1 **Evaluation of natural colonisation of cementitious materials: effect of**
2 **bioreceptivity and environmental conditions.**
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17 **Abstract**

18 Incorporation of living organisms, such as photosynthetic organisms, on the
19 structures envelope has become a priority in the area of architecture and construction
20 due to aesthetical, economic and ecological advantages. Important research efforts are
21 made to achieve further improvements, such as for the development of cementitious
22 materials with an enhanced bioreceptivity to stimulate biological growth. Previously, the
23 study of the bioreceptivity of cementitious materials has been carried out mainly under
24 laboratory conditions although field-scale experiments may present different results.
25 This work aims at analysing the colonisation of cementitious materials with
26 different levels of bioreceptivity by placing them in three different environmental
27 conditions. Specimens did not present visual colonisation, which indicates
28 environmental conditions have a greater impact than intrinsic properties of the material
29 at this stage. Therefore, it appears that in addition to an optimized bioreceptivity of the
30 concrete (i.e. composition, porosity and roughness), extra measures are indispensable
31 for a rapid development of biological growth on concrete surfaces. An analysis of the
32 colonisation in terms of genus and quantity of the most representative microorganisms
33 found on the specimens for each location was carried out and related to weather
34 conditions, such as monthly average temperature and total precipitation, and air quality
35 in terms of NO_x, SO₂, CO and O₃.
36 OPC-based specimens presented the higher colonisation regarding both
37 biodiversity and quantity. However, results obtained in a previous experimental
38 program under laboratory conditions suggested a higher suitability of Magnesium
39 Phosphate Cement-based (MPC-based) specimens for algal growth. Consequently,
40 carefully considering the environment and the relationships between the different
41 organisms present in an environment is vital for successfully using a cementitious
42 material as a substrate for biological growth.

43 **Key words:** *Bioreceptivity, intrinsic properties, natural colonisation, cementitious*
44 *materials, microorganisms.*

45 **1. Introduction**

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Nowadays, two major technologies are used to create vegetated wall surfaces, i.e. systems rooted in the ground and systems rooted in artificial substrates. The first group refers mainly to the use of climber plants with or without supporting systems. In contrast, the second group is characterized by its dependence on irrigation systems and addition of nutrients to the substrate (Perini et al., 2011). Both systems suffer from high investment and maintenance costs (Pérez Luque, 2010). A cheaper alternative would be to stimulate the development of colourful patinas of biological origin on the surface of building materials.

As concrete is one of the most used building materials in cities around the world, it represents an eligible substrate for development of green facades. Ordinary Portland cement, however, is not a feasible material for the rapid development of a biological patina. The high alkalinity (pH of about 12) and rather low porosity make it a material with a low bioreceptivity. Biological colonization only occurs once the pH has sufficiently dropped (pH of about 9-10), due to reaction of the cement matrix with atmospheric carbon dioxide, a process known as carbonation (Taylor, 1990). Ordinary Portland cement, however, is not the only binder used in construction. Cement with fly ash or silica fume addition present lower concentration of alkali and hydroxyl ions. Moreover, the aforementioned reduction would depend on the nature of the addition, the level of cement replacement, the alkali content of the cement and the age (Shehata et al., 1999; Erdem and Kirca, 2008).

At UPC (Barcelona, Spain), we have developed a multi-layered concrete panel for the development of green facades. This patented material is composed of four layers (Manso et al., 2013). The first layer consists of conventional concrete and is responsible for the structural function of the panel. The main function of the second layer is to protect the first layer from ingress of water and noxious substances. Furthermore, it acts as a bond layer between the inner and outer layer. The function of the third layer is to stimulate the development of the biological patina. It represents an anchorage site for airborne microorganisms and a niche for microbial growth. Finally, the fourth and last layer is a discontinuous one in order to allow different designs of the surface. Exit of water is then redirected to the areas without this fourth layer, promoting better local conditions for colonising organisms.

In our previous study, we have screened several mortar formulations for their effectiveness as a substrate for biological growth (Manso et al., 2014b). Six different mortar designs were subjected to an accelerated algal fouling test. Main results extracted from this work were two; first, chemical intrinsic properties seemed to have more influence than physical ones under laboratory conditions and, second, Magnesium Phosphate Cement (MPC) appeared to be the most promising binder for the third layer of the multi-layered concrete panel.

