Bioreceptivity optimisation of concrete substratum to stimulate biological colonisation

DOCTORAL THESIS

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A mis padres, Rafael e Isabel,

a mi hermano, Rafa, y mi cuñada, Carolina,

os quiero.

A la memoria de Luís Agulló Fité.

"Life is not easy for any of us. But what of that? We must have perseverance and above all confidence in ourselves. We must believe that we are gifted for something and that this thing must be attained"

"La vida no es fácil, para ninguno de nosotros. Pero... ¿y qué? Hay que perseverar y, sobre todo, tener confianza en uno mismo. Hay que sentirse dotado para realizar algo y que ese algo debe alcanzarse"

Marie Curie (1867-1934).

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SUMMARY

The lack of green areas into cities is caused by the increase in population and the urbanisation process. However, an increasing interest in city greenery was observed since the beginning of twentieth century. Several benefits are associated to green areas from a social, economic and ecologic point of view. Consequently, the solution to increase the urban green spaces lies in their inclusion on structures surfaces. In that sense, a great number of different technologies were developed grouped into green walls and green roofs. Unfortunately, existent systems for both green walls and green roofs present several disadvantages in terms of installation and maintenance costs, low integration with the structure, extra loads, limitations in their use in rehabilitation, and others.

The general objective of this dissertation is to provide a first approach to the possibility of using a structure surface as biological substratum. This was treated considering two different subjects or research lines. The first research line is the modification of the chemical and physical properties of the cementitious material, which will be used as substratum. Then, the second issue is the evaluation of materials' bioreceptivity under both laboratory and environmental conditions.

Regarding the material, chemical properties were firstly studied. pH was taken as a priority from the diversity of involved chemical properties. In that sense, two different ways to obtain a low pH cementitious material were studied. First, the reduction of the pH of the most common hydraulic binder, Ordinary Portland Cement (OPC), was attempted. Then, the characterisation of Magnesium Phosphate Cement (MPC) as a hydraulic binder of naturally low pH was carried out. Insufficient reductions in pH of OPC were obtained and properties such as flexural and compressive strengths were highly affected. In contrast, positive results were obtained regarding the use of MPC as hydraulic binder for the specific purpose to be used as a biological substratum.

Regarding the physical properties, porosity and roughness were main properties considered. For that purpose, modifications of the dosage of different samples were carried out by means of modifying the hydraulic binder, granular skeleton, the water to cement ratios and the amount of cement paste. The methodology used for the estimation of the cement paste content worked well for OPC specimens although MPC responded differently. The characterisation of the twenty-three initial materials' bioreceptivities provided significant results. Consequently, six different mix designs were selected to be exposed to colonisation.

Afterwards, the evaluation of the materials' bioreceptivity in terms of colonisation was studied under both laboratory and environmental conditions. Regarding the experimental program under laboratory conditions, an accelerated laboratory test was carried out to evaluate the behaviour of different specimens when they were exposed to colonisation. Magnesium Phosphate Cement specimens obtained better results than OPC mortars for algal colonisation under those particular conditions.

Finally, specimens were also evaluated under environmental conditions since conditions significantly differ from what happens under laboratory conditions. Furthermore, two different inclinations and three different locations were studied. Significant differences were observed between inclinations (horizontal and vertical) and between locations with different contamination levels (Barcelona city, Natural Park of Montseny and Ghent city). Horizontal specimens obtained better results in terms of predominant genus diversity as well as quantification. Moreover, specimens located in urban areas (Barcelona city) obtained also better results. However, results showed that further research should be considered for environmental experimental programs.

RESUMEN

La ausencia de espacios verdes en las ciudades es consecuencia del constante incremento poblacional y el proceso de urbanización. Sin embargo, existe un interés social creciente en aumentarlos desde inicios del siglo XX. Los beneficios asociados pueden englobarse en tres niveles: social, económico y ecológico. Por ello, la incorporación de elementos vegetales en los edificios se ha visto, principalmente, como una alternativa en aquellas ciudades con escasez de espacios disponibles para la construcción de áreas verdes. En este sentido, diversos sistemas han sido desarrollados tanto a nivel de fachadas como de cubiertas vegetales. Desafortunadamente, los sistemas existentes presentan diversos inconvenientes, los cuales se refieren a los costes de instalación y mantenimiento, a la baja integración entre los elementos naturales y la estructura, la carga adicional que conlleva y, en algunos casos, la limitación en su uso, entre otros.

El objetivo general de la presente tesis es demostrar que es posible utilizar el propio material cementicio como soporte biológico, para lo cual se han considerado dos líneas principales: modificar de forma controlada el material cementicio en base a sus propiedades químicas y físicas y, evaluar la bioreceptividad del soporte en muestras colonizadas.

En lo referente al material, primero se abordaron las propiedades químicas, fijándose como prioritario reducir el pH, para lo cual se abordaron dos vías: incorporación de ácidos en morteros en base a cemento Portland y, empleo de cementos en base a fosfato de magnesio. Las reducciones de pH alcanzadas para el cemento Portland no fueron significativas y sí, en cambio, la afectación a nivel de resistencias fue elevada. Por contra, los resultados obtenidos para el cemento en base a fosfato de magnesio fueron positivos, sin efectos secundarios.

Respecto a las propiedades físicas, se han considerado la porosidad y la rugosidad. Para la modificación controlada de estas propiedades se realizaron cambios entre dosificaciones en cuanto al tipo de cemento, al esqueleto granular, a la relación agua-cemento y a la cantidad de pasta de cemento. La metodología usada para la estimación de la cantidad de pasta de cemento funcionó correctamente para el mortero de cemento Portland. Sin embargo, el mortero en base a cemento de fosfato de magnesio presentó particularidades que hicieron cambiar los criterios de selección. Veintitrés tipos de mortero de diferente bioreceptividad fueron caracterizados y los resultados obtenidos permitieron reducir dicho número a seis, los cuales fueron expuestos a colonización.

En el proceso de evaluación del crecimiento biológico, las muestras fueron expuestas tanto a condiciones controladas de laboratorio, mediante un ensayo acelerado, como a condiciones ambientales, habiéndose obtenido los mejores resultados para las muestras en base al cemento de fosfato de magnesio.

Finalmente, las muestras fueron expuestas a colonización ambiental, ya que los resultados podrían diferir considerablemente. Además de evaluarse los diferentes grados de

bioreceptividad de las muestras entre sí, también se evaluó la influencia de la inclinación (horizontal y vertical) así como de la localización comparando tres emplazamientos (Barcelona ciudad, parque natural del Montseny y Gante ciudad). Diferencias significativas fueron observadas entre muestras con diferente inclinación así como entre las localizaciones con diferente grado de contaminación ambiental. Las muestras horizontales presentaron mejores resultados a nivel de diversidad y cuantificación. Y, además, las muestras colocadas en zonas urbanas obtuvieron mejores resultados que aquellas en emplazadas en una zona no contaminada. Sin embargo, los resultados muestran la necesidad de más investigación en condiciones ambientales así como por periodos más prolongados.

