Secondary bioreceptivity of granite: effect of salt weathering on subaerial biofilm growth

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Abstract

Salt crystallisation is a very common and powerful weathering agent that can modify the petrophysical properties of building stone such as granite. In addition, the weathering can affect the susceptibility of the stone to biological colonisation. The aims of the present study were to examine the properties of a granite weathered by sodium chloride crystallisation and to evaluate the effects of the weathering on the secondary bioreceptivity of the stone to subaerial phototrophic biofilms. For this purpose, granite samples were subjected to a laboratory-based accelerated salt weathering test, and changes in weight, open porosity, bulk density, capillary water content, abrasion pH and surface roughness of the samples were determined. Samples of both weathered and non-weathered granite were then inoculated with a multi-species phototrophic culture derived from a natural subaerial biofilm and incubated under standardised laboratory conditions for three months. The weight loss produced by the weathering process was consistent with significant changes in abrasion pH and surface roughness. The bioreceptivity of the stone was also altered. According to the bioreceptivity index (*BI*), the granite under study was characterised by 'mild primary bioreceptivity', but 'high secondary bioreceptivity' after the salt weathering process. Study of the secondary bioreceptivity of stone materials can provide very useful information about response to weathering effects, and the findings can be used to improve the selection of materials for building purposes.

Keywords

Cultural heritage; phototrophic biofilm; sodium chloride; stone.

1. Introduction

The term ‘bioreceptivity’ was introduced by Guillitte [1] as an alternative to the term ‘susceptibility’ for use in the field of building ecology. It is defined as ‘the aptitude of a material to be colonised by one or several groups of living organisms without necessarily undergoing any biodeterioration’. This definition implies an ecological interaction between the colonising organisms and the material, and the term can also be defined as ‘the totality of material properties that contribute to the establishment, anchorage and development of fauna and/or flora’. Guillitte [1] also established differences that depend on the degree of alteration of the material under study. Hence, when a material has not yet been exposed to colonisation, so that its properties remain very similar or identical to those in the initial state, the bioreceptivity will only be expressed in response to the appearance of the first colonising organisms and is termed ‘primary bioreceptivity’. In stone, primary bioreceptivity indicates the initial potential of freshly cut quarry rocks to be colonised. When the properties of a material evolve over time under the action of colonising organisms or other environmental factors, it may result in a different type of bioreceptivity, called ‘secondary bioreceptivity’, involving weathered rocks. When the properties of the material are artificially modified, as by the coatings or consolidation treatments commonly used to conserve stonework, ‘tertiary bioreceptivity’ can be induced.

Studying the bioreceptivity of lithotypes under laboratory conditions before using them as building stones is essential for appropriate selection of construction materials and thus for the preventive conservation of outdoor stone buildings and monuments. Several studies have investigated the bioreceptivity of stone materials (see [2] for a review). Most of these have assessed the primary bioreceptivity of different types of construction material [3-12] and the influence of different conservation treatments on the tertiary bioreceptivity [13-19]. These investigations have provided very valuable information concerning the stone characteristics influencing the susceptibility to biological colonisation, as well as the effects of some stone treatments. However, the relationships between the potential bioreceptivity of stone and the physical or chemical characteristics of the material have not been clearly established, although some properties such as surface roughness, open porosity and mineralogical characteristics are considered key factors [2]. In the particular case of granite, Prieto and Silva [4] demonstrated that the primary bioreceptivity of several types of granite varies due to the differences in some physical

properties. The extent of colonisation was mainly related to the surface roughness, in addition to four intrinsic properties: abrasion pH, bulk density, open porosity and capillary water. These researchers developed a simple, rapid method of investigating the potential bioreceptivity of granite to cyanobacteria, based on the characterisation of these intrinsic properties and use of an equation to estimate the expected amount of chlorophyll *a* (chl *a*) per cm^2. More recently, Vázquez-Nion et al. [12] carried out a comprehensive study of the primary bioreceptivity of eleven varieties of granitic rocks commonly used as construction material and ornamental stone. In this study, sample blocks were inoculated with a multi-species phototrophic culture and incubated under standardised growth conditions, revealing that growth of phototrophic biofilms is strongly enhanced by high open porosity, capillary water content and surface roughness, rather than by differences in the chemical and mineralogical composition of the granites.

The secondary bioreceptivity of stones has been much less widely investigated [20-28]. Silva et al. [21] examined the causes of an extensive colonisation on a granite building. These researchers concluded that the severe weathering of the raw granite used for construction, characterised by high porosity and capillarity -which favoured rapid absorption of large amounts of water- played a key role in the propensity of the material to be colonised. Papida et al. [22] induced artificial microbial and physical weathering in samples of two limestones and one dolomite and observed that the combination of physical and biological processes significantly enhanced the extent of decay relative to the sum of the individual actions of each process. Moreover, these processes favoured the selection of microbial consortia, as specific bacterial populations remained high throughout the experiment, even though the samples were not re-inoculated and no extra nutrients were added. Cámara et al. [24, 25] claimed that the weathering of dolostone samples depends on the fungal colonisation strategy, which is a direct consequence of the bioreceptivity of the lithic substrate. These researchers identified a sequence of fungal colonisation in which the prior presence and activity of endolithic microorganisms helped lichen symbiosis to become established during later stages of colonisation, favoured by previous biodeterioration processes. Marques et al. [27] compared the bioreceptivity of freshly quarried and naturally weathered schist to biofilm-forming cyanobacteria. They observed that the bioreceptivity of weathered schist was significantly higher than that of unweathered schist, in addition to differences in physical properties and abrasion pH related to the degree of weathering. In relation to how subaerial biofilms interact with

stone, an increasing number of researchers claim that in some cases biofilms have no impact on the integrity of building stones or may even be bioprotective [29, 30]. For instance, Gulotta et al. [28] observed that the chemical-physical weathering of carbonate stones influenced the growth of subaerial biofilms, but that, apart from altering the aesthetic appearance of the stone, biofilms were not primary damaging factors.

