

## **A procedure to evaluate the resistance to biological colonization as a characteristic for product quality of ceramic roofing tiles**

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## **Abstract**

Ceramic roofing tiles suffer deterioration through time due to environmental exposure. Biological colonization affects the appearance and integrity of building materials, such as roofing tiles. The resistance to biocolonization represents an important property affecting the product quality of ceramic roofing tiles. While natural colonization of roofing tiles by organisms is a progressive, heterogeneous, and slow process, laboratory assessment of this phenomenon requires a sensitive procedure that can be carried out within a reasonable period of time. Different microorganisms have been evaluated and the use of phototrophs, specifically the cyanobacterium *Oscillatoria*, presented several advantages such as good adherence, homogeneous growth on surfaces, and the chlorophyll-autofluorescence which can be used for a sensitive detection. Colonization by *Oscillatoria* on roofing tiles was assessed by measuring the autofluorescence of cells. This study proposes the use of specific cyanobacterial cells and a simple method for monitoring biofilm formation and biological colonization of roofing tiles.

**Keywords:** Ceramic roofing tiles, *Oscillatoria*, chlorophyll fluorescence, bioreceptivity, microbial colonization.

## 1. Introduction

The origin of clay roofing tiles can be traced back to China during the Neolithic Age (around 10,000 B.C). Ancient civilizations such as Egyptians, Babilonias, Greeks and Romans roofed their buildings with clay tiles and this practice continues throughout the world.

Time and exposure to the environment (including physical, chemical and biological factors) cause progressive transformations to building materials <sup>1</sup>. These changes are generally reported as deterioration. The processes leading to the deterioration of buildings have been the subject of numerous publications <sup>1-5</sup>. When time is considered, the role of the growth of organisms on these materials is the major factor affecting the conservation of their appearance and properties <sup>3</sup>. The effects of the development of organisms on building materials are closely related to physical and chemical factors. The development of plants and animals on buildings and construction materials has been reported although microorganisms appear to be the most destructive of living beings growing on buildings <sup>6</sup>. In fact, microorganisms are the first colonizers which are first attached and developing on the materials. This primary colonization usually represents aesthetic changes on the materials but it is a necessary step in the colonization process. During the primary colonization the initial conditions for the growth of other organisms are generated allowing a progressive succession of species (i.e., formation of mature microbial biofilms, lichens, mosses, ferns and vascular plants). Over time, the colonization process generally leads to significant physical and chemical damage of the materials as a result of the progressive biological development<sup>7</sup>. The colonization of ceramic roofing tiles by microorganisms have been reported to

diminish the product quality<sup>8,9</sup> although the literature on methods to determine the biological colonization on ceramic roofing tiles is barely inexistent.

The huge diversity of microorganisms existing in our planet<sup>10</sup> implies that they can produce transformations in nearly all naturally occurring materials. Most microorganisms, including bacteria, cyanobacteria, algae, protozoa, fungi and lichens, are able to produce specific compounds as a result of their metabolism. A common example is the production of acids and other chemicals which can accelerate the transformations of carbonate and silicate minerals<sup>3,6,11</sup> which constitute a significant portion of construction materials, including roofing tiles. While a single microorganism is likely to have null effects on any building, the typical development of these microscopic cells, generally forming biofilms constituted by millions of cells, can cover significant portions of the materials and present the potential for causing serious damage to building and man-made structures. Thus, the development of microbial biofilms can result in aesthetic as well as structural changes representing serious problems for the conservation of construction materials, for instance, ceramic roofing tiles.

Clay roofing tiles have one of the longest life expectancies among historic roofing materials, generally, above a hundred years. Because of this longevity, the exposure to environmental factors is translated in a higher risk potential to be colonized by microorganisms and, progressively by other organisms such as lichens and plants.