SAMENVATTING

Door de constante groei van de wereldbevolking en de toenemende urbanisatie, is een gebrek ontstaan aan groene ruimten in steden. Sinds het begin van de twintigste eeuw is er wel een toenemende interesse in de aanleg van stedelijk groen. Het incorporeren van groen in de stad brengt vele positieve effecten met zich mee, zowel vanuit socio-economisch als vanuit milieuperspectief. In vele Europese steden doen zich milieuproblemen voor, bijvoorbeeld op vlak van luchtkwaliteit, waterverbruik en energieverbruik. Daarom zijn inspanningen noodzakelijk om meer duurzame steden te bekomen, die alle sociale, ecologische en economische noden kunnen vervullen.

Initieel was de belangrijkste reden om levende organismen, voornamelijk planten, te introduceren in steden, de esthetische waarde. Nochtans hebben verschillende auteurs intussen de vele bijkomende voordelen geduid, zoals op vlak van menselijke gezondheid, reductie van CO_2-concentraties, zuurstofproductie, zuivering van lucht en water, geluidsreductie, en economische voordelen voor zowel de stad als de stedelingen. De stedelijke natuur vormt een sleutelelement voor de leefbaarheid en duurzaamheid van de stad.

Het concept van het “vergroenen” van de stad bracht ook het concept van het vergroenen van gebouwen met zich mee, vooral in steden waar de beschikbare ruimte voor aanleg van groene ruimten schaars is. Dit houdt in dat men meer groen in de stad bekomt door dit toe te voegen aan een gebouwoppervlak. Verschillende technieken werden ontwikkeld om vegetatie aan te brengen op de gebouwschil, zoals groene wanden en groene daken. Een groen dak, ook wel levend dak genoemd, is een conventioneel dak bedekt met vegetatie. Een groene wand bestaat uit een façade van een gebouw met vegetatie die direct op het oppervlak groeit, of op een afzonderlijke structurele eenheid die vrijstaand nabij de muur of bevestigd aan de muur kan zijn.

Helaas zijn er verschillende nadelen verbonden aan zowel groene muren als groene daken, op vlak van installatie- en onderhoudskosten, een laag niveau van integratie met de structuur, bijkomende belasting, beperkingen voor de aanwending bij renovatieprojecten, enzovoort. Enkel de installatiekosten van groene daken variëren reeds van 70 tot 220 €/m² en voor groene wanden van 30 tot 1200 €/m².

Anderzijds wordt ook soms een ongewenste biologische kolonisatie vastgesteld op constructies vervaardigd met cementgebonden materialen, zoals monumenten, historische gebouwen, of gewoon oudere gebouwen. Deze kolonisatie is het gevolg van drie geconnecteerde factoren, namelijk de aanwezigheid van pioniersorganismen in de omgeving, de omgevingsomstandigheden en de eigenschappen van het materiaal. De term bioreceptiviteit verwijst naar de laatste factor, namelijk de ontvankelijkheid van het materiaal voor kolonisatie door levende organismen. De studie van biodeterioratie vormt een belangrijk gevestigd onderzoeksdomein, waarin men tracht om de negatieve effecten van biologische groei op bouwmaterialen te definiëren en mechanismen om de biologische vervuiling tegen te gaan te

formuleren. De grens tussen biodeterioratie en bioprotectie is soms vaag, en deze kan bovendien verschuiven door wijzigingen in omgevingsomstandigheden, substraat en koloniserende organismen.

In dit doctoraat wordt getracht een antwoord te formuleren op sommige van de aangehaalde problemen. Het objectief van dit onderzoek was om een eerste mogelijkheid aan te brengen om een gebouwoppervlak te gebruiken als biologisch substraat. Hiertoe werden twee verschillende onderzoeksfasen gedefinieerd. Vooreerst werd getracht de chemische en fysische eigenschappen van het steenachtig materiaal, dat gebruikt zal worden als substraat, te modificeren. Vervolgens werd de bioreceptiviteit van het ontwikkelde materiaal geëvalueerd zowel via een versnelde proef in het laboratorium als door blootstelling aan een buitenomgeving. Het uitgevoerde werk combineert hierbij theoretische en experimentele componenten.

In de eerste fase van het onderzoek werden in twee stappen de chemische en nadien de fysische eigenschappen van het materiaal aangepast. Ondanks de talloze parameters die kunnen gewijzigd worden bij het ontwerp van een cementgebonden materiaal als biologisch substraat, werd prioritair de pH gewijzigd. Hierbij werden twee strategieën gevolgd. Enerzijds werd getracht om de pH van het meest gebruikte hydraulische bindmiddel, Portlandcement, te reduceren. Anderzijds werd een ander hydraulisch bindmiddel met een natuurlijke lage pH, magnesiumfosfaatcement, gekarakteriseerd.

Om een pH-reductie bij beton met Portlandcement te bekomen, werden zuren toegevoegd aan het mengsel. De pH van de verse en verharde mengsels, de gewichtsvariaties, de buigtrek- en druksterkte, de transmissiesnelheid van ultrasoongolven, en de chemische en mineralogische samenstelling met behulp van FTIR en XRD werden bepaald. Er werd echter een te beperkte reductie van de pH bekomen en eigenschappen zoals buigtrek- en druksterkte werden sterk beïnvloed. Wat betreft de aanwending van magnesiumfosfaatcement als bindmiddel voor biologische substraten, werden echter wel beloftevolle resultaten bekomen.

Wat betreft de modificatie van de fysische eigenschappen, werden porositeit en ruwheid als de belangrijkste parameters aanzien. De samenstelling van het beton werd aangepast om proefstukken te produceren met verschillende niveaus van porositeit en ruwheid. De microporositeit werd gewijzigd door verschillende water/cementverhoudingen toe te passen en de macroporositeit werd gevarieerd door in te spelen op de granulaatgrootte en het cementpastavolume. Naast de bepaling van de bekomen porositeit en ruwheid – als maatstaf voor de bioreceptiviteit – werden ook buigtrek- en druksterkte bepaald, en werden calorimetrie en thermogravimetrische analyse toegepast om de materialen verder te karakteriseren.

De methodologie die werd aangewend voor de inschatting van de optimale hoeveelheid cementpasta, functioneerde goed voor Portlandcement proefstukken, maar de monsters met magnesiumfosfaatcement reageerden anders. Voor het verdere onderzoek werd het aantal

samenstellingen gereduceerd van 23 naar 6, om enkel proefstukken met significant verschillende eigenschappen bloot te stellen aan biologische kolonisatie.

Vervolgens werd de bioreceptiviteit van de ontwikkelde materialen bestudeerd, zowel in het laboratorium als in buitenomgeving. In het laboratorium werd een versnelde proef uitgevoerd, waarbij de omstandigheden voor kolonisatie door algen optimaal waren. Het is gekend dat levende organismen in een dergelijke versnelde proef niet op dezelfde wijze reageren als in hun natuurlijke omgeving, vooral door het verschil in klimatologische condities en door de competitie tussen soorten. Deze test had echter als doel om het gedrag van proefstukken met verschillende eigenschappen onder gecontroleerde omstandigheden te vergelijken. Bovendien konden zo resultaten bekomen worden in een relatief korte tijdspanne.

