then every 14 days thereafter and were shown to have stabilised, giving three consistent readings, for all three stone types by week 8 (Figure 3). Initial higher levels of growth were observed in week 1, but these were not stable and most likely due to the growth medium used for applying the inoculum. All stones were capable of supporting an algal colony under the growth conditions used.

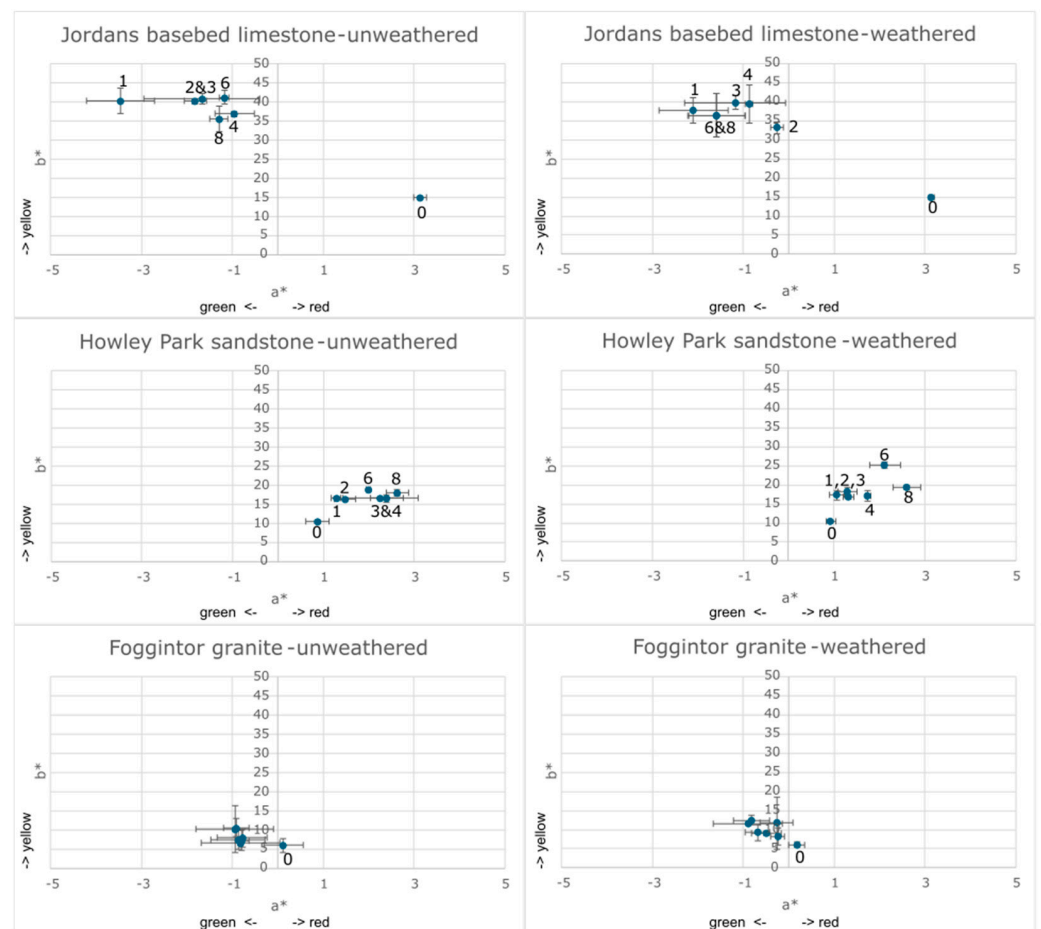


**Figure 3.** Chlorophyll a extraction results from weeks 1–8 for unweathered and weathered stone samples.

Each stone type demonstrated a significantly ( $p < 0.05$ ) higher level of algae present on artificially weathered samples compared to the unweathered equivalent based on the amount of chlorophyll extracted per  $\text{cm}^2$ . Jordans Basebed limestone supported the highest level of growth, with  $1.44\times$  higher chlorophyll a on weathered compared to unweathered stone. Foggintor granite and Howley Park sandstone supported lower initial levels of growth but showed a greater proportional change after weathering ( $2.71\times$  and  $2.57\times$  higher chlorophyll a on weathered compared to unweathered stone, respectively).