Numerous research groups have been investigating natural colonisation of building materials. Studies mainly focus on the identification of the organisms on those surfaces, how to prevent their colonisation by means of the development of surficial treatments or methodologies of evaluation. For instance, Gaylarde and Gaylarde (2005) identified *Chlorophyceae* and *Cyanophyceae* as the most prevalent microorganisms found, where algal growth depends on humidity and porosity of the material and *Cyanophyceae* was mainly observed on concrete walls subjected to dry

periods. De Muynck et al. (2009) compared different strategies for the prevention of algal fouling on two types of concrete and stated the performance of the surface treatments is dependent on the bioreceptivity of the concrete. Prieto et al. (2005) compared three different methodologies of biofilm quantification on stone: quantification of chlorophyll *a*, determination of fluorescein diacetate hydrolysis and determination of the total colour difference. The authors concluded differences in colour can be used since it is statistically robust, easy, quick, non-destructive and can be used in situ and on site.

The use of accelerated laboratory tests is common when evaluating the intrinsic properties of a material (Guillitte and Dreesen, 1995; Dubosc et al., 2001; Escadeillas et al., 2007; De Muynck et al., 2009; Tran et al., 2012). However, colonisation of materials by living organisms depends not only on the bioreceptivity of the material but also on the climate conditions and the organisms present in the environment (Guillitte, 1995). Therefore, the necessity of field-scale experiments has gained more interest when studying biofouling of cementitious materials, although only few publications presented field-scale results. For instance, Tran et al. (2014) stated no correlation between laboratory and field-scale tests should be done due to dissimilarities between those two experimental scales. The authors suggested the necessity of modifying laboratory accelerated tests to approach the configuration to a real situation.

The aim of this research is evaluating different cementitious materials' designs to stimulate biological growth under different environmental conditions. For this purpose, a comparison between three different locations was carried out. First, the selection of the methodology to remove the microorganisms from the specimens was done. Then, identification and quantification of the predominant culturable microorganisms present on the specimens was performed for all three locations. Finally, weather and air quality data were also analysed to link materials' properties, microorganisms present in the environment and weather conditions to the colonisation.

2. Materials and methods

2.1. Mortar specimens and setup

Different hydraulic binders (Ordinary Portland Cement, OPC, and Magnesium Phosphate Cement, MPC), silica aggregates sizes (0/2 and 2/4 mm), water to cement ratios (ranging between 0.15 and 0.28 and between 0.3 and 0.6 for MPC and OPC specimens, respectively) and amounts of cement paste were used to produce mortar specimens with different bioreceptivity.

The type of OPC used was type I 52.5R while MPC was composed by magnesium oxide (MgO), monoammonium phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$) and borax acting as a retarder ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$). Water to cement ratios for OPC specimens were selected based on the standard UNE-EN 196-1:2005 and for MPC specimens based on the characterisation developed in a previous work (Manso et al., 2014a). Variations on the amount of cement paste were applied starting from an estimated reference value. The aforementioned reference value (C) corresponded to the minimum amount of cement paste required to join all the aggregates for OPC specimens (Manso et al., 2014b). The composition of the mortars is given in Table 1.

Table 1. Specimens' composition.

OPC and MPC specimens were cast into 80 x 80 x 20 mm³ polyurethane elastomer moulds, demoulded after 24 h and 1 h respectively and cured at 22 ± 2 °C and 95 ± 5% relative humidity for 28 days.

Expanded polystyrene plates of 3 cm thickness were cut in order to randomly embed the specimens. Then, support plates were produced to place some of the samples horizontally and others vertically, oriented to the north, as shown in Figure 1. This type of setup was produced per triplicate in order to place specimens in three different environmental conditions in terms of weather and air pollution. Accordingly, fifteen replicates per dosage and environmental conditions were produced and randomly placed.

Figure 1. Setup of specimens exposed to environmental conditions: (a) General view, (b) vertical specimens and (c) horizontal specimens.

2.2. Locations

Locations selected for the current experimental program were Barcelona city in Spain, the Natural Park of Montseny (60 km from Barcelona) and Ghent city in Belgium. Maintenance of the setup areas was carried out in order to avoid interference of surrounding plants. Specimens were exposed for 1 year in all locations.

The first location in Barcelona city corresponds to a contaminated area with Mediterranean climate, the second one in Montseny to similar climate conditions but in a non-contaminated area and the third in the surroundings of the city of Ghent with oceanic climate. Mediterranean climate is characterised generally by moderate temperatures with hot, dry summers and wet winters with an average temperature of around 10° C. Moreover, countries included in the Mediterranean region lie between 30° and 45° north and south of the Equator. In contrast, the temperate maritime climate influenced by the North Sea and the Atlantic Ocean, has cool summers (average temperature less than 22° C) and moderate winters (average temperatures warmer than -3° C). Furthermore, the rainfall is usually distributed throughout the year (Lionello et al., 2006; Barry and Chorley, 2009).