De proefstukken met zes verschillende niveaus van bioreceptiviteit, werden beoordeeld via colorimetrische analyse, beeldverwerking en biomassa kwantificatie. De eerste twee technieken verstrekten informatie over de intensiteit van de biofouling, de bedekte oppervlakte en andere parameters zoals de helderheid en kleur van het (gekoloniseerde) materiaal. De biomassametingen werden uitgevoerd met behulp van een niet-destructieve test waarbij gebruik gemaakt werd van PAM fluorometrie. Bij deze laboratoriumproef bleek dat proefstukken gemaakt met magnesiumfosfaatcement duidelijk sneller gekoloniseerd werden door algen dan proefstukken met Portlandcement.

Tenslotte werden de proefstukken ook geëvalueerd in buitenomgeving, waarbij de omstandigheden significant kunnen verschillen van de condities bij het laboratoriumexperiment. Hierbij verschillen niet enkel de proefstukken onderling, maar ook de omgevingsomstandigheden en aspecten die betrekking hebben op de levende organismen. Twee verschillende opstellingen van de proefstukken (horizontale en verticale positie) en drie verschillende locaties werden onderzocht (het stadscentrum van Barcelona, het natuurgebied van Montseny nabij Barcelona en de stadsrand van Gent). Voor de verschillende proefstukken en locaties werd het predominante genus van de koloniserende micro-organismen vastgesteld en de aanwezige micro-organismen werden gekwantificeerd in termen van aantal kolonievormende eenheden. Dit werd in verband gebracht met data betreffende weersomstandigheden en luchtkwaliteit voor de verschillende locaties. Er werden significante verschillen in kolonisatie vastgesteld tussen proefstukken met horizontale en verticale opstelling en tussen proefstukken op de drie locaties die gekenmerkt worden door een duidelijk verschillende contaminatie. Hierbij waren het de horizontaal opgestelde proefstukken die een grotere diversiteit aan dominante genera vertoonden en ook kwantitatief gezien een sterkere kolonisatie. Verder vertoonden de proefstukken opgesteld in stedelijk gebied (centrum van Barcelona) duidelijk betere resultaten qua kolonisatie. Het werd echter ook duidelijk dat het testprogramma in buitenomgeving langer zal moeten opgevolgd worden om tot sluitende conclusies te komen.

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SIMBOLS

a*	Green-red light component
A_{Agg}	Aggregate area (m²)
$Agg_{\%}$	Percentage of aggregate retained in a mesh (%)
$Agg_{max\%}$	Percentage of aggregate retained in a mesh of the bigger diameter considered (%)
b*	Blue-yellow light component
$B_4O_7^{2-}$	Borate
$B(OH)^-_4$	Tetrahydroxiborate
C	Estimated amount of cement paste considered as the reference value
$Ca[B(OH)_4]_2$	Calcium diborate
$CaC_2O_4{\cdot}H_2O$	Calcium oxalate
$CaCO_3$	Calcium carbonate
CaO	Calcium oxide
$Ca(OH)_2$	Calcium hydroxide (portlandite)
CO	Carbon monoxide
CO_2	Carbon dioxide
$(COOH)_2{\cdot}2H_2O$	Oxalic acid
$C_2O_4^{2-}$	Oxalate
C_2S	Dicalcium silicate (belite)
C_3S	Tricalcium silicate (alite)
D	Pore diameter (m)
DF	Weight of a dried filter (mg)
DFA	Weight of a dried filter with the filtrated algae after being dried at 105° C until constant mass (mg)
DW	Dry weight (mg/L or mg/cm²)
F_m	Maximum fluorescence intensity
F_0	Minimum fluorescence intensity
F_v	Difference between the maximum and the minimum fluorescence intensity

F_v/F_m	Maximum quantum efficiency of photosystem II (PS II)
FI	Fouling intensity
G	Gain
g	Gravity (m/s²)
h	Heigh (m)
$HC_2O_4^-$	Hydrogen oxalate ion
$H_2C_2O_4$	Oxalic acid
H_2O	Water
H_3O^+	Hydronium ion
H_3BO_3	Boric acid
H_2PO_4	Dihydrogen phosphate ion
HPO_4	Hydrogen phosphate ion
L*	Lightness factor
Log DI	Differential intrusion logarithm
m_{Agg}	Aggregate mass (kg)
$Mg_3(PO_4)_2{\cdot}4H_2O$	Bobierrite
$MgHPO_4{\cdot}3H_2O$	Newberyite
M_n	Value of the mesh n (correspond to the maximum particle size not retained in the mesh)
M_{SiO2}	Amount of SiO_2 (mole)
ML	Measuring light intensity
N	Nomber of the piston stroke
NH_4^+	Ammonium ion
$NH_4H_2PO_4$	Ammonium Dihydrogen Phosphate
$NH_4MgPO_4{\cdot}H_2O$	Dittmarite
$NH_4MgPO_4{\cdot}6H_2O$	Struvite
$(NH_4)_2Mg(HPO_4)_2{\cdot}4H_2O$	Schertelite
$(NH_4)_2Mg_3(HPO_4)_4{\cdot}8H_2O$	Hannayite
$Na_2B_4O_7{\cdot}10H_2O$	Borax
NO	Nitrogen monoxide
NO_2	Nitrogen dioxide

NO_x	Nitrogen oxides
O_2	Oxigen
O_3	Ozone
P	Preassure (Pa)
PM_{10}	Particulate matter of diameters ranged from 2.5 to 10 µm
PO_4^{2-}	Phosphate ion
r_a	Average radius (mm)
R	Reflectance
R_a	Centre-line roughness (mm)
R_q	Root mean square roughness (mm)
R_2O_3	The sum of aluminium oxide (Al_2O_3) and iron oxide (Fe_2O_3)
SA_{Agg}	Surface area of the aggregates (m^2)
SiO_2	Silicon dioxide
SO_2	Sulfur dioxide
SO_3	Sulfur trioxide
SSA	Specific surface area (m^2/kg)
t_e	Equivalent thickness (m)
Ta	Total area (m^3)
T_{max}	Maximum temperature (° C)
V	Volume of the mould (m^3)
V_{Agg}	Aggregate volume (m^3)
V_{cp}	Volume of the cement paste (m^3)
V_s	Volum of the specimen (m^3)
W	Piston weight (kg)
W_{cp}	Weight of cement paste (g)
W_{ds}	Weight of the dry specimen (kg)
W_h	Hydrostatic weight (kg)
W_i	Initial weight (g)
W_{is}	Immersed weight (g)
W_{sds}	Saturated specimen with dry surface (g)
Y	Quantum yield

Δa^*	Green-red light colour difference
Δb^*	Blue-yellow light colour difference
ΔC^*	Chromatic variations
ΔE^*	Total colour difference
ΔH^*	Changes in hue
ΔL^*	Lightness difference
Δt	Time differential
ΔT	Temperature differential
ρ_{Agg}	Aggregate density (kg/m³)
ρ_f	Density of the fluid (kg/m³)
ε_c	Compaction energy (kg·cm/cm³)
γ	Surface tension of mercury (N/m)
Ø	Pore diameter
α-β SiO_2	Transformation of siliceous aggregates, which occurs at around 575° C
ϑ	Contact angle between mercury and the solid surface