Microbial colonization causes changes in coloration and in the chemical and physical properties of the roofing tiles. Consequently, there is a need to preserve these materials for the longest time possible and a requirement to evaluate the resistance of these materials to be colonized by microorganisms. Guillite<sup>7</sup> defined the concept of

‘bioreceptivity’ as the capacity of a material to be colonized by living organisms. The bioreceptivity represents a useful parameter for the comparison of roofing tiles of different composition. Roofing tiles showing poor bioreceptivity will be desirable over those showing high bioreceptivity, which relate to the life expectancy of roofing tiles and aesthetic changes of building roofs.

Microbial diversity developing on buildings and monuments have been studied <sup>11,12</sup> although little has been proposed on effective procedures for the quantification of biological colonization <sup>13</sup>. A previous study on the organisms that naturally colonized roofing tiles reported the presence of biofilms constituted mainly by photosynthetic microorganisms, a diverse set of bacteria, and they even showed the development of lichens and plants in abundantly colonized roofing tiles <sup>14</sup>. Previous colonization assessments on stone materials <sup>5</sup> also presented the negative aspects of too long exposure times, heterogeneous colonizations and the use of complex communities, difficult to reproduce, for the evaluation of growth development. The time required for the growth of these complex biofilms and communities on roofs is above the expected requirements for any evaluation or test to be carried out for the comparison of building materials as demanded by related industries.

While natural colonization of roofing tiles by microorganisms is a progressive and slow process, laboratory assessment of this phenomenon requires a procedure than can be carried out within a reasonable period of time. In this study, we evaluate different microorganisms for their suitability to be used as models for colonization experiments and propose a reproducible procedure to evaluate the bioreceptivity of roofing tiles to

simulate a colonization process under laboratory conditions within a reasonable timeframe.

## **2. Experimental**

### *2.1. Ceramic materials*

Industrial ceramic roofing tiles were prepared from raw materials based on kaolinite-illite clay, quartz, feldspar and carbonates. The processes involved in the manufacturing of the ceramic roofing tiles were: shaping, extrusion (20-22 wt% water), drying (chamber dryer,  $t=16\text{h}/T_{\text{max}}=80^{\circ}\text{C}$ ) and firing (tunnel kiln,  $t=24\text{h}$ ) at a maximum temperature of  $950^{\circ}\text{C}$ .

Five different ceramic roofing tiles were used. Among them one was unglazed and another one glazed (hereinafter named unglazed and glazed roofing tile). The chemical composition of the unglazed roofing tiles and the layer of the glazed roofing tile are shown in Table 1. The characteristics of these two ceramic roofing tiles, such as porosity and roughness, are described in Table 2. The mineralogy of the unglazed roofing tile is shown in the diffractogram of Figure 1 where the major and minor crystalline phases are detailed. The other ceramic roofing tiles were represented by three different commercial products, named 1, 2 and 3, which presented specific coatings to provide differential aspects to these commercial types of roofing tiles. These samples present the same chemical composition, described in Table 1, but each of them has a different coating, and so different appearance. These coatings are called engobe, which are prepared mainly with crystalline raw materials, ceramic frits and pigments. The surface of these ceramic roofing tiles are very heterogeneous, in visual appearance,

porosity and roughness, because of the refractory properties of the material used to cover them. Table 3 shows the chemical composition of the surface of these commercial products, and Table 4 details other characteristics such as porosity and roughness. In this type of samples it is not possible to determine the porosity (% water absorption) as it was carried out in the unglazed roofing tile. So, in order to have a value of the surface porosity a qualitative assay was performed. This assay consists of dropping a certain volume of water on the surface and measuring the time taking to be absorbed by the surface.

The roofing tiles were cut in 4 cm x 4 cm coupons which were used for the colonization experiments performed in this study. These pieces were sterilized in an autoclave at 121°C for 20 min just before the colonization experiment.