2.3. Sampling and tests

A comparison between two biological growth removal methodologies was accomplished in order to select one. Quantification of total viable microorganisms was obtained by means of the use of cotton swabs and by immersion of the samples in sterile Ringer. First, specimens were disinfected with alcohol until complete evaporation. Then, inoculation of the specimens was carried out with microorganisms often found on cementitious materials in a liquid culture. Afterwards, specimens were incubated and removal of the microorganisms for quantification was achieved after 24 hours and 7 days by means of both methodologies:

- Cleaning of the surface with sterile cotton swabs, inoculation of microorganisms and incubation before total plate counting.

- Immersion of the specimens in sterile Ringer under stirring for 10 minutes. Suspensions were then centrifuged to obtain the pellets, which were sown and incubated before the quantification.

Regarding field samples, they were analysed once after one year of exposition. Three replicates per dosage for each inclination and environmental condition were randomly chosen and crushed for analysis by means of scanning electron microscope (SEM) and three other replicates were used to collect the biological growth.

The removal of the biological growth was carried out by means of sterile cotton swabs. Those samples were then inoculated in different culture media to detect bacteria, algae and fungi as detailed in Table 2. Furthermore, a unique sample of environmental microorganisms present for each one of the three areas were also obtained by exposing two different culture media, one containing Tryptic Soy Agar and the other one containing Sabouraud Agar with antibiotic. Petri dishes were maintained open next to the setup during 10 minutes to obtain the sample.

Table 2. Culture media and incubation regimes.

After specific incubation (see Table 2), different classical assays were carried out in order to provide information about the microorganisms. Identification of bacteria was determined by means of the Gram stain method, the spores staining for Gram positive *Bacillus*, the catalase and oxidase test and the microorganisms sown in API gallery according to the presumptive genera. Then, identification of fungi was obtained by means of fresh samples observation in lactofen blue and evaluation of macroscopic as well as microscopic characteristics. Finally, yeasts identification was achieved by means of fresh samples observation in lactofen blue, methylene blue stain and Analytical Profile Index (API) 20C AUX gallery (Cowan, 1974; Atlas, 2010).

Moreover, climate data were obtained from the nearest climate station. Data corresponding to the monthly average temperatures (°C) and total precipitation per month (mm) will be presented.

3. Results and discussion

3.1. Evaluation of the suitability of the microorganisms' removal methodology

The use of cotton swabs for the removal of the microorganisms is a widely used methodology (Hallander et al., 1975; Evans and Stevens, 1976). However, quantification of the microorganisms may be influenced by the type of substrate such as the mortars here studied. According to Table 1 and considering the biological growth observed on the specimens, the different porosity of the specimens could be affecting the results. Consequently, a comparison between the two methodologies described in the materials and methods section has the purpose of verifying the suitability of the method.

Table 3 shows the quantification results after 24 hours and 7 days of culture on the specimens' surface by means of both methodologies. Results corresponding to the first quantification (1 day) presented unclear trends, since no correlation between method and growth can be established. Quantification was higher for half of the samples when using the cotton swabs. However, results of quantification after 7 days showed a

clearer trend. For organisms who were established and anchored to the material for at least one week, results indicate the use of cotton swabs as a more suitable technique to remove microorganisms than the immersion in sterile Ringer.

Table 3. Comparison between the use of cotton swabs and immersion in sterile Ringer for microorganisms' extraction (quantification after 1 and 7 days).

The hypothesis was that more microorganisms would be obtained when immersing them in sterile Ringer, since all the ones present inside the material, in the pores, would be then also in contact with the solution. However, the results showed lower amounts, which may lead to two different conclusions. Either no more microorganisms are extracted by immersion, or the survival of the microorganisms is lower when using that method.

3.2. Identification of microorganisms

Environmental samples

Table 4 shows the bacterial and fungal genera identified from the environmental samples of Barcelona city, the Natural Park of Montseny and Ghent city. The most remarkable difference observed in the environmental samples of Ghent was the identification of the yeast *Saccharomyces sp.*, which is common in that region (Martens et al., 1997).

Table 4. Bacterial and fungal genera identified from environmental samples.

The results presented above correspond to a unique sampling and provide an idea about the microorganisms presents in each environment at that moment. In general, bacteria are usually less present in the atmosphere than fungi and they are usually associated with other particles. Concentration as well as variability of aerobacteria change between day and night and also between seasons. According to Tong and Lighthart (1997), pigmented bacteria are more numerous during the day due to their solar radiation protection. Furthermore, they also state that Gram positive bacteria with sporulation capability decrease during the night and Gram negative increase while the opposite occurs during the day (Lighthart and Shaffer, 1995). Concerning to spreading of fungi, sporulation and spore dispersal depend on biological, climatic and physical processes (Hjelmroos, 1993). According to McCartney and Lacey (1991), rain and wind are the most important carriers geographically.