In summary, chemical-physical weathering processes can alter properties that affect the susceptibility of stone to biological colonisation; however, the extent of these effects and also the potential variability induced by different types of weathering and in different lithotypes remain poorly understood. Salt weathering is a very powerful and widespread deterioration agent of building materials, including granite [31-35]. Mechanical action of dissolution-crystallisation cycles can exert pressures capable of producing a weight loss, a change in the size of the grains, a degree of splitting, a change in the size of the pores and visible surface deterioration [36]. Salt weathering can therefore modify the petrophysical properties of the stone, including those related to the bioreceptivity. Thus, the aims of the present study were as follows: i) to assess the alteration in the properties of granite produced by sodium chloride crystallisation; and ii) to evaluate the effects of this type of weathering on the secondary bioreceptivity of the stone to subaerial phototrophic biofilms.

2. Materials and methods

2.1. Lithotype studied

The stone used in the study was Campo Lameiro granite (hereafter CL) (Figure 1). CL granite is characterised by fine to medium grains and the presence of two micas (biotite and muscovite) in equal proportions or by being rich in muscovite crystals, which can reach a diameter of up to 1 cm. The size distribution of grains is equigranular, although some feldspar megacrystals may occur. Fissures are very common in this granite, mainly inside the plagioclase crystals. The essential components are quartz (often fractured), K-feldspar (microcline), plagioclases (acidic albite-oligoclase), biotite, late magmatic muscovite, sillimanite and andalusite. Chlorite, apatite, zircon, tourmaline, rutile and scarce opaque grains occur as accessory minerals.

[Figure 1 approximately here]

2.2. Laboratory-induced salt weathering of granites

Nine cubic blocks (5x5x5 cm^3) of granite CL were subjected to a salt crystallisation test modified from [37] using sodium chloride. The weathering process comprised 15 cycles, each consisting of three steps: i) immersion of the blocks in a saline solution (NaCl 16 % w/w) at room temperature (~ 20 ºC) for 4 hours; ii) removal of the blocks from the solution and drying in an oven at 60 ºC for 16 h; iii) cooling of the blocks at room temperature for 4 h before the next cycle was started. Every five cycles (ie after 5, 10 and 15 cycles), three granite blocks were removed for analysis, and the NaCl solution was changed. At the end of the procedure, the salts were extracted from the stone blocks by successive washing with distilled water until the electrical conductivity in the washing water was below 0.5 $\mu S\ cm^{-1}$. The blocks were then dried until they reached a constant weight and the dry weight loss (%) was calculated. Three additional blocks of granite CL not subjected to the salt crystallisation process were used as controls (non-weathered samples) in the subsequent experimental procedures.

2.3. Characterisation of weathered and non-weathered granite

The properties of the granite (both weathered and non-weathered) related to the bioreceptivity were analysed to assess the effects of the laboratory-based salt crystallisation test. Abrasion pH values were measured after grinding 20 g of rock sample in 40 mL of distilled water for 2.25 min and allowing the solution to settle for another 2 min [38]. The samples were also physically characterized by determination of the bulk density and open porosity according to [39]. As water can be a limiting factor in biological colonisation, the maximum amount of water absorbed by capillary suction (capillary water content) was also determined according to [40]. All determinations were carried out in triplicate.

The granite blocks were cut with a diamond blade to produce samples of size 5 x 2.5 x 1 cm^3. The samples were classified as surface (cut from the outer part of the blocks) and inner (from the 1 cm-inner part of the blocks), as depicted in Figure 2. In order to study the possible variations in the surface roughness of the stone due to the salt weathering process, 1 cm^2 areas of both types of samples were examined by White Light Optical Interferometry (WLOI) (Wyko NT1100) in Vertical Scanning Interferometry (VSI) mode. The results were reported as *Sa* values (µm), representing the mean roughness of the surface.

[Figure 2 approximately here]

2.4. Procedure for biofilm formation

A multi-species phototrophic culture, derived from a natural biofilm grown on a granite building (San Martín Pinario Monastery, Santiago de Compostela, NW Spain) and composed by several taxa, including Bryophyta (*Syntrichia ruralis* protonemata), Charophyta (*Klebsormidium* sp.), Chlorophyta (*Bracteacoccus* sp., *Chlamydomonas* sp., *Chlorella* sp. and *Stichococcus bacillaris*) and Cyanobacteria (*Aphanocapsa* sp. and *Leptolyngbya cebennensis*), was used as the inoculum for forming biofilms in laboratory. This culture and the original biofilm have previously been characterized in detail via high-throughput sequencing and microscopic observations [41, 42].

For both weathered (5, 10 and 15 cycles) and non-weathered (0 cycles) stone, 1 mL of culture (equivalent to 1.5 mg dry weight biomass), in exponential growth phase, was inoculated on the upper surface of each of ten previously autoclaved 5x2.5x1 cm^3 samples cut from the blocks. Five of these were surface samples, used to study the effect of possible variations in roughness on the secondary bioreceptivity, and five were inner samples, used to study the effect of the other stone properties without having to consider possible variations in surface roughness (Figure 2). A total of 40 samples were inoculated.