## 2.2. *Microorganisms*

Different microorganisms were assayed for their capacity to colonize the coupons and for their simplicity of detection. Heterotrophic bacteria were previously tested by Laiz et al.<sup>14</sup> but the present study focuses on the colonization of roofing tiles by photosynthetic microorganisms. An Eukaryote, the unicellular algae *Chlorella* sp. (Chlorophyta) isolated by the authors from naturally colonized rock substrates, and three Cyanobacteria from the culture collection at the 'Instituto de Biología Vegetal y Fotosíntesis' (CSIC, Seville, Spain) were used in this study to evaluate their colonization on the studied roofing tiles. The three tested cyanobacterial strains were a Nostocales, *Nostoc* sp. PCC 9203, and two Oscillatoriales, *Leptolyngbya* sp. PCC 9324 and *Oscillatoria* sp. PCC 9325. *Nostoc* is a typical sheath forming Cyanobacteria<sup>15</sup> while *Oscillatoria* is a sheathless microorganism.

### 2.3. Colonization experiments and bioreceptivity tests

Cultures of these phototrophic microorganisms were performed in BG11 medium <sup>16</sup>.

This medium composition was (per liter): Na<sub>2</sub>CO<sub>3</sub>, 0.02 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.075 g; CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.036 g; K<sub>2</sub>HPO<sub>4</sub>, 0.04 g; NaNO<sub>3</sub>, 1.5 g; citric acid, 0.006 g; ferric ammonium citrate, 0.006 g; ethylenediaminetetraacetic acid disodium salt, 0.001 g; trace metal solution, 1 ml. The composition of the trace metal solution was (per liter): H<sub>3</sub>BO<sub>3</sub>, 2.86 g; MnCl<sub>2</sub>·4H<sub>2</sub>O, 1.81 g; Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O, 0.39 g; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.22 g; CuSO<sub>4</sub>·5H<sub>2</sub>O, 79 mg; CoCl<sub>2</sub>·6H<sub>2</sub>O, 26 mg. Medium was esterilized and adjusted to pH 7.1. The cultures were incubated at 28°C for one week under illumination with 16h/8h light/dark cycles. Stock cultures were used to inoculate roofing tiles placed vertically in a covered glass container containing BG11 medium. Under these conditions, different phototrophic microorganisms were analyzed to examine their capacity to develop on roofing tiles using unglazed tiles placed horizontally on the culture medium.

To comparatively evaluate the bioreceptivity of ceramic roofing tiles, the coupons were placed vertically in a covered glass container in which the medium covered up to 0.5 cm of the lowest side of the roofing tiles (Figure 2). These tests were repeated twice. The same conditions described above were used in these bioreceptivity experiments except that in this case the incubation was allowed to continue for one month. *Oscillatoria* sp. PCC 9325 was used in these experiments. After the incubation period, the development of the cyanobacteria was monitored by 2D scanning of the surface of the roofing tiles quantifying the fluorescence from the chlorophyll of *Oscillatoria* using a pulse-amplitude modulated chlorophyll fluorometer (Heinz Walz GmbH, Effeltrich, Germany) as previously suggested <sup>17</sup>. The fiberoptic was placed perpendicular to the surface at 2 mm above the coupon. Chlorophyll fluorescence over the scanned area was



plotted using the analytical software package SigmaPlot v.8.0 (SPSS Inc., Chicago). Bioreceptivity was quantified by using the distance and fluorescence per unit of area colonized by the *Oscillatoria* strain in a given time period. The colonization space was estimated from the vertical distance where the fluorescence intensity measured over the roofing tile coupon surface showed its maximum negative derivative. A phase-contrast and fluorescent microscope, Zeiss Axio imager 2 (Carl Zeiss, Germany), was used to obtain comparative microphotographs under white light illumination and the autofluorescence when illuminated with blue light using conventional filter sets.

### **3. Results and Discussion**

#### *3.1. Development of bioreceptivity procedure by using different microorganisms*

In order to evaluate the bioreceptivity of ceramic materials, such as roofing tiles, it is required to use a microorganism that it is able to develop on these substrata and it is easily detected even at relatively low abundances or early stages of colonization. In this study we have evaluated some microorganisms on these premises and proposed a procedure to comparatively test the level of bioreceptivity of ceramic roofing tiles and the ability of microorganisms to colonize these materials.