ABBREVIATIONS

a:c:w	Aggregates:cement:water ratio
ADP	Ammonium Dihydrogen Phosphate
AF	Calcium sulfoaluminate hydrates (Alumina, ferric oxide, mono-sulfate)
Aft	Calcium sulfoaluminate hydrates (Alumina, ferric oxide, tri-sulfate)
BA	Boric acid
CCAP	Culture Collection of Algae and Protozoa
CFUs	Colony Forming Units
Chl a	Chlorophyll a
CIELab	Colour space defined by the Commission Internationale de l'Eclairage
CSH	Calcium silicate hydrate gel
DTA-TG	Differential thermal analysis and thermogravimetry
FTIR	Fourier Transform Infrared Spectroscopy
MgO	Magnesium oxide, periclase
MIP	Mercury Intrusion Porosimetry
MPC	Magnesium Phosphate Cement
OA	Oxalic acid
ocw	Over cement ratio
OPC	Ordinary Portland Cement
P:M	ADP to MgO ratio
PAM	Pulse Amplitude Modulation
PCR	Polymerase Chain Reaction
PET	Polyethylene terephthalate
RH	Relative humidity
SEM	Scanning Electron Microscopy
SPS	Sulfite Polymixin Sulfadiazine Agar
TSA	Tryptic Soy Agar
TSN	Tryptic Sulfite Neomycin Agar
WHO	World Health Organisation
w/c	water to cement ratio
XRD	X-Ray Diffraction

GLOSSARY

Autotroph: An organism capable of making nutritive organic molecules from inorganic sources via photosynthesis (involving light energy) or chemosynthesis (involving chemical energy).

Biodegradation: A process by which microbial organisms transform or alter (through metabolic or enzymatic action) the structure of chemicals introduced into the environment.

Biodeterioration: Any undesirable change in the properties of a material caused by the vital activities of living organisms.

Biodiversity: The number and variety of living organisms found within a specified geographic region or location.

Biofilm: Aggregate of bacteria held together by a mucuslike matrix of carbohydrate that adheres to a surface. Biofilms can form on the surfaces of liquids, solids and living tissues.

Biofouling: The accumulation of living organisms on wetted surfaces.

Biomass: The total amount of living material in a given habitat, population, or sample. Specific measures of biomass are generally expressed in dry weight (after removal of all water from the sample) per unit area of land or unit volume of water.

Biophilia: Innate tendency to focus on life and lifelike processes.

Bioprotection: Active or passive microbially induced or mediated consolidation, cleaning, and/or protection of stone materials affected by chemical, physical, and/or biological weathering phenomena.

Bioreceptivity: The aptitude of a material (or any other inanimate object) to be colonised by one or several groups of living organisms without necessarily undergoing any biodeterioration.

Chlorophyll: The green pigment found in the chloroplasts of higher plants and in cells of photosynthetic microorganisms, which is primarily involved in absorbing light energy for photosynthesis.

Colonisation: The action or process of setting up colonies of living organisms.

Colony forming units (CFUs): A measure of viable cells in which a colony represents an aggregate of cells derived from a single progenitor cell.

Counting chamber: a device for counting microscopic objects suspended in fluid, as cells and platelets in dilute whole blood or bacteria in broth culture. It consists of a microscope slide containing a shallow cavity of uniform depth the floor of which is ruled with a grid and which, when closed with a cover glass, holds a precise volume of fluid.

Dry weight: The dry matter of a sample or of an object when completely dried. It is a reliable measure of the biomass.

Enzimatic process: Any chemical reaction or series of reactions catalysed by enzymes, which are usually proteins with large complex molecules whose action depends on their particular

molecular shape. Some enzymes control reactions within cells and some, such as the enzymes involved in digestion, outside them.

Erlenmeyer: Type of laboratory flask which features a flat bottom, a conical body and a cylindrical neck.

Evapotranspiration: Loss of water by evaporation from the soil and transpiration from plants.

Heterotroph: An organisms that is unable to synthesize its own organic carbon-based compounds from inorganic sources, hence, feeds on organic matter produced by, or available in, other organisms.

Inoculate: Introduce cells or organisms into a culture medium.

Isopolar: Pollen grains have identical proximal and distal poles.

Pellet: The material concentrated at the bottom of a centrifuge tube after centrifugation.

Petri dishes: A shallow circular dish with a loose-fitting cover, used to culture bacteria or other microorganisms.

Photosynthetic electron transport chain: A group of compounds that pass electron from one to another via redox reactions coupled with the transfer of proton across a membrane to create a proton gradient that drive ATP synthesis.

Photosystem: A multisubunit complex found mainly in the thylakoid membranes of plants and algae, and in the cytoplasmic membranes of photosynthetic bacteria. It is primarily involved in capturing light to cause a series of redox reactions.

Plate count: An estimate of the number of viable cells in a culture. A plate count of a bacterial culture is made by inoculating the culture plate with a dilute solution of the microorganisms and counting the number of cells that appear in the resultant culture.

Polymerase Chain Reaction (PCR): A system for in vitro amplification of DNA.

Spore: A dormant, reproductive cell formed by certain organisms. It is thick-walled and highly resistant to survive under unfavourable conditions so that when conditions revert to being suitable it gives rise to a new individual.

Sporulation: Process of forming spores.

Stomata: Plural of stoma, which refers to any of various small apertures, especially one of the minute orifices or slits in the epidermis of leaves, stems, etc., through which gases are exchanged.

Sunshine duration: Time that direct radiation is higher than 120 W/m^2.

Taxonomy: The classification of organisms in a hierarchical system or in taxonomic ranks (e.g. kingdom, phylum or division, class, genus, specie) based on shared characteristics or on phylogenetic relationships inferred from the fossil record or established by genetic analysis.

Tricolpate: Pollen grain with a combination of colpus and porus per triplicate.

1. INTRODUCTION

1.1. SCOPE OF THE RESEARCH

The lack of green areas into cities is caused by the constant increase in population and the urbanisation process. In that sense, the United Nations (2009) stated the world population is supposed to increase from 6.7 billion in 2007 to 9.2 billion in 2050. Moreover, the aforementioned increase correspond to an increase of the urban population with 3.1 billion and a decrease of the rural population with 0.6 billion between 2007 and 2050. In total, the world urban population in 2050 is expected to be twice as large as in 2007. However, the rate of growth of is expected to decrease from 1.8 % for the period 2007-2025 to lower than 1.3 % for the period 2025-2050.

In 1920 less than 30 % of the developed countries population was living in urban areas although the percentage increased to more than 50 % in 1950. In 2007 more than 80 % of the Australian, New Zealand and North American population were living in urban areas, which is expected to increase until 90 % in 2050. Accordingly, the urban population in Europe is predicted to increase from 72 % in 2007 to 83 % in 2050. Consequently, the continued increase of urban population combined with the pronounced deceleration of rural population growth lead to a sustained urbanisation process as is shown in Figure 1.1. In that figure, real data regarding the period comprised between 1950 and 2007 as well as expected values until 2050 are provided.