In order to promote biofilm formation, the inoculated samples were subjected to favourable growth conditions, as previously described [42]. The samples were placed in Petri dishes, which were periodically filled with sterilized distilled water and were incubated in a climatic chamber under stationary conditions of 23 °C, 95 % relative humidity and a 12 h light/dark photoperiod (~20 µmol photon m^{-2} s^{-1}) for three months. Lightning was provided by fluorescent lamps (OSRAM L 36W/765). The position of each block was semi-randomly varied to prevent the effects of possible micro-climatic variations in the chamber.

2.5. Assessment of biofilm growth

Pulse Amplitude Modulated (PAM) fluorometry was used to assess biofilm growth on the stones after the three-month incubation period. Fluorescence signals were measured at different wavelengths in a Phyto-PAM (Heinz Walz GmbH) equipped with a Phyto-EDF fiberoptics emitter-detector unit, which allows measurement on surfaces via a 50 mm long and 4 mm diameter perspex rod [43]. Inoculated blocks were kept in darkness for 20 min prior to the measurements, carried out with the tip of the rod directly in contact

with the block/biofilm surface. The fluorescence parameters recorded were F_0, the minimal fluorescence signal of dark-adapted cells, and F_m, the maximal fluorescence signal after a saturating light pulse in dark-adapted cells. The values of these two parameters can be used to calculate the maximum quantum yield as $Yield = (F_m - F_0)/F_m$, a measure of the maximum photochemical efficiency of PSII that can be considered an indicator of the general level of fitness of the photosynthetic organisms. The $F_{0,665nm}$ signal was expressed as µg chl *a* cm^{-2} by using a previously developed equation [12] and used as indicator of the phototrophic biofilm biomass. The $F_{0,470nm}$ / $F_{0,645nm}$ ratio was considered an indicator of the dominance of green algae (high values) or cyanobacteria (low values) in the biofilms [12]. A total of 6 readings were taken at different zones on each block, and the mean value was calculated.

Biofilm growth after the three-month incubation period was also assessed by colour measurements obtained with a portable spectrophotometer (Konica Minolta CM-700d). A total of 6 readings were made in different zones on each block under the following conditions: illuminant D65, observer 2° and a 10 mm diameter target area [44]. The colour of the blocks was measured directly on the surface of the humid samples before inoculation and at the end of the three-month incubation period. The colour measurements were made within the CIELAB colour space, and the total colour difference ΔE^*_{ab} was calculated [45].

2.6. Statistical analyses

The data on the properties of both weathered and non-weathered stones and the parameters used to assess the biofilm growth at the end of the three-month incubation period were subjected to analysis of variance (ANOVA) and post-hoc Tukey HSD tests, in order to study the effect of the weathering process on the bioreceptivity. The relationships between the different variables were also determined by two-tailed Bivariate Spearman's correlations. Statistical analyses were implemented using SPSS Statistics v19.0 (IBM) software, and differences were considered statistically significant at $p \leq 0.05$.

The chl *a* content and ΔE^*_{ab} values for the phototrophic biofilms developed on the stones were also used to calculate the bioreceptivity index (*BI*) developed by Vázquez-Nion et al. [46].

3. Results and discussion

3.1. Weathering of granite by salt crystallisation

The properties related to the bioreceptivity measured in the weathered and non-weathered granite, as well as the results of the ANOVA, are shown in Table 1. For sound granite (ie non-subjected to the salt crystallisation weathering process) the values obtained were 2.52 ± 0.02 g cm^{-3} for bulk density, 4.82 ± 0.84 % for open porosity, 0.20 ± 0.03 g cm^{-2} for capillary water content and 6.76 ± 0.22 for abrasion pH. In previous studies, the values of the properties associated with the bioreceptivity of four [4] and eleven [12] different varieties of granite (sound and weathered) were between 2.4 and 2.9 g cm^{-3} for bulk density, between 0.5 and 11.0 % for open porosity and between 5.98 and 9.58 for abrasion pH. The relatively high values of open porosity and low values of bulk density and abrasion pH found in the CL granite reveal a certain degree of alteration in the granite prior to the samples being subjected to the laboratory-based salt weathering test [4]. This stone is therefore expected to be susceptible to salt crystallisation weathering [36].

[Table 1 approximately here]

After the laboratory-based salt weathering test, the granite samples suffered significant weight loss (Table 1). The loss of material increased with the number of weathering cycles applied, up to 1.08 ± 0.25 % after 15 cycles. This weight loss was not accompanied by significant changes in bulk density, open porosity or capillary water content. However, the laboratory-based weathering process significantly altered the abrasion pH and the surface roughness of the granite. Abrasion pH significantly decreased from an initial value of 6.76 ± 0.22 to 5.86 ± 0.08 after 10 cycles of weathering. The application of additional 5 cycles (ie after 15 cycles) did not produce any subsequent significant change. *Sa* values increased continuously throughout the weathering process, from 18.90 ± 4.36 µm in the non-weathered granite up to 52.37 ± 4.85 µm after 15 cycles. The weight loss observed after the alteration of the granite can therefore be mainly attributed to the loss of material from the surface of the stone blocks (Figure 3).

[Figure 3 approximately here]

The loss of stone material was mainly from the surface and not within the blocks, as revealed by the non-significant modification ($p > 0.05$) of properties such as bulk density, open porosity and capillary water content, which are more closely related to the inner

porous system, after the salt crystallization tests. Changes in the bioreceptivity could therefore be only induced on the surface of the weathered blocks but not the inner part. In order to check this possible effect, samples from 1 cm inside the blocks (Figure 2) were also studied in the subsequent bioreceptivity assay. The roughness of the inner samples, obtained by cutting the blocks with a diamond blade, was assumed to be the same for either weathered and non-weathered granite, measured as 9.56 ± 3.10 µm (*Sa*, Figure 3C).