A previous study on microorganisms isolated from naturally colonized roofing tiles<sup>14</sup> proposed the use of an Actinobacteria belonging to the genus *Streptomyces* as a potential strain for colonization assays of roofing tiles. This microorganism produced heterogeneously distributed white colonies on the tiles which are relatively easy to visualize. A Fungi (*Fusarium*) was also tested<sup>14</sup> but growth produced thin hiphae which did not lead to clear images of colonization above all during its early stage of

development. Uneven distributions of colonizing biofilms represent a serious drawback for the monitoring and quantification of colonization processes<sup>17-19</sup>.

The use of specific photosynthetic microorganisms presents a clear advantage against heterotrophic microorganisms due to the possibility of detecting the autofluorescence of chlorophyll<sup>20</sup> when illuminating phototrophs with blue light (Figure 3). The use of fluorescence allows for a higher sensitivity in the detection of the colonizing microorganisms even at early stages of colonization<sup>17</sup>. Thus, using fluorescence from the chlorophyll contained in the phototrophic microorganisms, colonization can be detected in base to a relatively low number of cells. This implies a significant reduction of the time required to detect a colonization process during bioreceptivity tests.

At this respect, a previous report<sup>14</sup> has proposed the use of a mix, complex microbial community obtained from a naturally colonized roofing tile as other authors did with a variety of materials, for instance, limestone<sup>5</sup>. In Laiz et al.<sup>14</sup>, the community was dominated by the development of the green algae *Chlorella* although the use of complex, undefined microbial communities presented serious problems of reproducibility due to potential variations of the structure of such community.

Variations in microbial community structure certainly affect the results of future analyses and their reproducibility among different institutions. Ideally, a bioreceptivity test should be performed with a single organism which is available to everyone and will allow a standardization of procedures. Besides, the use of a photosynthetic microorganism (i.e., containing chlorophyll) will facilitate its detection at early colonization stages.

As potential models of photosynthetic microorganisms for assays of bioreceptivity of ceramic roofing tiles, four strains were tested (Figure 4). The eukaryote *Chlorella* showed slow and poor growth on the coupons forming a heterogeneous distribution. The Cyanobacteria *Leptolyngbya* was unable to clearly develop on the roofing tiles under the provided conditions. The sheath-forming Cyanobacteria *Nostoc* showed slow growth although clearly visible, but presented a highly heterogeneous distribution forming clumps unable to spread over the tile surface throughout time and covering only small portions of the coupon area. Thus, the patches of the Cyanobacteria *Nostoc* and the alga *Chlorella* growing on roofing tiles makes difficult the assessment and quantification of the colonization process since they did not meet a requirement for an homogenous colonization<sup>17</sup>. *Oscillatoria* showed the fastest growth, forming significant biofilms on the roofing tile coupons. Besides, *Oscillatoria* presented a clear trend to grow spreading all over the tile surface which was progressively and homogeneously covered by the filaments. The Cyanobacteria *Oscillatoria* was chosen for the bioreceptivity tests due to its capacity to colonize faster and more homogeneously than the other strains. Besides, when colonizing a coupon in vertical position, *Oscillatoria* showed the property of forming a biofilm which spreads upwards over time as a result of the growth of *Oscillatoria* filaments along the surface of the coupons and this development was dependent on the material under evaluation (Figure 5).