Colourimetry was used to measure the colour of the stone over the eight-week period of algal growth. The first month of data for limestone and granite showed a shift towards green colouration, which corresponds to the initial surge of algal growth, probably due to the combination of growth media with the stone. The green colour then stabilised as the algae equilibrated with the surface. Figure 4 shows the average and range of  $a^*$  (green–red) and  $b^*$  (blue–yellow) measured during each sampling point on the three stone types.

Colourimetry readings started to overlap (representing the colour reaching a relatively stable level) earlier than was seen with the chlorophyll *a* extraction due to greater variability in the coverage of the surface by the algae in these early stages. In the case of Foggintor granite, which already had a green component in some of the surface minerals, this resulted in overlapping results for all inoculated readings. After eight weeks, only Foggintor granite demonstrated a significant difference (Student's *t*-test,  $p < 0.05$ ) between the colourimetry measurements of algal growth on artificially weathered and unweathered samples. The overlap in weekly readings for Foggintor granite, combined with colourimetry being unable to significantly characterise the difference between unweathered and artificially weathered samples, suggests that colourimetry alone is not a suitable measure of bioreceptivity, since chlorophyll *a* levels demonstrated significant differences in algal growth between artificially weathered and unweathered samples.



**Figure 4.** Comparison of  $a^*$  and  $b^*$  colour readings for inoculated stone over weeks 0–8. Week 0 shows the average  $a^*$  and  $b^*$  of the saturated non-inoculated stone. With the exception of Foggintor granite, for which the data points are too highly clustered and all fall within each other's error bars, the data points are annotated by the week they represent. Error bars are standard deviation.

Colourimetry data for Howley Park sandstone showed that any green shift due to algae was masked completely by a shift towards red (Figure 4). Petrographic data demonstrate that the sandstone samples showed a change in the mineral phase of the iron upon artificial weathering, specifically conversion from iron oxide to the iron-associated members of the chlorites and to iron sulphide; these data are available from our Historic England technical report [63]. This suggests that the redshift may be due to the chemical alteration of the iron minerals present in the stone due to the test environment.

A control sample of each stone type was run without an algal inoculum to determine how much of the colour change was due to the presence of algae and how much was due to the natural patination of the stone, which occurs in a damp environment. In the case of Howley Park sandstone, between weeks 2 and 4, the algal growth drives the red colour change at a faster rate than the uninoculated sample, but by week 6 the presence of the algae begins to reduce the redness of the stone. This implies that the presence of algae may also be interacting with the stone to increase or alter the rate of the redshift. The tested limestone and granite did not show a similar redshift. This natural change in colour is not accounted for in the bioreceptivity index calculation proposed by Vázquez-Nion et al. [34], demonstrating that, as published, their method will not be suitable for all stone types. In the next section, we discuss the required modifications to the method in order to account for natural colour changes in stones.

### 3.3. Bioreceptivity Index

Results from the colourimetry and chlorophyll levels allow for the calculation of the three bioreceptivity indexes using the equations derived by Vázquez-Nion et al. [34], which they suggested could be applied to other stone types beyond granite. Primary bioreceptivity is derived from unweathered stone results, and secondary bioreceptivity is calculated from the readings taken from weathered stone. Their proposed methodology for calculating  $BI$  and  $BI_{colour}$  relies on a colourimetry reading prior to inoculation being compared with a reading after the stone has been incubated for three months. This does not account for stones that undergo colour change due to mineralogical alteration in the presence of water, such as Howley Park sandstone. This can cause an artificial amplification or reduction of the total  $BI$  and the  $BI_{colour}$ , depending on the mineralogical composition of the stone. Table 2 shows calculations for the three types of bioreceptivity index for Howley Park sandstone. The two left-hand columns of the table show results when using the calculations exactly as proposed by Vázquez-Nion et al. [34]. In order to overcome inaccuracies introduced by the colour change due to water exposure, we have incorporated a non-inoculated control, which is exposed to the same conditions as the sample with algae growing on it. The  $L^*a^*b^*$  readings from the non-inoculated control at each individual sample point are used for  $L1/a1/b1$  to calculate  $\Delta E^*ab$  for  $BI_{colour}$ . This produces a different bioreceptivity index result from the one calculated using Vázquez-Nion et al.'s [34] methodology, as their control was a wetted stone that had not been placed in a climate chamber alongside algal growth samples. This change in how the control sample is prepared means that any natural colour change in the stone that falsely alters the bioreceptivity index is now accounted for (Table 2, the two right-hand columns).