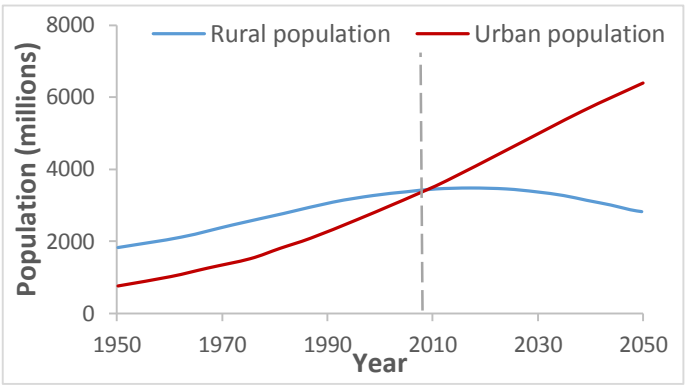

Figure 1.1.- Urban and rural populations in the world for the period comprised between 1950 and 2050 (adapted from United Nations, 2009)

However, the growth of the urban population is associated with a modification of the urban landscape (McDonnell et al., 1997; Paul and Meyer, 2001). According to Czech et al. (2000) and McKinney (2002), urban development has a high responsibility for local extinction rates and loss of native species and has more influence on the environment than any other human activity. Grimm et al. (2008) stated cities are hot spots that drive environmental change to multiple scales. Those changes refer to five main points: land-use, biogeochemical cycles, climate change, hydrologic systems and biodiversity.

According to the World Health Organisation (WHO) and other organisations, one of the parameters involved in the society's quality of life are the green areas surface per habitant (Priego, 2011). The minimum value accepted by the WHO is 10 m² of green areas per habitant and that value is not achieved in a high number of European cities. Figure 1.2 shows information regarding the percentage of green areas of some European cities and the surface of green spaces in the entire country per person. Points, which corresponds to cities, are coloured according to the percentage of urban green spaces within the city and countries are coloured according to the surface of green area per capita. As the figure shows, southern and eastern countries recorded the lowest surfaces of green areas per capita. However, it is also remarkable that the global situation of the country does not necessarily fit with the situation of its main cities.

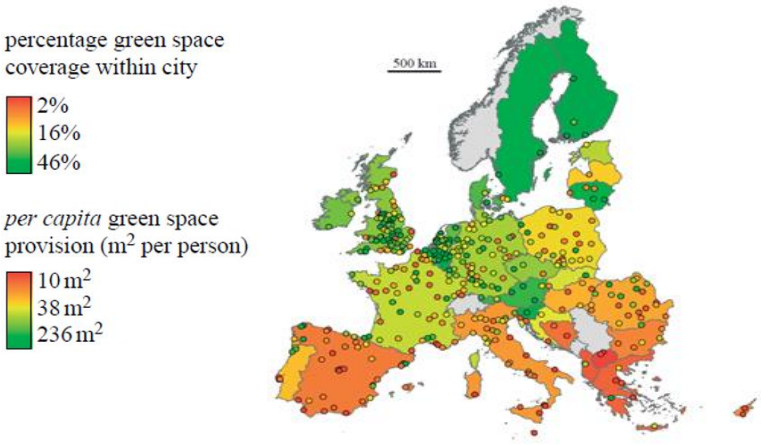

Figure 1.2.- Urban green spaces in Europe. (Fuller and Gaston, 2009).

Additionally, Figure 1.3 shows the evolution of those urban green areas for two different periods: 1990-2000 and 2000-2006. According to the authors, an increase of urban green spaces was recorded for the Western and Southern cities in the second period. In contrast, Eastern cities recorded a negative trend characterised by the increase in red dots in Figure 1.3. For instance, taking into account the recommendations given by the WHO in which a city should have a green space of 10 to 15 m² per person, only 15 Spanish provincial capitals from 50 comply with it (OSE, 2009).

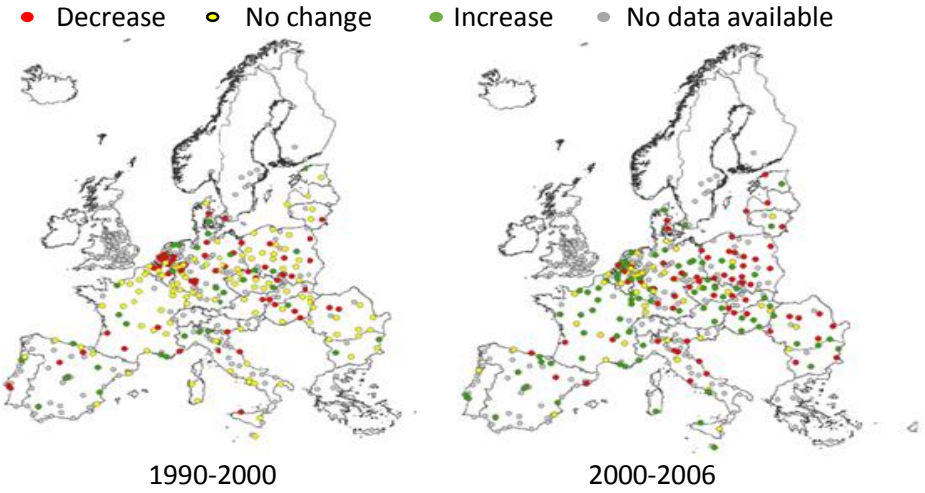

Figure 1.3.- Evolution of urban green areas in Europe (adapted from Kabisch and Haase, 2013).

Kabisch and Haase (2013) analysed the development of urban green spaces of 202 European cities and studied the correlation between density of population, the total city area and the urban green spaces for the period between 2000 and 2006. Accordingly, Figure 1.4 shows the absence of correlation between the urban green space per capita and the density of population. However, that figure also gives information about the amount of European cities that do not achieve the minimum area of urban green spaces recommended by the WHO.

Consequently, the solution to increase the urban green spaces lies in their inclusion on structures surfaces. In that sense, a great number of different technologies were developed to include vegetation as a building envelope such as green walls and green roofs. However, systems developed until now, present several inconveniences in terms of installation and maintenance costs, low integration with the structure, extra loads, limitations in their use in rehabilitation, and others. Therefore, the current research proposes a new concept of vegetal envelope in which some of the aforementioned limitations would be successfully overcome.

1.2. MOTIVATIONS

Incorporation of urban green elements in cities to improve the environmental situation as well as human health has been increasingly demanded. In that sense, the greenery of the structures' envelope is drawing the attention of both society and industry. Developed systems address the issue in two different ways: extern systems such as green walls and green roofs and

intern ones such as porous panel concretes. However, construction industry is searching for innovative solutions to overcome existing limitations of those systems.