### 3.2. Comparative analysis of the bioreceptivity of different roofing tiles

Experiments were carried out for the comparative analysis of the bioreceptivity of different roofing tiles and the level of colonization was evaluated by an increase of the intensity of chlorophyll autofluorescence as well as the distance or height colonized by

*Oscillatoria* during its growth on different roofing tiles. An example of this colonization is shown in Figure 5. Figure 6 shows the quantification of fluorescence intensity over the surface of two roofing tiles. Previous analyses have reported a direct relationship between chlorophyll content and fluorescence measurements using pulse-amplitude modulated fluorometers<sup>17</sup>. Herein, the procedure provided with quantitative information on the development of the Cyanobacteria *Oscillatoria* growing on ceramic roofing tiles (Figure 6) although it is also useful to assess differences in bioreceptivity between a variety of materials. In order to quantify and compare the bioreceptivity of different materials two parameters can be utilized: fluorescence intensity (i.e., scale of colors in Figure 6) and growth distance or height (Y axes in Figure 6). Figure 7 schematically shows the estimation of the colonization distance on a roofing tile. In the examples of Figure 6, one can easily observe differences in both parameters useful for the comparison of two different roofing tiles. The unglazed roofing tile coupon allowed a much more intense colonization as judged by the higher levels of fluorescence than in the glazed coupon. The glazed coupon presented low bioreceptivity as shown by the poor growth of *Oscillatoria* (average height, 3 mm; standard deviation 0.4). The colonization by *Oscillatoria* on the unglazed coupon covered over a centimeter (average 18.5 mm; standard deviation 0.5) (Table 2) showing a relatively high bioreceptivity and presenting chlorophyll fluorescence closed to 10-fold the measurements obtained on the glazed roofing tiles.

The three commercial roofing tiles showed lower bioreceptivity than the unglazed tile due to the effect of their proprietary coatings to provide different aesthetic aspects to these ceramics (Figure 5). For instance, the commercial type 1 showed scarce colonization (average 0.5 cm; standard deviation 0.1). Commercial type 2 presented an

intermediate (average 3.3 cm; standard deviation 0.6) bioreceptivity between the types 1 and 3. The third type of commercial roofing tile showed the highest observed colonization (average 7.8 cm; standard deviation 0.7) among the three coated ceramic roofing tiles of commercial origin (Table 4). Commercial types 1 and 3 have a dark coating which might preclude the visualization of early stage colonization. However, the use of a fluorescent detection method of the chlorophyll content in the proposed cyanobacterium overlooks that potential problem because autofluorescence measurements are not affected by the coloration of the tile surface. Besides, the proposed procedure has resulted in the ability to statistically distinguish ceramic roofing tiles in base to the bioreceptivity (i.e., in terms of biological colonization) of these ceramics.

Ceramic roofing tiles can show distinct composition or manufacturing characteristics. In the example provided in this study, we differentiated the colonization of glazed and unglazed roofing tiles and three commercial types with a surface coating. Glazing leads to a resistance to colonization and so to low bioreceptivity. Unglazed roofing tiles allowed the growth of *Oscillatoria* resulting in elevated bioreceptivity which could be a consequence of its higher porosity. Coatings result in a surface with lower porosity than the unglazed roofing tile and it may explain the observed differences in bioreceptivity. Roughness and surface chemical composition showed similar values for the three commercial roofing tiles so these two factors could not account for the differences in bioreceptivity. Primary stages of the colonization process are highly related to the capacity of microorganisms to attach to the materials to be colonized. In this process, the ability of microorganisms to produce exopolymeric substances is decisive for their adhesion to the substrate<sup>11</sup>. The anionic nature of these polymers can adsorb cations and

stabilize dust particles. Microbial cells embed in these polymeric substances and the process leads to the formation of stable biofilms. Primary colonization and the adhesion of microorganisms can be facilitated by an elevated porosity and water absorption of the material surface. Further research is in progress to understand the relationships between bioreceptivity and the chemical and physical properties of roofing tiles as some studies have suggested for other materials <sup>21,22</sup>.

High bioreceptivity of a type of roofing tile implies an elevated chance of being colonized by microorganisms and so a potentially much higher risk of leading to changes in the external aspect of the tiles. Besides, aesthetic changes, intense colonization of roofing tiles and construction materials can result in transformations of the material properties and so, it can affect negatively its performance and durability. Evaluating the potential bioreceptivity of roofing tiles is essential to ensure the maintenance of their quality properties (including physical, chemical and aesthetic characteristics). An essential concept for the marketing of materials, including ceramic roofing tiles, is to warrant those characteristics and to pass standardized quality controls, mainly when competitiveness is looked at in today's markets.