Bacteria on specimens

Regarding the bacteria identified on the specimens' surface, a genus of bacteria, which was not found on environmental samples was identified, *Streptococcus*. Table 5 shows the most representative genera of bacteria isolated from all specimens. According to previous hypothesis, the horizontal position was the more suitable since gravity favours deposition of bacteria, spores, organic matter, and others. Furthermore, climate conditions such as rain and incident wind will affect also the maintenance of the organisms on the surface. Previous hypotheses also consider that wind would provoke the detachment of the most surficial organisms on vertical specimens while rain would do it mainly to the ones on horizontal specimens, considering a higher impact of the raindrops on the specimens' surface.

Table 5. Isolated bacteria classified per bioreceptivity level, inclination and location of the specimens.

The highest biodiversity of bacteria was found for specimens placed horizontally in Barcelona city. In addition, the genus *Bacillus* was identified from all the samples, which was expected from the results of the environmental samples. When bacteria like *Bacillus* are present in the environment, it is normal to find cells everywhere due to their specific characteristics. The aforementioned genus has the capability to form endospores, which are remarkably resistant to heat, desiccation, radiation and chemical attack. Sporulation is usually induced by nutrient starvation although it is not an immediate process. Different responses can happen before such as the activation of the flagellar motility to search for new food sources, production of antibiotics in order to destroy competitors and others. In fact, sporulation is their last survival attempt (Stephens, 1998). SEM images are presented in Figure 2.

Figure 2. Detail of Pa40-1C surface specimen with bacteria (b), which may belong to genus *Aerococcus* (a) and *Bacillus* (b).

Fungi on specimens

Regarding the identification of fungal growth from the specimens' surface, three more genera of fungi (*Epicoccum*, *Gilmaniella* and *Mucor*) were identified. Table 6 shows the most representative genera of fungi isolated from all specimens. One of the most interesting results obtained was the majoritarian presence of yeast on specimens placed in Ghent when comparing them with the results from the other two locations. The aforementioned results agreed with the environmental samples detailed above. Moreover, a higher biodiversity was observed on specimens placed in Ghent than in the other two locations. SEM images are presented in Figure 3.

Table 6. Isolated fungi classified per bioreceptivity level, inclination and location of the specimens.

Figure 3. Detail of fungal hyphae (with arrow) on Pa40-1C (a) and Ma20-0.75C (b,c) specimens.

3.3. Quantification of microorganisms

Bacteria

Figure 4 and 5 show the colony-forming units (CFUs) of bacteria isolated from the horizontal and vertical surface of the specimens, respectively. Both graphs present the distribution of CFUs comparing the different bioreceptivity designs and locations by means of box plots analysis.

Figure 4. Box plot of bacterial quantification for horizontal specimens.

The higher level of biodiversity for bacteria was found in Barcelona city and quantification results show also a higher amount of bacteria for the specimens placed horizontally in that location. The lowest amount of bacteria was found on specimens placed in Ghent. Regarding MPC specimens, differences between the three different bioreceptivity designs were not evident as Figure 3 shows, especially for specimens placed in Barcelona city. However, OPC specimens showed differences between the different designs although the most bioreceptive design could not be established.

However, results obtained for specimens placed vertically did not present the same trend (see Figure 5). In that case, the city of Ghent was the location in which specimens, especially OPC, presented the higher amount of bacteria. Moreover, the comparison between results obtained for specimens placed in Barcelona city and Montseny shows higher amount of bacteria for the first location. This fact corresponds with the expectations since bacterial presence is lower in rural areas than in cities. Concentration of aerobacteria in forests is estimated between 385 and 1200 CFUs/m³, while for urban areas the values are between 540 and 7200 CFUs/m³ (Jones and Cookson, 1983; Laine et al., 1999).

Figure 5. Box plot of bacterial quantification for horizontal specimens.

Accordingly, Figure 6 shows data corresponding to the air quality for the city of Barcelona and the Natural Park of Montseny. When comparing the data corresponding to the air quality between these two locations, the difference is clear. Barcelona city registered higher amounts of all pollutants (SO₂, NO, NO₂, NO_x and CO) and lower amounts of ozone (O₃).

Figure 6. Air quality of Barcelona city (a,b) and Natural Park of Montseny (c,d).