**Table 2.** Comparison between Vázquez-Nion et al.'s methodology and our modified methodology used on the sandstone samples demonstrating the correction in  $BI$  and  $BI_{colour}$  after using a non-inoculated colour control.

|               | Howley Park Sandstone Bioreceptivity as per Vázquez-Nion et al. [34] Calculation |           | Howley Park Sandstone Bioreceptivity with Non-Inoculated Control Based on Revised Methodology |           |
|---------------|----------------------------------------------------------------------------------|-----------|-----------------------------------------------------------------------------------------------|-----------|
|               | Unweathered                                                                      | Weathered | Unweathered                                                                                   | Weathered |
| $BI_{growth}$ | 0.18                                                                             | 0.47      | 0.18                                                                                          | 0.47      |
| $BI_{colour}$ | 4.50                                                                             | 4.76      | 1.30                                                                                          | 1.66      |
| $BI$          | 1.62                                                                             | 1.90      | 0.56                                                                                          | 0.87      |

The results of applying our revised bioreceptivity calculation to all three stone types are shown in Table 3. These data are the first time secondary bioreceptivity has been calculated for any stone type in a lab-based setting and also provide the first full characterisation of primary and secondary bioreceptivity for any stone type. Of the stones tested, Jordans Basebed limestone has the highest total bioreceptivity ( $BI$ ), mainly driven by the high  $BI_{colour}$

reading, with the other stone types falling into a very low bioreceptivity category. All stone types show an increase in bioreceptivity on weathering, i.e., secondary bioreceptivity readings are higher than primary bioreceptivity.

**Table 3.** Bioreceptivity index (*BI*) results, including growth ( $BI_{growth}$ ) and colour measurements ( $BI_{colour}$ ), calculated using the revised methodology for all three stone types tested.

|               | Jordans Basebed Limestone |                          | Howley Park Sandstone    |                          | Foggintor Granite        |                          |
|---------------|---------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|               | Primary<br>(Unweathered)  | Secondary<br>(Weathered) | Primary<br>(Unweathered) | Secondary<br>(Weathered) | Primary<br>(Unweathered) | Secondary<br>(Weathered) |
| $BI_{growth}$ | 1.20                      | 1.73                     | 0.18                     | 0.47                     | 0.27                     | 0.73                     |
| $BI_{colour}$ | 9.06                      | 9.25                     | 1.30                     | 1.66                     | 0.91                     | 1.24                     |
| <i>BI</i>     | 3.82                      | 4.24                     | 0.56                     | 0.87                     | 0.48                     | 0.90                     |

### 3.4. Relationship between Stone Characteristics and Bioreceptivity

In order to better understand the relationship between the change in stone characteristics and bioreceptivity measurements, Pearson's correlation coefficient was used. Datasets for water absorption by capillarity, open porosity and surface roughness were compared to the level of chlorophyll a extracted at the eight-week point at equilibrium for both weathered and unweathered stone (Table 4). These properties were evaluated, as they have been shown in past studies to directly correlate to the bioreceptivity of the surface [3,4,11]. These were compared to the chlorophyll a levels extracted from the stone, as this is a direct measure of the amount of biological growth on the surface.