The test proposed in this study represents a significant step forward to allow the comparison of the bioreceptivity in ceramic roofing tiles by applying a laboratory evaluation of the behavior of these materials within a logical experimental timeframe. This procedure is relevant to the primary phase of colonization when the first microorganisms initiate their attachment and growth on the studied materials. The monitoring of this process requires a standardized protocol to assess and compare the performance of the roofing tiles. The proposed procedure has been tested for roofing

tiles although it can be applied to a variety of materials. A number of studies is available on the causes and damage than biodeterioration induces in buildings and monuments<sup>3,5,11,13</sup>. Most colonization studies have been carried out on stone buildings and related materials<sup>2,12,13</sup>. Detailed, quantitative and easily standardizable procedures, which could be implemented by any interested party, to monitor and evaluate colonization processes on a variety of materials and their bioreceptivity remained to be proposed<sup>5</sup>.

#### **4. Conclusions**

The study allows several conclusions to be drawn:

- 1) A novel procedure is proposed to assess the bioreceptivity of roofing tiles following an standardizable methodology. A protocol for the quantitative comparison of the colonization of roofing tiles and the use of a model microorganism for this purpose (*Oscillatoria*) are introduced and tested to allow the evaluation and analysis of ceramics. The use of biofilm-forming microorganisms to proceed with strict quality controls is a prerequisite of modern industry. Novel procedures, such as the one proposed in this study, need to be designed and described to fulfill those demands and meet the required certifications.
- 2) The unglazed roofing tile coupon allowed a much more intense colonization as judged by the higher levels of fluorescence than in the glazed coupon. The glazed coupon presented low bioreceptivity as shown by the poor growth of *Oscillatoria* probably due to the low porosity (% water absorption) of this type of material.

- 3) The three commercial roofing tiles showed lower bioreceptivity than the unglazed tile due to the effect of the coatings used to provide different aesthetic aspects to these ceramics. Differences in porosity may explain most of the observed differences in bioreceptivity.

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## Figure legends

**Figure 1.** Crystalline phases in the unglazed ceramic roofing tiles.

**Figure 2.** Assembly scheme used in the bioreceptivity tests.

**Figure 3.** Examples of *Chlorella* (A, B) and *Oscillatoria* (C, D) cells visualized under phase-contrast microscopy (A, C) and under epifluorescence microscopy (B, D) using blue excitation light. In B and D, the red color of the emitted fluorescence can be observed allowing a sensitive detection of these cells. Bars represent 10  $\mu\text{m}$ .

**Figure 4.** Examples of biofilms formed by the growth of *Chlorella* (A), *Nostoc* (B) and *Oscillatoria* (C) on unglazed roofing tiles. The case of *Leptolyngbya* is not shown since it did not form visible biofilms.

**Figure 5.** Biofilm formation of *Oscillatoria* on roofing tile coupons during bioreceptivity tests comparing glazed (A) and unglazed (B) roofing tiles, as well as three different commercial types 1 (C), 2 (D), and 3 (E). Incubation time was one month. Arrows indicate the level reached by the surface of the culture medium. F shows a microphotograph of the *Oscillatoria* cells colonizing an unglazed roofing tile (A) by fluorescence microscopy under blue excitation light. Bar (F) indicates 20  $\mu\text{m}$ .

**Figure 6.** Graphical representation of the 2D measurements of fluorescence from the growth of *Oscillatoria* biofilms during the bioreceptivity test of the glazed

(A) and unglazed (B) roofing tiles shown in Figure 5. Arrows indicate the location of the surface of the culture medium. A colored scale is shown corresponding to fluorescence intensity.