Atmospheric pollution levels also affect the colonisation and development of organisms (Nuhoglu et al., 2006). Sulphur dioxide (SO₂) is a gaseous component, which is one of the major constituents of polluted atmospheres in urban areas. Sulphur dioxide forms sulphuric acid due to oxidation, which favours the formation of gypsum on concrete surfaces. During the process of crystallisation, different particles present in the environment are accumulated on the surface and provide a good substratum to microorganisms' colonisation and development (Saiz-Jimenez, 1997). Furthermore, dry deposition of nitrogen oxides (NO_x) also promotes the oxidation of the sulphur dioxide (Johanson et al., 1988). The above is applicable to OPC specimens although not to MPC since the chemical composition is completely different. Concerning NO_x, Mancinelli and McKay (Mancinelli and McKay, 1983) state that NO has a bacteriostatic effect, although NO₂ decreases the effect of air pollution on MPC.

In Barcelona, NO_x levels ranged from 27 µg/m³ to 65.4 µg/m³ with a monthly average of 46.2 µg/m³. Furthermore, recorded NO₂ levels were significantly higher than NO levels and low SO₂ levels were obtained. In contrast, lower values than 5 µg/m³ of NO_x and SO₂ were recorded in the Natural Park of Montseny. Therefore, that fact may stimulate a higher colonisation and development on specimens in Barcelona city. Figure 7 illustrates plenty of particles attached on the surface specimens by means of SEM images. Based on previous works (Nuhoglu, 2006), all those particles may be consequence of the environmental pollutants.

Figure 7. Surface particles (white arrows) on Ma20-0.75C specimen due to air pollution of Barcelona city.

Additionally, clear differences when comparing OPC and MPC specimens were only observed for the specimens placed in Ghent city. In that case, specimens produced with OPC presented the higher amount of bacteria, suggesting the pH of carbonated OPC to be more suitable for bacteria in this environment. Differences in

quantification due to the physical properties of the specimens such as superficial roughness and porosity did not show a uniform trend for all locations.

Fungi

Figures 8 and 9 shows the colony-forming units of fungi isolated from the surface of the horizontal and vertical specimens, respectively, represented in box plots. A higher number of CFUs for specimens placed in the city of Ghent was observed, according to the location of the highest biodiversity of fungi. That fact is visible for OPC specimens, where the quantity is significantly higher, and not for the MPC ones. This may be consequence of the great presence of yeasts in the environment as mentioned in the subsection 3.1. However, according to the results related to the bacterial growth, it was not possible to state differences between specimens for the same location, with some exceptions (MPC specimens placed in Ghent city and specimens indicated with black arrows). Then, differences between the different locations was evident although it was not possible to determine the most suitable design.

Figure 8. Box plot of fungal quantification for horizontal specimens.

In general, most fungal genera grow in environments with pH between 5 and 7 (Azmi and Seppelt, 1997). Consequently, a higher fungal growth in MPC specimens than in OPC ones was expected. Then, although MPC has a more suitable pH for those organisms, the presence of bacteria may have caused a local drop in the pH producing a suitable substrate for the yeasts (Cole, 1982).

Furthermore, genera *Acremonium*, *Alternaria* and *Cladosporium* were identified on the majority of the specimens. According to Onions and Brandy (1987), *Acremonium* can grow in a wide range of pH, pH 6 being the optimum and growing better at a pH 9 than a pH 3. However, the morphology of the mycelium is different going from branched mycelial clumps to unbranched filaments at pH 6 and 9 respectively. Genus *Alternaria* has also an optimal growth pH between 6 and 7. The genus *Phoma* was only identified on MPC specimens and that fact may be due to the optimal growth pH of that genus, which is between 3 and 6, making MPC specimens more suitable as a support than OPC specimens.

Figure 9 shows results corresponding to the fungal quantification of the vertical specimens. According to results of the horizontal specimens, the highest colonisation was observed on OPC specimens placed in Ghent city. Moreover, less CFUs were quantified on the vertical specimens as seen in the results related to bacterial growth. Unfortunately, combination of horizontal and vertical results for both, bacterial and fungal growth, do not provide homogeneous results.

Figure 9. Box plot of fungal quantification for vertical specimens.

Finally, weather conditions are important to better understand the aforementioned results. Figure 10 shows the available data corresponding to all three locations for the period of the study. Although other weather conditions such as wind direction, wind speed or sunshine duration may be interesting, in this study only temperature and precipitation were analysed. Identification of the most representative genus did not reveal the presence of photosynthetic organisms on the specimens, which indicated

that it was not necessary to analyse the photosynthetic active radiation (PAR). Moreover, specimens were oriented to the north in order to get the most suitable conditions for the growth.