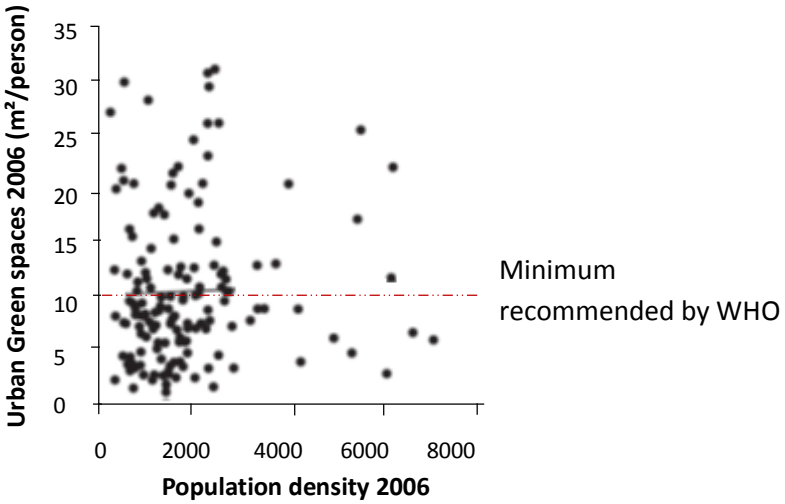

Figure 1.4.- Urban green spaces per capita of European cities in 2006 (adapted from Kabish and Haase, 2013).

The most recent systems, which correspond to the second system mentioned above, assimilate some of the drawbacks of previous developments. However, several aspects still need to be further studied and completely different perspectives should be considered. Within the remaining problems, the ones considered as more important are the low level of integration between the biological growth and the stone material, the limitation on the application system and the maintenance costs. Therefore, the proposal of an innovative solution is considered as essential to move forward. The motivation here presented are discussed in detail in Chapter 2.

1.3. GENERAL OBJECTIVE

This PhD thesis aims to provide answers to some of the problems indicated in the previous section. Taking that into account, the general objective of this dissertation is to provide a first approach to the possibility of using a structure surface as biological substratum. The objective was treated considering two different subjects or research lines. The first research line is the modification of the chemical and physical properties of the stone material, which will be used as substratum. Then, the second issue is the evaluation of biological colonisation in both laboratory and environmental conditions.

1.4. SPECIFIC OBJECTIVES

In order to accomplish the aforementioned general objective, several specific objectives were proposed. The main ones are presented in Table 1.1.

Table 1.1.- Specific objectives.

Subject	Specific objectives
Cementitious material	Evaluate the decrease of the pH of a conventional hydraulic binder by using additions. Evaluate magnesium phosphate cements to be used as biological substratum in construction. Propose a cement formulation to obtain a stone material with a pH near the neutrality to be used as biological substratum in construction. Define a dosage criterion to produce different controlled bioreceptivities of the mortars. Characterise different mix designs and select different mortar types.
Biological growth	Evaluate different bioreceptivities of the mortars exposed to colonisation by pioneer microorganisms under both laboratory and environmental conditions. Assess the aptness of different methodologies to evaluate biofouling. Define the most suitable mixture or mixtures for pioneer's colonisation in terms of time, amount and homogeneity of growth. Assess differences in colonisation due to specimens' inclination and locations. Determine the necessity of extra treatments to further accelerate the colonisation.

1.5. METHODOLOGY

The work here presented combines both theoretical and experimental components and has been developed in two Universities: Polytechnical University of Catalonia and Ghent University. The experimental programs mix different approaches both active and passive and result in this thesis, which is structures as detailed below.

The thesis is subdivided in six parts as shown in Figure 1.5. The current chapter, **Chapter 1**, corresponds with a brief scope of the research in which the topic is presented. Moreover, the objectives to be achieved in the current research are also shown.

A brief description of the state of the art is presented in **Chapter 2**. This chapter was the base of all works conducted and aims to establish the motivation in a global context starting with the environmental problems caused by urbanisation. Moreover, a brief analysis of some of the positive and negative interactions between living organisms and constructions is provided, since incorporation of green areas in buildings is considered as one of the solutions for the preservation of natural areas in cities.

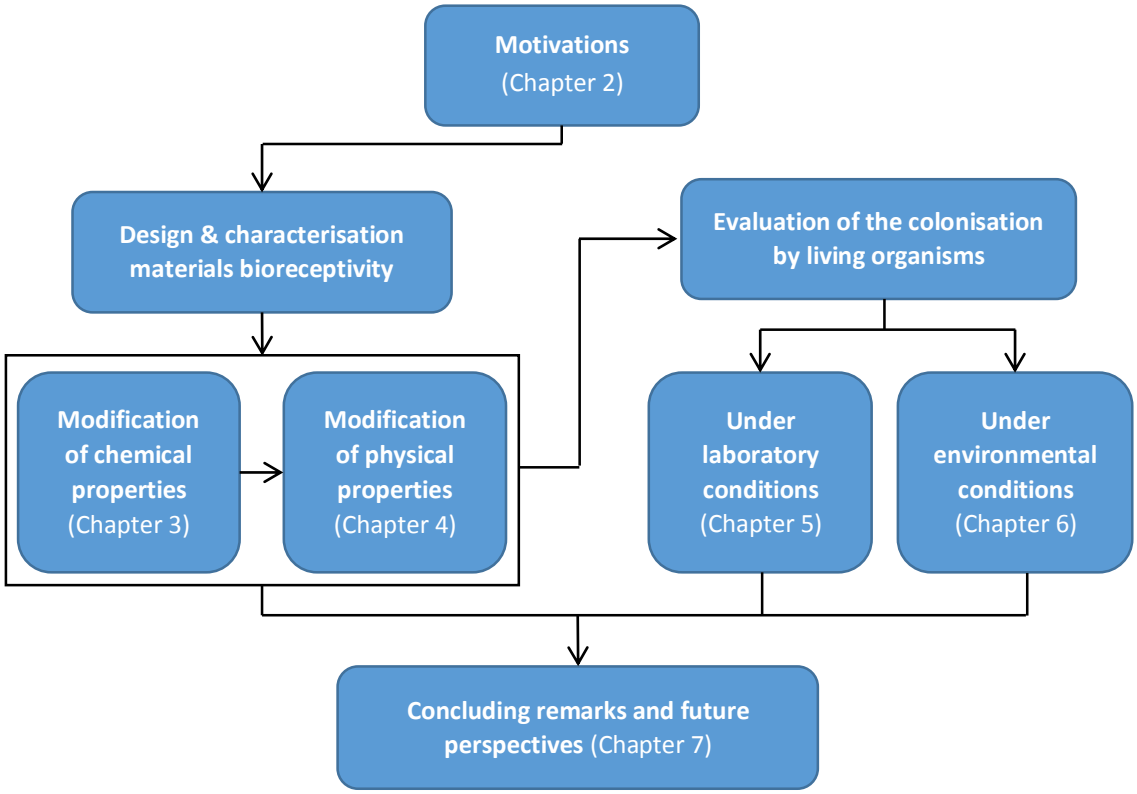

Figure 1.5.- Outline of the thesis.

Chapter 3 corresponds to the first experimental program, which main purpose is the modification of the chemical properties of a cementitious material in order to obtain a low pH substratum. Two different ways were evaluated: first, use of additions in Ordinary Portland Cement mixtures and characterization of different formulations of Magnesium Phosphate Cement.

In **Chapter 4**, the second experimental program regarding the modification of the physical properties of the mortar is carried out. In that chapter, a dosage criterion is stipulated in order to produce specimens with a certain controlled roughness and porosity. In that sense modifications of the type of hydraulic binder, the granular skeleton, the water to cement ratios and the amount of cement paste were made.