3.2. Assessment of biofilm growth on the weathered and non-weathered granite

Samples of non-weathered granite and granite weathered by the salt crystallization process, from the surface and from the inner part of the original blocks (ie with the same surface roughness) were subjected to the previously described inoculation and incubation protocol. After the three-month growth period, phototrophic biofilms were visible on all samples studied (Figure 4).

[Figure 4 approximately here]

The extent of the colonisation on the stone samples, assessed by Phyto-PAM and colour measurements, is shown in Figure 5. The chl *a* contents determined in the different cases studied (Figure 5A), used as proxy for biofilm growth quantification, ranged from 0.91 ± 0.28 (5 cycles-weathered inner samples) to 2.70 ± 0.40 µg cm^{-2} (15 cycles-weathered surface samples). Considering the surface samples, granite CL showed similar amounts of chl *a* in non-weathered blocks and blocks weathered for 5 cycles, but an increase in the colonisation achieved in blocks subjected to 10 and 15 cycles of weathering. These results are consistent with those of previous studies in which a higher degree of surface roughness and lower abrasion pH (Table 1) are related to higher bioreceptivity in granitic rocks [4, 12]. Considering the inner samples (all with the same degree of surface roughness) biofilm growth was similar, independently of the number of weathering cycles applied. The lower degree of roughness derived from cutting the blocks with a diamond blade, maintaining the rest of variables at the same levels, undoubtedly decreased the bioreceptivity of these samples, as in all cases the inner part of the block was less susceptible to colonisation than the surface, but those were not affected by the weathering. Thus, the ANOVA of these data (Table 2) revealed that both the weathering process and the surface of the block inoculated (the surface or in the inner part of the original block) significantly affected the chl *a* content. The interaction between these two factors did not significantly affect the bioreceptivity of the stone samples.

[Figure 5 and Table 2 approximately here]

Regarding the other Phyto-PAM parameters measured in the biofilms formed on the granite studied (Table 2), no significant effects were observed for the maximum quantum yield, but the weathering degree and the surface inoculated significantly affected the $F_{0,470nm}$ / $F_{0,645nm}$ ratio. The maximum quantum yield of the microorganisms grown on granite CL is similar for both weathered and non-weathered samples and for both inner and surface samples, with values around 0.55 (Figure 5B). However, a continuous decrease, from 0.63 ± 0.03 (non-weathered) to 0.58 ± 0.03 (15 cycles-weathered) for the $F_{0,470nm}$ / $F_{0,645nm}$ ratio was observed in the surface samples (Figure 5C). This was not observed in the inner samples, which suggests better adaptation of the cyanobacteria present in the inocula, than that of green algae, to the increased surface roughness of the stone derived from the laboratory-induced weathering. Differences in how microbial communities under the same environmental conditions adapt to changes in the physical properties of colonised stones have previously been reported. Papida et al. [22] observed that, from a mixed microbial population, halotolerant heterotrophic bacteria survived particularly well on an artificially weathered limestone, probably due to the higher porous structure of the stone, which enhanced the water content. Cámara et al. [25] found different distribution patterns of microbial colonization in a dolostone quarry related to different biodeterioration degrees. They suggest that the presence and activity of pioneering endolithic microorganisms could have helped lichens to settle, and the loss of some endolithic fungi in more developed communities could be related to the chemical and physical effects of the lichen thalli on the substrate.

The total colour differences caused by biofilm growth on the granite were only significantly affected by the surface inoculated (Table 2). Thus, ΔE^*_{ab} values remained constant independently of the cycles of weathering applied for both surface and inner slabs, although the values of the former were always higher (Figure 5D). Thus, the differences in chl *a* contents of surface and inner samples probably caused the differences in the ΔE^*_{ab} values, but the differences in the chl *a* contents due to the weathering process in the surface slabs were not reflected by colour measurements.

The correlations between the properties of the granite studied, both weathered and non-weathered, and the parameters used to assess biofilm growth in the different cases may help to explain these data. The chl *a* contents were significantly correlated with the

surface roughness and the abrasion pH of the samples tested (Table 3). The correlation between the chl *a* content and surface roughness can be attributed to the general decrease in chl *a* in the inner samples relative to the surface samples. Moreover, the increased surface roughness of the granite brought about by the salt crystallisation promoted biofilm growth. In addition to the increase in the surface roughness, the granite weathering also decreased the abrasion pH. The photosynthetic performance (*yield*) of the biofilm-forming microorganisms was not correlated with any of the stone properties studied, although the surface roughness was significantly correlated with the microbial composition of the biofilms (as ratio $F_{0,470nm}$ / $F_{0,645nm}$). As previously noted, greater surface roughness seems to be related to the proliferation of cyanobacteria rather than green algae. The presence of anchoring sites and micro-refuges for the attachment and settlement of biological colonization in rougher surfaces [2] may have favoured the cyanobacterial growth due to their smaller cell size [47]. The significant correlation between ΔE^*_{ab} and surface roughness can mainly be attributed to the general decrease of these values in the inner samples relative to the surface samples.

[Table 3 approximately here]

3.3.Secondary bioreceptivity of granite

The values of the bioreceptivity index (*BI*) [46] were calculated for the granite studied (Table 4). The *BI* values, derived from the chl *a* contents and ΔE^*_{ab} values measured on the stones after the application of the standardised biofilm growth conditions, fitted to a 0-10 scale, enable classification of the granites according to their bioreceptivity. Thus, low values of *BI* correspond to stone that is least susceptible to biological colonisation while high values indicate that the stone is highly bioreceptive. The *BI* obtained for non-weathered granite CL, ie for describing its primary bioreceptivity, was 5.3, thus classifying this granite as having 'mild bioreceptivity'. The application of 5 salt weathering cycles did not modify the classification. However, after 10 and 15 weathering cycles, the bioreceptivity of the granite was substantially increased (to respectively 6.2 and 6.5), thus classifying the weathered granite CL as 'highly bioreceptive'.