Figure 10. Monthly average temperature and cumulative precipitation for the locations of Barcelona city (a), Natural Park of Montseny (b) and Ghent city (c).

Comparing the monthly average temperature between Barcelona city and the Natural Park of Montseny, the data are pretty similar although slightly lower for the second location. The aforementioned lower temperatures were mainly consequence of the different altitudes of the locations (175 m versus 732 m). A one-year cycle was accomplished for all three locations although the setup was installed at different moments for the Ghent city location than for the other two locations. Consequently, maximum temperatures corresponding to the summer periods do not correspond to the same point in the graphs. In general, temperature for the location of Ghent city was lower than for the Spanish locations.

Related to the precipitation data, higher precipitation was recorded in Ghent followed by the Natural Park of Montseny. In contrast, the city of Barcelona recorded lower values than for the other locations. Rain and low-speed wind favour the deposition of aerial particles (microorganisms, dust, and others). Moreover, high relative humidity provides moisture, which stimulates the growth of microorganisms. Then, temperatures and sunshine will also affect the growth of the microorganisms. For instance, growth of *Alternaria* is favoured by low sunshine intensity, which would suggest a higher growth of that genus on vertical specimens (with the inconvenience of the inclination) or in locations with low sunshine intensity and duration.

4. Conclusions

Regarding predominant microorganisms, a lower diversity of bacteria was found on specimens placed in the Natural Park of Montseny than in Barcelona city. Slight differences were found in terms of biodiversity since the genera found for both locations were similar. Moreover, a lower presence of microorganisms on specimens placed vertically was noticed in all locations. In contrast, the city of Ghent presented a higher biodiversity as well as amount of fungi than the other two locations. That fact is mainly due to the high presence of yeasts in that environment.

Data corresponding to the air quality as well as the weather conditions in terms of precipitation and average temperatures contribute to justify the results obtained in the experimental program. Moreover, the results corresponding to the city of Ghent, in which environmental conditions favour the biological growth, showed higher amounts of microorganisms on OPC specimens. Based on the results obtained in the accelerated algal test from a previous work (Manoso et al., 2014b), those results correspond to the expected ones. In that previous work, MPC specimens presented the better properties to allow algal growth, specifically for *Chlorella vulgaris*.

The aforementioned hypothesis correspond to the fact that some bacteria can inhibit the growth of algae by producing algicidal compounds (Berger et al., 1979; Cole, 1982; Afi et al., 1996; Fukami et al., 1997) and some algae such as *Chlorella vulgaris* produce antibiotics. Chlorellin is the name of the compound produced by *Chlorella*

vulgaris, which is effective against some species of bacteria including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis* and *Pseudomonas aeruginosa* (Cole, 1982; Pratt et al., 1944). Consequently, under environmental conditions, the suitability of a material to enhance natural colonisation as well as the succession of the colonising organisms will depend on the environment.

Finally, the suitability of the use of cotton swabs was verified for the removal of the microorganisms to study the composition and quantifying them. However, other comparisons between methodologies may be interesting.

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Table 1. Specimens' composition.

Specimens	Composition	Compressive strength (MPa) (n = 6)	Porosity (%)	Roughness R_a (μm) (n = 12)	pH
Pa40-1C	Sand 0/2 mm a:c:w ¹ = 4.41:1:0.4	15.83 $\pm$ 0.7	22.97	0.03 $\pm$ 0.00	$\approx$ 9
Pa60-1.75C	Sand 0/2 mm a:c:w ¹ = 3.22:1:0.6	46.81 $\pm$ 1.1	12.27	0.03 $\pm$ 0.01	
PA30-1C	Sand 2/4 mm a:c:w ¹ = 3.8:1:0.3	27.52 $\pm$ 2.0	10.60	0.16 $\pm$ 0.02	
Ma20-0.75C	Sand 0/2 mm a:c:w ² = 4.81:1:0.2	9.82 $\pm$ 0.1	18.20	0.04 $\pm$ 0.00	6.7
Ma28-1C	Sand 0/2 mm a:c:w ² = 4.03:1: 0.28	24.45 $\pm$ 1.4	2.47	0.06 $\pm$ 0.00	
MA15-0.5C	Sand 2/4 mm a:c:w ² = 6.6:1:0.15	9.18 $\pm$ 0.8	13.15	0.15 $\pm$ 0.01	

¹CEM I 52.5R; ² Cement made by NH₄H₂PO₄, MgO and borax. NH₄H₂PO₄:MgO ratio = 1:1.75 and the addition of borax amounted to 6 % by weight of the sum of NH₄H₂PO₄ and MgO weights; a:c:w is the ratio aggregates:cement:water; n is the number of replicates. Labels of specimens make reference to the binder (P: OPC; M: MPC), aggregate size (a: 0/2 mm; A: 2/4 mm), w/c ratio (e.g.: 20 means w/c = 0.2 and 60 0.6), cement paste content (factor multiplying the minimum amount of cement paste, C, needed to join all the aggregate particles) (Klein et al., 2012).