**Figure 7.** Example of fluorescence intensity along the vertical position of an unglazed roofing tile colonized by *Oscillatoria* during the experiments described in this study. The position of the level of medium in the experiment is shown and the maximum negative slope is used to locate the end of the colonized area and to estimate the colonization distance for this example. Data points correspond to three different measurements along a y-axis.

**Table 1.** Chemical composition of the glazed and unglazed roofing tiles used in this work.

<b>Oxides</b>	<b>Concentration (% by weight)</b>	
	<b>Unglazed roofing tile</b>	<b>Layer of glazed roofing tile</b>
SiO <sub>2</sub>	60.3	54.9
Al <sub>2</sub> O <sub>3</sub>	15.6	12.0
B <sub>2</sub> O <sub>3</sub>	<0.15	2.6
Fe <sub>2</sub> O <sub>3</sub>	5.66	0.95
CaO	3.25	10.1
MgO	2.73	0.3
Na <sub>2</sub> O	0.37	0.86
K <sub>2</sub> O	3.95	3.7
TiO <sub>2</sub>	0.74	0.03
MnO	0.05	<0.01
P <sub>2</sub> O <sub>5</sub>	0.08	<0.01
BaO	<0.01	6.6
ZnO	<0.01	7.8
LOI (1000°C)	7.12	-

**Table 2.** Comparison of different ceramic roofing tiles evaluated based on the colonized height during the experimental procedure described in the text over a 30 days period. The characteristics of the ceramic roofing tiles used in this study are also shown.

	<b>Colonization height (mm)<sup>1</sup></b>	<b>95% Confidence interval</b>	<b>Porosity (% water absorption)</b>	<b>Roughness (Ra, nm)</b>
Unglazed roofing tile	18.5 (0.5)	(17.3, 19.7)	10	3400
Glazed roofing tile	0.3 (0.4)	(0, 1.2)	<1	1200

<sup>1</sup> Average  $\pm$  sd.



**Table 3.** Chemical composition of the surface of the commercial roofing tiles used in this study.

Oxides	Concentration (% by weight)		
	Commercial roofing tile 1	Commercial roofing tile 2	Commercial roofing tile 3
SiO <sub>2</sub>	60.2	60.7	48.3
Al <sub>2</sub> O <sub>3</sub>	18.0	19.0	12.3
Fe <sub>2</sub> O <sub>3</sub>	7.3	3.6	24.9
CaO	6.2	8.2	0.83
MgO	0.6	0.5	0.54
Na <sub>2</sub> O	0.9	1.2	0.79
K <sub>2</sub> O	3.6	4.3	4.12
TiO <sub>2</sub>	1.1	0.6	0.35
MnO	1.4	0.1	6.58
P <sub>2</sub> O <sub>5</sub>	0.2	0.5	0.24
BaO	0.1	0.1	0.39
ZnO	0.05	0.3	0.05
ZrO <sub>2</sub>	0.06	0.4	0.05
Cr <sub>2</sub> O <sub>3</sub>	0.05	0.1	0.06
S	0.07	0.11	-

**Table 4.** Comparison of commercial ceramic roofing tiles evaluated based on the colonized height during the experimental procedure described in the text over a 30 days period. The characteristics of the ceramic roofing tiles used in this study are also shown.

	<b>Colonization height (mm)<sup>1</sup></b>	<b>95% Confidence interval</b>	<b>Porosity (min/100<math>\mu</math>L)</b>	<b>Roughness (Ra, nm)</b>
Commercial roofing tile 1	0.5 (0.1)	(0.2, 0.8)	>100	5900
Commercial roofing tile 2	3.3 (0.6)	(1.9, 4.7)	8.9	5700
Commercial roofing tile 3	7.8 (0.7)	(6.1, 9.5)	6.1	5400
Unglazed roofing tile	18.5 (0.5)	(17.3, 19.7)	3.5	3400
Glazed roofing tile	0.3 (0.4)	(0, 1.2)	>100	1200

<sup>1</sup> Average  $\pm$  sd.