[Table 4 approximately here]

For fresh cut quarry stones used for building purposes, granite CL is therefore expected to be 'mildly' susceptible to colonisation by subaerial phototrophic biofilms. This level

of primary bioreceptivity is similar to that reported for other granites commonly used as construction materials [46]. However, if structures made from this granite are subjected to the weathering process described here due to the effect of salt (sodium chloride) crystallisation, the bioreceptivity may increase. Salt crystallisation is a very common and powerful weathering agent of building materials, including granite, particularly in marine environments and under mild climatic conditions [32, 35, 48-51]. Dissolution-crystallisation cycles can alter the surface roughness and the abrasion pH of granites, but also can change the size of the grains and pores, which may modify its petrophysical properties and produce visible surface deterioration [31-34, 36, 52]. As an increasing number of cycles in the laboratory-induced salt weathering process produced a more intense weathering (Table 1), the number of cycles applied to the granite could represent the intensity and/or the exposure time of a natural salt weathering process. Thus, if the granite is moderately weathered by salts in the environment (here represented by 5 cycles of weathering), the bioreceptivity may be expected to remain constant. However, if the weathering process continues (here represented by 10-15 cycles of weathering) so that the properties of the stone are substantially altered (surface roughness and abrasion pH), the granite is expected to become 'highly bioreceptive'. Under this new scenario, the biological colonisation of the granite may increase, which may ultimately affect the treatments used to conserve the stone building or monument.

4. Conclusions

The laboratory-based salt weathering test used in the present study led to weight loss in the granite samples under study. The weight loss was not accompanied by significant changes in bulk density, open porosity or capillary water content, but the abrasion pH and surface roughness of the stone were significantly altered. Assessment of biofilm formation on the weathered and non-weathered granite revealed different levels of susceptibility to biological colonisation. As expected, the increased surface roughness and decreased abrasion pH derived from the weathering promoted biofilm growth. Moreover, as changes in the microbial composition were detected in the different biofilms formed, the different microorganisms present in the multi-species phototrophic culture used as the inoculum seem to vary in their capacity to adapt to the changes in the stone properties. Thus, greater proliferation of cyanobacteria relative to green algae can be expected in salt-weathered granite.

Calculation of the bioreceptivity index (*BI*) for the weathered and non-weathered granite revealed that the salt weathering process led to an increase in the bioreceptivity of the stone. Thus, granite CL was characterised by mild primary bioreceptivity, but high secondary bioreceptivity derived from the salt weathering, which could affect subsequent processes of biodeterioration or bioprotection. These results show that the study of the secondary bioreceptivity of granites and other construction materials that have been subjected to weathering processes can provide useful information about the expected responses in the medium to long term and can therefore improve the selection of materials for building purposes.

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6. Compliance with Ethical Standards

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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Tables

Table 1. Results of ANOVA of the properties of the granite studied before and after different cycles of the laboratory-based salt crystallisation weathering test. Values are expressed as means ± standard deviation of three replicates. Different superscript letters indicate significant differences between the number of weathering cycles applied. *P*-values ≤ 0.05 are indicated in bold type.

Number of cycles	Weight loss (%)	Bulk density (g cm^{-3})	Open porosity (%)	Capillary water (g cm^{-2})	Abrasion pH	Surface roughness, *Sa* (µm)
0	0.00 ± 0.00^{a}	2.52 ± 0.02	4.82 ± 0.84	0.20 ± 0.03	6.76 ± 0.22^{a}	18.90 ± 4.36^{a}
5	0.23 ± 0.05^{ab}	2.52 ± 0.01	4.29 ± 0.46	0.18 ± 0.02	6.22 ± 0.14^{ab}	31.50 ± 1.99^{ab}
10	0.55 ± 0.05^{b}	2.49 ± 0.02	5.25 ± 0.63	0.21 ± 0.01	5.86 ± 0.08^{b}	38.09 ± 4.57^{bc}
15	1.08 ± 0.25^{c}	2.51 ± 0.02	4.67 ± 0.77	0.20 ± 0.04	5.89 ± 0.10^{b}	52.37 ± 4.85^{c}
ANOVA	$F = 38.9$ **$p < 0.001$**	$F = 1.3$ $p = 0.330$	$F = 1.0$ $p = 0.448$	$F = 0.3$ $p = 0.813$	$F = 16.6$ **$p = 0.010$**	$F = 23.0$ **$p = 0.006$**

Table 2. Results of ANOVA of the Phyto-PAM parameters and colour measurements used to assess the biofilm growth on the different blocks studied, considering the number of weathering cycles applied and the surface of the sample inoculated (the surface or the inner part of the cubic blocks) as factors. *P*-values ≤ 0.05 are indicated in bold type.

Factor	Chl *a* (µg cm^{-2})	*Yield*	$F_{0,470nm} / F_{0,645nm}$	ΔE^*_{ab}
Weathering	**0.037**	0.195	**0.026**	0.240
	$F = 3.2$	$F = 1.7$	$F = 3.5$	$F = 1.5$
Surface	**< 0.001**	0.848	**0.001**	**< 0.001**
	$F = 48.3$	$F < 0.1$	$F = 12.2$	$F = 30.8$
Weathering x Surface	0.266	0.353	0.129	0.699
	$F = 1.4$	$F = 1.1$	$F = 2.0$	$F = 0.5$

Table 3. Bivariate Spearman's correlation matrix for the properties of the granite studied and the parameters used to assess the biofilm growth at the end of the incubation period (n = 40). Significant correlations ($p \leq 0.05$) are indicated in bold type.