**Table 4.** Calculated correlation between extracted chlorophyll a ( $\text{mg}/\text{cm}^2$ ) measurements at eight weeks and stone properties. All correlations were significant, with a *p*-value of  $< 0.05$ .

|                           |             | Water Absorption Coefficient<br>by Capillarity | Open Porosity | Surface Roughness |
|---------------------------|-------------|------------------------------------------------|---------------|-------------------|
| Jordans Basebed limestone | Unweathered | 0.99                                           | 0.93          | 0.97              |
|                           | Weathered   | 0.99                                           | 0.87          | 0.91              |
| Howley Park sandstone     | Unweathered | 0.96                                           | 0.99          | 0.98              |
|                           | Weathered   | 0.94                                           | 0.88          | 0.93              |
| Foggintor granite         | Unweathered | 0.93                                           | 0.99          | 0.81              |
|                           | Weathered   | 0.95                                           | 0.99          | 0.99              |

There was a positive correlation between chlorophyll a levels and water absorption by capillarity, open porosity and surface roughness for all stone types, whether unweathered or weathered. This corresponds with the findings in the bioreceptivity literature and supports the validity of this test method.

There were no significant correlations between colourimetry and chlorophyll levels on any stone type. This is not unexpected and is consistent with findings by other researchers [4,34].

## 4. Discussion

We have characterised an English sandstone, granite and limestone for both primary and secondary bioreceptivity. This is the first time the bioreceptivity properties of any UK stones have been characterised in this way and furthermore provides the first lab-based comparison of primary and secondary bioreceptivity.

Measurements of surface roughness, open porosity and water absorption by capillarity of the stone are required in order to understand the overall stone properties and how they

may influence bioreceptivity. As with other studies [4], we have also demonstrated the positive correlation between surface roughness, open porosity and water absorption by capillarity, as well as algal growth on the stone. While these characteristics are not required for calculating the bioreceptivity index, they are important in understanding and contextualising the observed changes between primary and secondary bioreceptivity and, as such, should be recorded together with the bioreceptivity measurements in future studies.

The artificial weathering methodology we developed for the measurement of secondary bioreceptivity has been demonstrated to create statistically significant changes to the stone matrix, with all three lithotypes demonstrating an increase in water absorption by capillarity and open porosity (although not all showed changes to surface roughness). A petrographic analysis was used to further demonstrate that our chosen ageing protocol had been successful in causing changes to the stone. The analysis of the samples clearly showed physical and in some cases, mineralogical, changes in the stones after artificial weathering. As with natural weathering, the change in properties is dependent on the stone type.

The standardised and optimised methodology for measuring bioreceptivity that we have developed builds on previous work in the field by Vázquez-Nion et al. [34]. Bioreceptivity measurements can be carried out using algal species, chemicals and equipment that are all readily available to others carrying out the tests. Through using standardised algal cultures and taking measurements of the same factors (chlorophyll a and colourimetry), further work in this field can be cross-compared between different research groups.

Our research demonstrates that the methods used provide a suitable lab-based model for measuring and calculating secondary bioreceptivity, in addition to primary bioreceptivity. Artificial weathering resulted in a statistically significant increase in the bioreceptivity of the stones, compared to the unweathered samples. The changes in the material properties of the stone caused by the artificial weathering process resulted in a higher capacity to support growth in a similar fashion to the natural evolution of material properties, which results in environmental secondary bioreceptivity [2].

We have demonstrated that the bioreceptivity index calculation proposed by Vázquez-Nion et al. [34] can be applied to calculate primary and secondary bioreceptivity for a range of stone types, although we have modified the methodology to ensure that the inoculum has reached growth equilibrium before the index is calculated, and to take into account stones that undergo an alteration in their natural colour due to mineralogical changes in the presence of water. To address lithic colour change, we have calculated the bioreceptivity index using colour readings based on a non-inoculated control exposed to the same conditions as the inoculated stone to prevent misleading readings.