Table 2. Culture media and incubation regimes.

Microorganisms	Culture media	Incubation regime
Aerobic bacteria	Tryptic Soy Agar (TSA)	25°C – 24 to 48 hours
Anaerobic bacteria	Tryptone Sulfite Neomycin Agar (TSN Agar)	42°C – 24 to 48 hours
	Sulfite Polymixin Sulfadiazine Agar (SPS Agar)	37°C – 24 to 48 hours
Enterobacteriaceae	MacConkey Agar	37°C – 24 to 48 hours
Fungi	Sabouraud Agar with antibiotic	25°C – 3 to 4 days

Table 3. Comparison between the use of cotton swabs and immersion in sterile Ringer for microorganisms' extraction (quantification after 1 and 7 days).

Specimen	1 day quantification		7 days quantification	
	Cotton swabs	Immersed in sterile Ringer	Cotton swabs	Immersed in sterile Ringer
Pa40-1C	62	Non-detected	$> 10^6$	Non-detected
Pa60-1.75C	$> 10^6$	$> 10^6$	952	80
PA30-1C	264	$> 10^6$	$> 10^6$	Non-detected
Ma20-0.75C	154	$> 10^6$	$> 10^6$	4
Ma28-1C	52	Non-detected	$> 10^6$	Non-detected
MA15-0.5C	648	53	68	74

Table 4. Bacterial and fungal genera identified from environmental samples.

Bacteria	Fungi
<i>Aerococcus</i> (<i>Aerococcus</i> sp.)	<i>Acremonium</i> (<i>Acremonium strictum</i>)
<i>Bacillus</i> (mainly <i>B. subtilis</i>)	<i>Alternaria</i> (<i>A. alternate</i>)
<i>Flavobacterium</i> (<i>Flavobacterium</i> sp.) ¹	<i>Aspergillus</i> (mainly <i>A. flavus</i>)
<i>Kocuria</i> (<i>K. kristinae</i> , <i>K. lutea</i> , <i>K. rhizophilum</i>)	<i>Aureobasidium</i> (<i>A. pullulans</i>)
	<i>Cladosporium</i> (<i>C. herbarum</i>)
	<i>Epicoccum</i> (<i>Epicoccum</i> sp.) ²
	<i>Fusarium</i> (<i>F. monoliforme</i>)
	<i>Penicillium</i> (<i>Penicillium</i> sp.)
	<i>Phoma</i> (<i>P. herbarum</i>)
	<i>Rhizopus</i> (<i>Rhizopus</i> sp.)
	<i>Rhodotorula</i> (<i>R. glutinis</i>)
	<i>Saccharomyces</i> (<i>Saccharomyces</i> sp.) ³

¹Not found in the Natural Park of Montseny sample; ²Only found in the Natural Park of Montseny sample; ³Only found in Ghent city.

Table 5. Isolated bacteria classified per bioreceptivity level, inclination and location of the specimens.