	Chl *a*	*Yield*	$F_{0,470nm}$ / $F_{0,645nm}$	ΔE^*_{ab}
Bulk density	-0.264	0.018	0.073	-0.131
Open porosity	0.229	-0.054	0.066	0.210
Capillary water	0.262	-0.158	-0.024	0.203
Abrasion pH	**-0.392**	0.080	0.301	0.021
Surface roughness, *Sa*	**0.745**	-0.132	**-0.640**	**0.529**

Table 4. Bioreceptivity index (*BI*) [46] calculated for the weathered and non-weathered granite CL.

Weathering cycles	*BI*	Classification
0	5.3	Mild bioreceptivity
5	5.2	Mild bioreceptivity
10	6.2	High bioreceptivity
15	6.5	High bioreceptivity

Figures

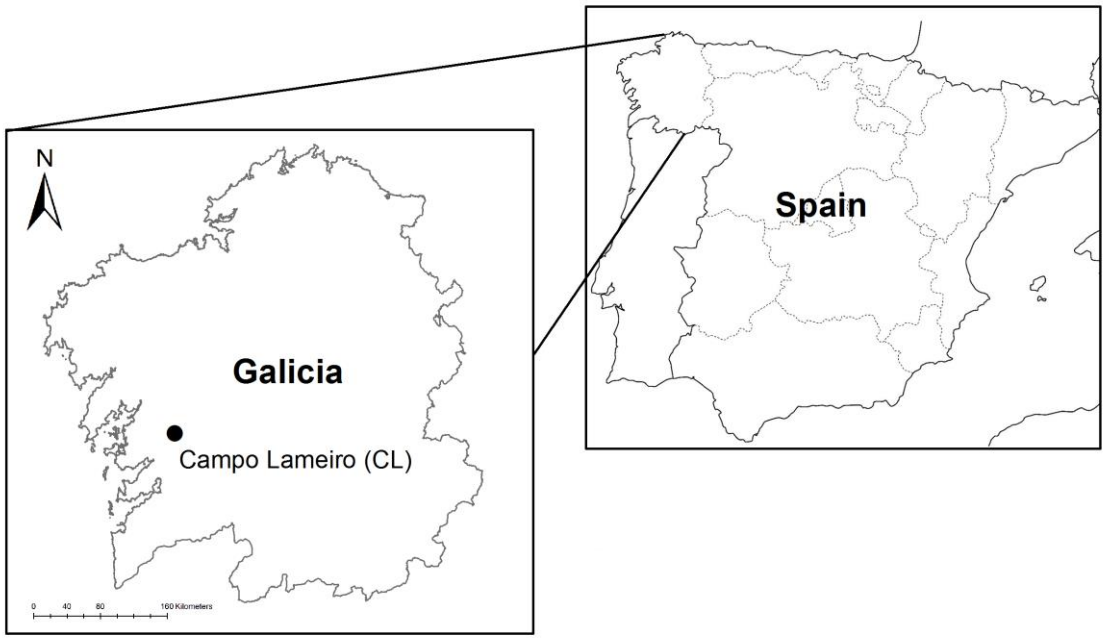

Figure 1. Location of the granite quarry where the material was obtained.

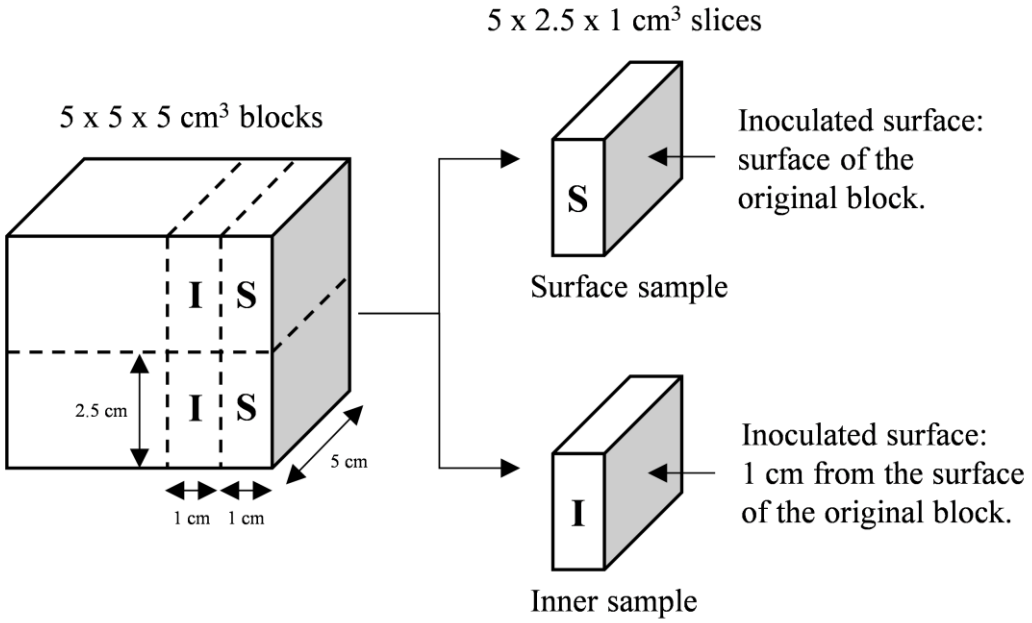

Figure 2. Cutting procedure for obtaining samples for subsequent inoculations from the weathered and non-weathered blocks.