Based on the categorisations of bioreceptivity proposed by Vázquez-Nion et al. [34], we can divide the stone types into bioreceptivity levels based on their total *BI* reading. Jordans Basebed limestone has a low primary bioreceptivity and a moderate secondary bioreceptivity (the term originally proposed for this category was mild bioreceptivity; however, we suggest moderate is a more internationally recognised term). The other two stone types, Howley Park sandstone and Foggintor granite, both demonstrated very low bioreceptivity, Table 5.

**Table 5.** Categorisation of tested stones into bioreceptivity categories proposed by Vázquez-Nion [27].

| Bioreceptivity Level | Qualitative Description  | Primary Bioreceptivity                     | Secondary Bioreceptivity                   |
|----------------------|--------------------------|--------------------------------------------|--------------------------------------------|
| 0–1.99               | Very low bioreceptivity  | Howley Park sandstone<br>Foggintor granite | Howley Park sandstone<br>Foggintor granite |
| 2–3.99               | Low bioreceptivity       | Jordans basebed limestone                  |                                            |
| 4–5.99               | Moderate bioreceptivity  | Jordans Basebed limestone                  |                                            |
| 6–7.99               | High bioreceptivity      |                                            |                                            |
| Greater than 8       | Very high bioreceptivity |                                            |                                            |

It is notable that the bioreceptivity results for the granites tested by Vázquez-Nion et al. [34] are generally higher than those found on the stones in this study. However, it must be viewed with caution, as they used a different (environmentally derived) culture, which may have resulted in higher readings, or the physical characteristics of the stones may have been the main drivers for these differences. This is an area that we intend to investigate as we continue our characterisation of stones for primary and secondary bioreceptivity.

The bioreceptivity index proposes the use of two-increment divisions to categorise primary bioreceptivity. From our data, it appears that this scale is also applicable to secondary bioreceptivity. However, further data from different stone types will be needed to determine whether the two-unit increment for categorisation is reasonable, for example, it is possible that there may be a high proportion of stones clustered in one area, and perhaps further definition between stages may be required.

## 5. Conclusions

Bioreceptivity measurements are an important aid in decision-making when choosing stones for construction, repair and replacement. In addition to other properties, such as chemical composition, mineralogy, porosity and permeability, heritage professionals also need to consider the initial and longer-term appearance of new stone as an additional part of their selection criteria [64]. Variations in bioreceptivity will impact the appearance of the building or structure if a replacement stone becomes greener than the surrounding masonry, necessitating increased resources for its cleaning and conservation. In order to determine how a new stone might compare to a weathered stone, information regarding both the primary and secondary bioreceptivity of a broad range of stone types is needed. At present, bioreceptivity measurements by different research groups are not comparable due to differences in approach. We have developed a lab-based methodology for determining these measurements, which can be used as a repeatable protocol for others working in the field. This methodology can also be used for the determination of tertiary and quaternary bioreceptivity and could be used to test new cleaning or coating products in a reproducible way before progressing the most effective products to field trials.

No UK stone has previously been characterised for primary and secondary bioreceptivity. Our selection of these three common building stones as case studies both validates and demonstrates the application of the methodology and adds new information for the selection of stone types in heritage projects and will, in time, allow us to build a global database of the bioreceptivity of building stones. We encourage others to also adopt this methodology and collaboratively share data to improve our understanding of this aspect of heritage stone conservation. As many of the original quarries for heritage stone have ceased to operate, finding close matches to stone becomes increasingly important [65]. An international database of bioreceptivity and related stone properties will support this process and may be useful in advocating for the limited reopening of heritage quarries by helping to confirm that there is a clear gap in the availability of alternatives.

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**Data Availability Statement:** The datasets regarding petrography in this article are available in the Historic England Technical report ‘Characterisation of Primary and Secondary Stone Bioreceptivity’, which is available from their Research Reports database (<https://historicengland.org.uk/research/results/reports/> accessed on 13 September 2024). All other datasets presented in this article are not readily available because the data are part of an ongoing study. Requests to access the datasets should be directed to the corresponding author.

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