After the development of materials with different bioreceptivity, in **Chapter 5** an evaluation of the colonisation of the specimens under laboratory conditions was achieved. Methodologies already used for biofouling of stone materials analyses were used and the evaluation consisted in both qualitative and quantitative analysis by means of non-destructive tests.

Chapter 6 aims to show the significant differences of results related to test conditions. Consequently, specimens' exposition to environmental conditions was carried out in order to

analyse the colonisation depending on the location and the weather conditions. In that sense, preliminary studies for a period of less than a year are presented although the necessity of thorough experimental programs for longer periods of time is clear.

Finally, **Chapter 7** describes the conclusions of each of the subjects addressed in the thesis and presents the future perspectives of the research.

2. STATE OF THE ART

2.1. INTRODUCTION

An increasing interest in city greenery was observed since the beginning of twentieth century. Biophilia is the term defined by Wilson (1984), which refers to the "*innate tendency to focus on life and lifelike processes*". According to the author, both environmental and genetic components are involved in the positive reaction humans have towards living organisms. Related to that fact, the incorporation of nature into cities is increasingly popular although environmental benefits should be also taken into account. Consequently, the main reason to include living organisms, mainly plants, initially was to provide an aesthetical value. Johnston and Newton (2004) state life is more pleasant when green elements are added, understanding green elements as plants and other greenery components.

This fact was evident since earlier times, when incorporation of climbing plants was considered as providing an added aesthetical value (Laurie, 1977) and the first environmental reaction to industrialisation (Kaltenbach, 2008). Moreover, European cities have been constantly growing as the United Nations stated in 2002 and 2009. They estimated that the proportion of people living in urban areas between 2000 and 2050 would rise from 46.6 % to 69.6 % with the consequent problems in environmental pollution, heat island effects and climate change. The above results in a general lack of green areas inside cities, such as parks and gardens. Consequently, building greenery is also an attempt to overcome this situation.

During the past years, perception of urban greenery has been changing. As mentioned before, the aesthetical value has been clear from the start of the urban greenery trend. However, presence of natural elements in cities has effect at three different levels such as the social, the ecologic and the economic levels (Heidt and Neef, 2008). Those causes a change in professionals' interest, which encourages them to use sustainability criteria in the design of construction elements (Pérez, 2011).

Regarding the social effect, health benefits have been widely described by several authors (Ulrich, 1993; Dilani, 2001; Nielsen and Hansen, 2007; Lee and Maheswaran, 2011). In general, different researches provide information regarding both physical and psychological benefits linked to presence of green areas. Among them, Pretty et al. (2003) stated green areas provide a natural space for the sports practice, which may stimulate physical activity contributing to different benefits such as better cardio- and cerebra-vascular state, resistance against depression, and others. Furthermore, Stigsdotter et al. (2010) stated green spaces positively affect stress and quality of life.

The ecological benefits of green spaces are mainly the absorption of CO_2 and production of oxygen due to photosynthetic organisms (Jo, 2002), purification of the local air and water, regulation of the microclimate and reduction of noise (Bolund and Hunhammar, 1999) as well as maintenance of the biodiversity (Attwell, 2000). Finally, from an economic point of view, general benefits are reported such as reduction in costs related to improvement of local areas. For instance, the decrease of air pollution would lead to economic benefits for both cities and citizens. The above may lead to reduced costs of measures to reduce pollution or prevention actions (Chiesura, 2004).

Afterwards, the concept of greening cities involved also the concept of greening buildings, especially in cities in which available spaces for green areas construction are scarce. Ecological engineering was born as an applied and interdisciplinary science to integrate human activities and the environment (Ottelé, 2011). According to the general benefits presented above, incorporation of green living elements in the exterior of buildings also implies benefits such as energy conservation or the provision of a more aesthetical environment (Getter and Rowe, 2006).

This chapter aims to review the current situation and necessity of integrating nature into the cities and more specifically the application on structures envelopes. In that sense, both positive and negative interactions between living organisms and structures is reviewed. Moreover, issues that still require improvement are identified. Hence, this state of the art pursues two main goals: to identify the motivations for the thesis and briefly cover literature concerning the subjects that will be addressed in this dissertation.

2.2. ENVIRONMENTAL SITUATION OF THE MAJOR EUROPEAN CITIES

The Urban Ecosystem Europe report (2007) analysed 32 European cities, considering the main socio-economic data. The air quality, wastewater, water consumption, green areas, the use of public transport, energy, waste production and waste management were considered in that report, which put in evidence the main problems of those European cities (Figure 2.1). The analysis was carried out considering the values to be reached in 2010.

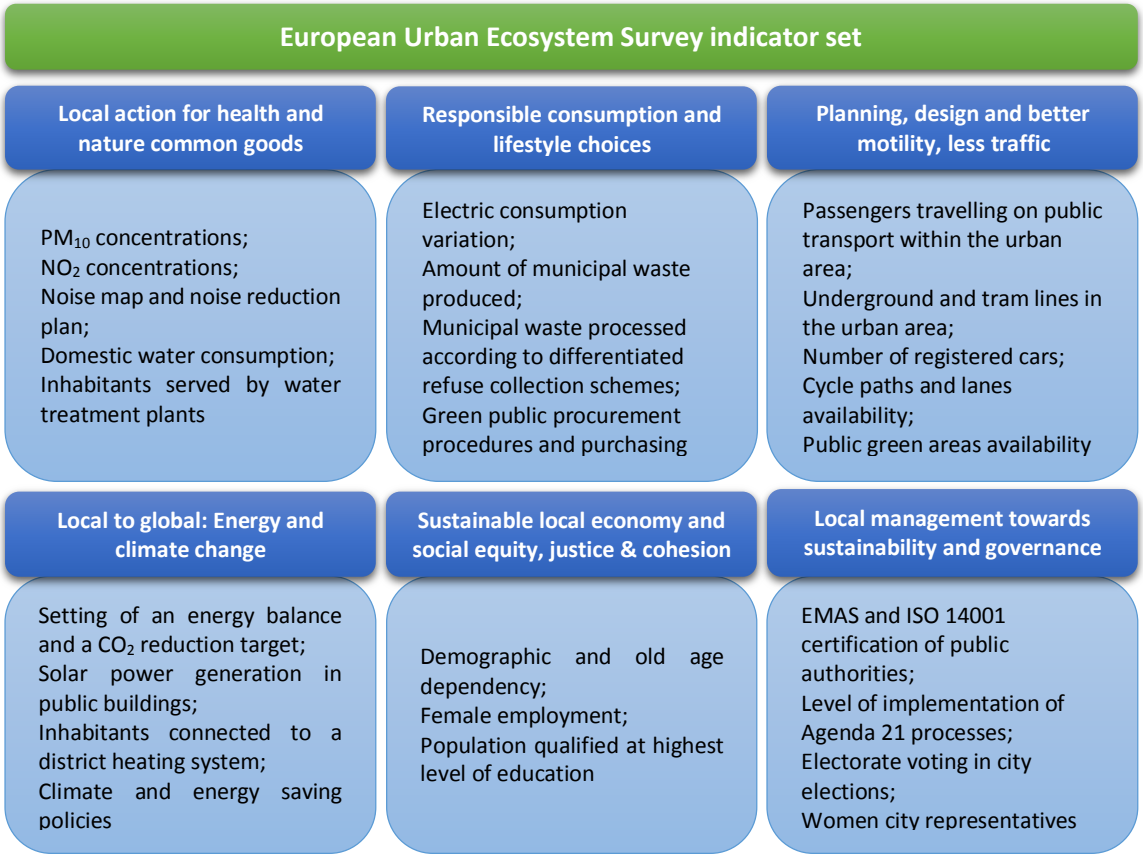

Figure 2.1.- The indicator set used in the Urban Ecosystem Europe report 2007.