Specimen	Horizontal			Vertical		
	Barcelona city	Montseny	Ghent city	Barcelona city	Montseny	Ghent city
Pa40-1C	<i>Aerococcus</i>	<i>Bacillus</i>	<i>Bacillus</i>	<i>Bacillus</i>	<i>Bacillus</i>	<i>Bacillus</i>
	<i>Bacillus</i>	<i>Kocuria</i>	<i>Koccuria</i>		<i>Kocuria</i>	<i>Kocuria</i>
	<i>Kocuria</i>		<i>Streptococcus</i>			<i>Streptococcus</i>
Pa60-1.75C	<i>Aerococcus</i>	<i>Bacillus</i>	<i>Bacillus</i>	<i>Aerococcus</i>	<i>Aerococcus</i>	<i>Bacillus</i>
	<i>Bacillus</i>	<i>Flavobacterium</i>	<i>Koccuria</i>	<i>Kocuria</i>	<i>Bacillus</i>	<i>Kocuria</i>
	<i>Flavobacterium</i>	<i>Kocuria</i>	<i>Streptococcus</i>		<i>Kocuria</i>	<i>Streptococcus</i>
PA30-1C	<i>Aerococcus</i>	<i>Aerococcus</i>	<i>Bacillus</i>	<i>Aerococcus</i>	<i>Aerococcus</i>	<i>Bacillus</i>
	<i>Bacillus</i>	<i>Bacillus</i>	<i>Kocuria</i>	<i>Kocuria</i>	<i>Bacillus</i>	<i>Kocuria</i>
	<i>Flavobacterium</i>	<i>Flavobacterium</i>	<i>Streptococcus</i>			<i>Streptococcus</i>
Ma20-0.75C	<i>Aerococcus</i>	<i>Aerococcus</i>	<i>Bacillus</i>	<i>Aerococcus</i>	<i>Bacillus</i>	
	<i>Bacillus</i>	<i>Bacillus</i>	<i>Streptococcus</i>	<i>Bacillus</i>		-
	<i>Flavobacterium</i>	<i>Kocuria</i>		<i>Kocuria</i>		
Ma28-1C	<i>Aerococcus</i>	<i>Bacillus</i>	<i>Bacillus</i>	<i>Aerococcus</i>	<i>Aerococcus</i>	<i>Streptococcus</i>
	<i>Bacillus</i>	<i>Kocuria</i>	<i>Koccuria</i>	<i>Bacillus</i>		
	<i>Kocuria</i>		<i>Streptococcus</i>	<i>Kocuria</i>		
MA15-0.5C	<i>Aerococcus</i>	<i>Bacillus</i>	<i>Bacillus</i>	<i>Bacillus</i>	<i>Aerococcus</i>	<i>Bacillus</i>
	<i>Bacillus</i>	<i>Kocuria</i>	<i>Koccuria</i>	<i>Kocuria</i>	<i>Bacillus</i>	
	<i>Kocuria</i>		<i>Streptococcus</i>		<i>Kocuria</i>	
	<i>Streptococcus</i>					

Table 6. Isolated fungi classified per bioreceptivity level, inclination and location of the specimens.

Specimen	Horizontal			Vertical		
	Barcelona city	Montseny	Ghent city	Barcelona city	Montseny	Ghent city
Pa40-1C	Acremonium	Cladosporium	Alternaria	Alternaria	Aureobasidium	Alternaria
	Alternaria	Epicoccum	Cladosporium	Cladosporium	Fusarium	Cladosporium
	Cladosporium		Fusarium			Fusarium
			Rhodotorula			Rhodotorula
Pa60-1.75C	Acremonium	Acremonium	Alternaria	Acremonium	Alternaria	Alternaria
	Alternaria	Alternaria	Cladosporium	Alternaria	Aureobasidium	Cladosporium
	Cladosporium	Cladosporium	Fusarium	Cladosporium	Cladosporium	Fusarium
			Rhodotorula	Phoma	Phoma	Rhodotorula
PA30-1C	Acremonium	Acremonium	Alternaria	Cladosporium	Acremonium	Alternaria
	Cladosporium	Alternaria	Cladosporium	Mucor	Cladosporium	Cladosporium
	Epicoccum	Cladosporium	Fusarium	Phoma	Rhodotorula	Rhodotorula
			Rhodotorula			Saccharomyces
Ma20-0.75C	Acremonium	Alternaria	Fusarium	Acremonium	Alternaria	Alternaria
	Alternaria	Cladosporium	Saccharomyces	Cladosporium	Fusarium	Cladosporium
	Cladosporium	Fusarium				Fusarium
	Gilmaniella	Penicillium				Saccharomyces
Ma28-1C	Alternaria	Alternaria	Alternaria	Acremonium	Aureobasidium	Cladosporium
	Cladosporium	Fusarium	Cladosporium	Alternaria	Cladosporium	Fusarium
	Phoma		Mucor	Cladosporium		
				Phoma		
MA15-0.5C	Alternaria	Alternaria	Cladosporium		Aureobasidium	Alternaria
	Cladosporium	Aspergillus	Fusarium		Cladosporium	Cladosporium
	Phoma	Aureobasidium	Saccharomyces	-	Phoma	
		Cladosporium				
		Penicillium				
		Rhodotorula				

Figure 1

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Figure 2

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Figure 4
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Figure 4. Box plot of bacterial quantification for horizontal specimens.

Figure 5
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Figure 5. Box plot of bacterial quantification for horizontal specimens.

Figure 6. Air quality of Barcelona city (a,b) and Natural Park of Montseny (c,d).

Figure 7
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Figure 8
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Figure 8. Box plot of fungal quantification for horizontal specimens.

Figure 9
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Figure 9. Box plot of fungal quantification for vertical specimens.

Figure 10. Monthly average temperature and cumulative precipitation for the locations of Barcelona city (a), Natural Park of Montseny (b) and Ghent city (c).