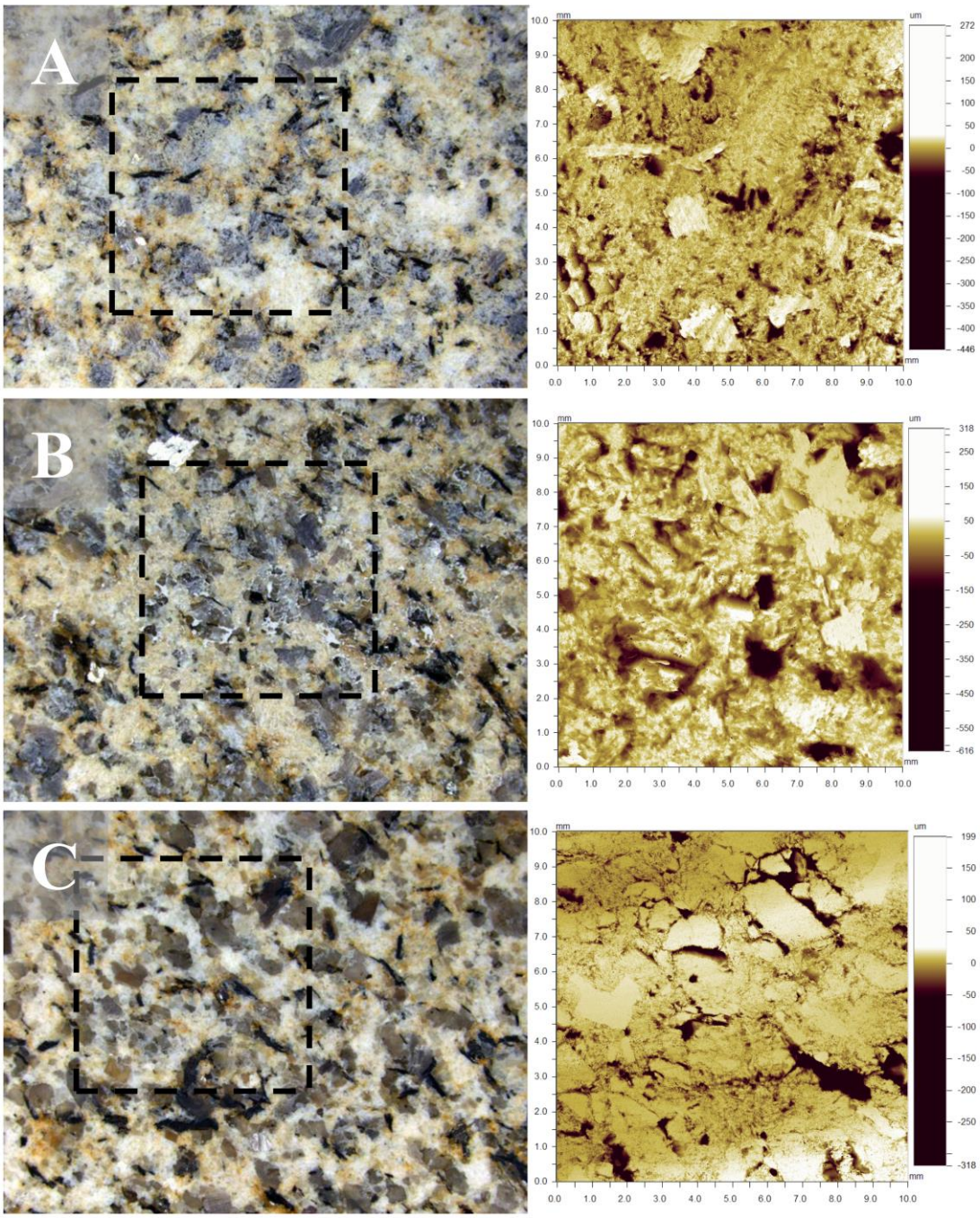

Figure 3. Optical microscope images of the granite CL. The areas outlined by dotted lines (10 x 10 mm^2) indicate where the WLOI measurements were made to quantify the surface roughness. A) Non-weathered granite, surface sample; B) weathered granite after 15 cycles, surface sample; C) weathered granite after 15 cycles, inner sample. The respective images derived from WLOI (colour scale indicates the variation in surface roughness) are shown on the right of each image.

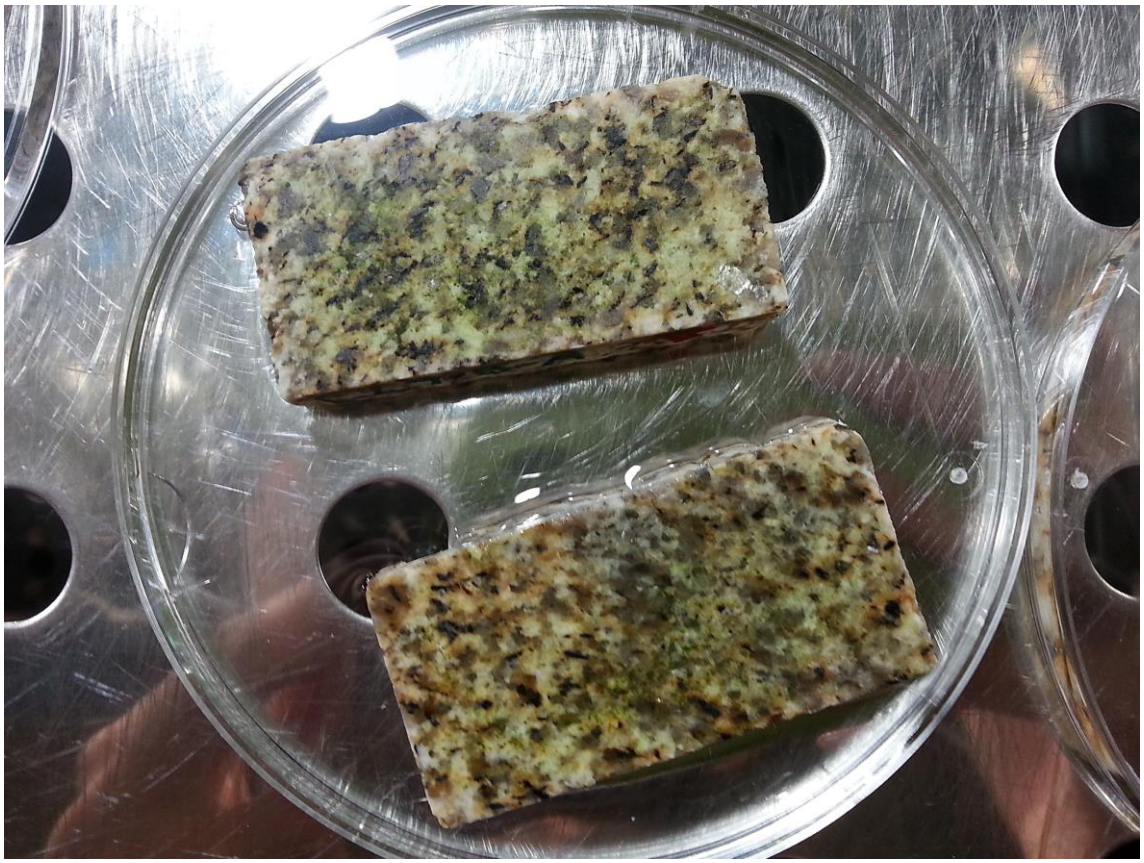

Figure 4. Appearance of samples after the three-month incubation period. Upper: 10 cycles-weathered surface sample; lower: non-weathered inner sample.

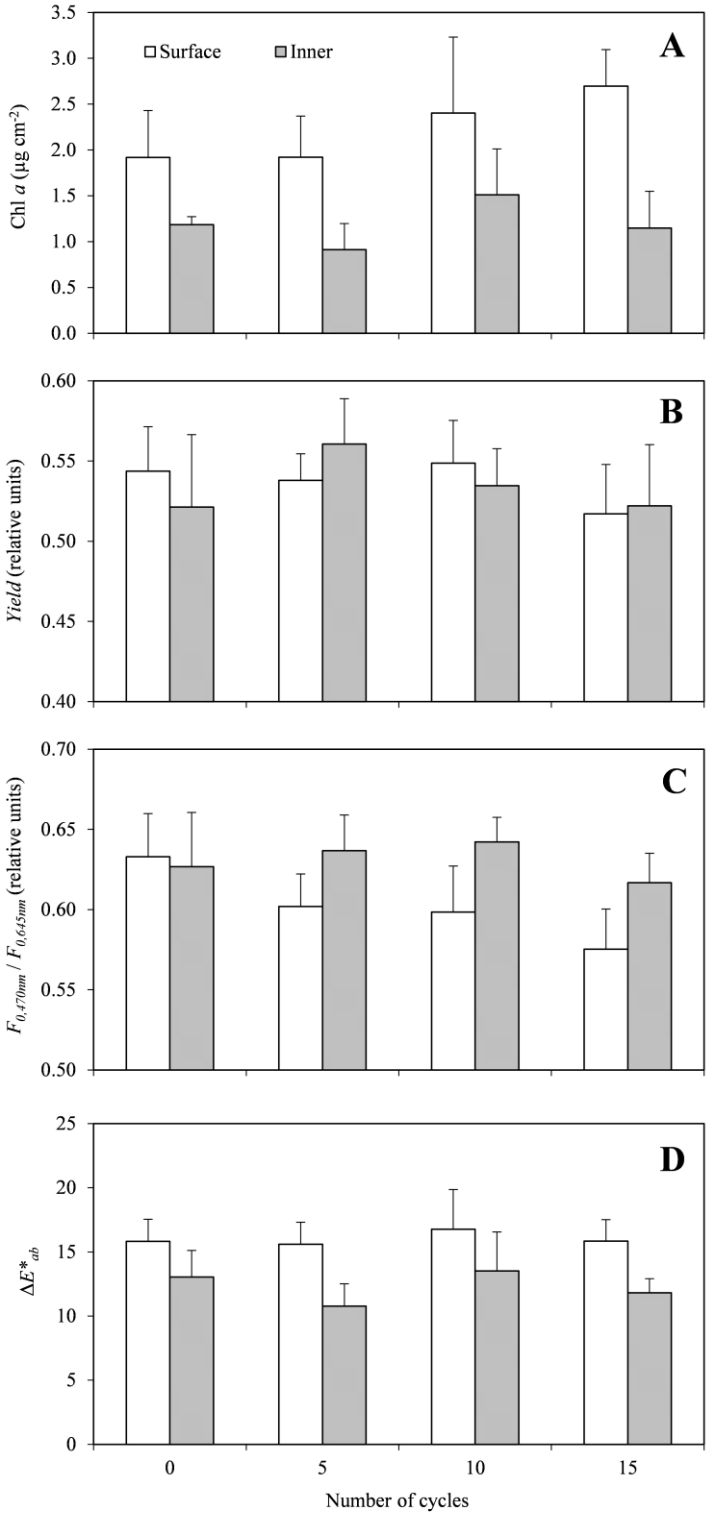

Figure 5. Results of Phyto-PAM analysis: A) chl *a* content, B) maximum quantum yield and C) $F_{0,470nm}$ / $F_{0,645nm}$ ratio; and colour measurements: D) ΔE^*_{ab}, used to assess the biofilm growth on the different blocks studied at the end of the three-month incubation period, expressed as mean values of five replicates (error bars indicate standard deviations).