Regarding air quality, at least 45 % of the analysed cities exceeded once per year the limit value of 40 µg/m³ of particulate matter of diameters ranged from 2.5 to 10 µm (PM_{10}). The situation was more critical in big cities, since 65 % of them exceeded the limit in the district with denser traffic. Regarding intense pollution episodes, the maximum number of days per year allowed for exceeding the limit of daily concentrations of 50 mg/m³ was fixed at 35, and 84 % of the studied cities exceed it. Concerning to NO_2 values 90 % of the cities were far from the limit of 40 µg/m³ and 60 % does not respect the limit of 2005 of 50 µg/m³.

According to the Ambiente Italia Research Institute (2007), more than 95 % of the cities exceeded the waste production of 400 kg/year and some of those exceeded the 700 kg/year. However, some administrations were reducing those amounts thanks to good recycling programs. Administrations respect the Kyoto Protocol and prove of that fact is the concern they

have for CO_2 emissions. Furthermore, some cities such as Barcelona, Hannover, Munich, Oslo or Rome defined highly innovative local regulation to improve energy efficiency and central and southern cities also increased the use of solar panels.

Later, the Green City Index quantified and compared environmental performance of 120 cities around the world considering thirty indicators. On one hand, pollution is usually initially associated with the economic development. Regarding infrastructures, high costs are commonly associated with high-quality green infrastructures, which only can be afforded by wealthy governments. Consequently, the economic resources are one of the most important factors to consider. However, there are other aspects that should be considered such as historical attitudes, culture or local available resources.

The thirty indicators are grouped in 8 main groups, covering the following topics: CO_2, energy, buildings, transport, waste and land use, water, air quality and environmental governance. Figure 2.2 shows a detailed scheme of all the indexes considered, which are composed of either 16 quantitative and 14 qualitative indexes.

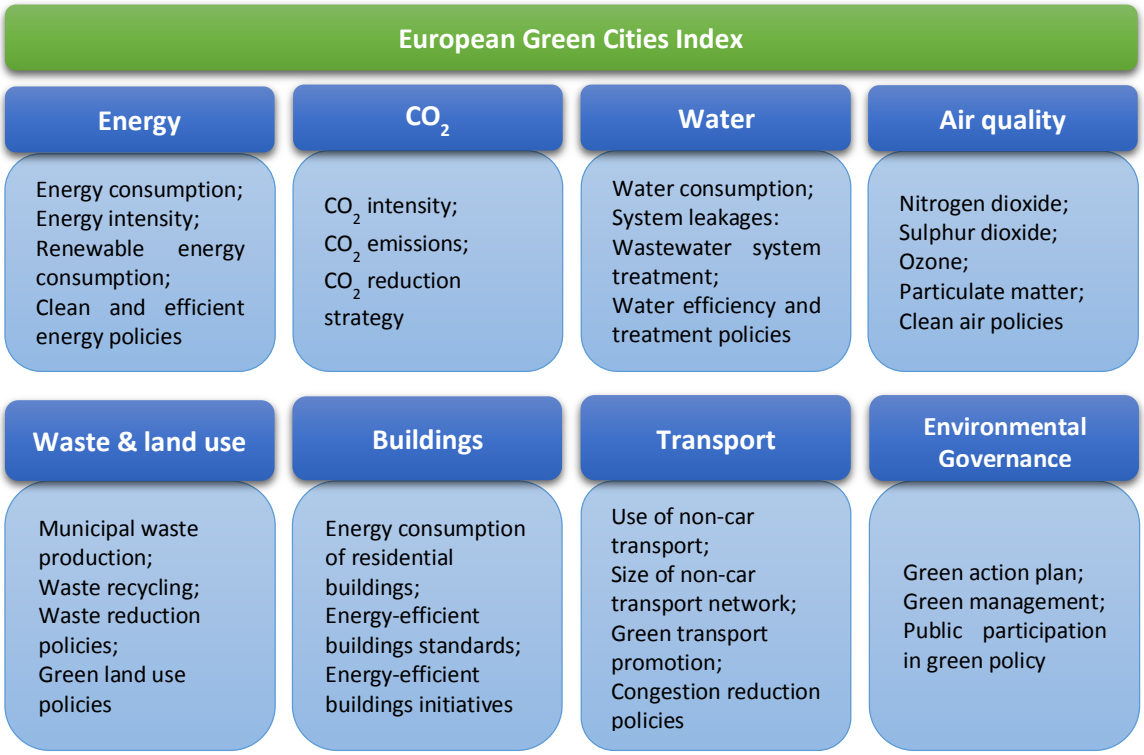

Figure 2.2.- Indexes considered for the European Green City Index (Economist Intelligence Unit, 2012).

Focusing the analysis on European cities, 8 of the 30 cities analysed by the Economist Intelligence Unit were selected as examples. Those cities are Amsterdam (AMS), Athens (ATH), Berlin (BER), Brussels (BRU), London (LON), Madrid (MAD), Paris (PAR), and Rome (ROM). Then, from all the indicators shown in Figure 2.2, only data concerning to CO_2 emissions, energy

consumption, renewable energy consumption, water consumption and share of waste recycled, were considered (Table 2.1).

Table 2.1.- Data for some indicators affecting the Green Cities Index corresponding to 2009

	Population (millions)	O_2 emissions (t/person)	Energy consumption (GJ/person)	Renewable energy consumption (%)	Annual water consumption (m^3/person)	Share of waste recycled (%)
AMS	0.74	6.66	74.51	5.80	53.47	43.00
ATH	3.40	5.92	88.77	2.66	106.88	10.00
BER	3.40	6.57	77.70	1.84	55.55	35.00
BRU	1.05	3.91	86.88	0.58	54.04	23.68
LON	7.60	5.84	77.96	1.20	57.59	20.00
MAD	6.10	4.08	80.28	2.78	71.37	9.88
PAR	11.70	5.04	96.65	2.30	109.50	19.00
ROM	4.00	3.50	84.57	18.69	87.03	19.50

According to the inquiry of 2009, the aforementioned cities correspond to the 5th, 22nd, 8th, 9th, 11th, 12th, 10th and 14th positions in the general ranking, respectively. However, there is a constant concern to improve the environmental quality of cities. Some of the indicators in terms of energy, CO_2, water, air quality and buildings could be improved by means of incorporation of more green areas into the cities. In that sense, values of CO_2 emissions may be reduced due to its absorption by plants and all photosynthetic organisms during the photosynthesis (Hess, 1980; Salisbury and Ross, 1992; Lodish et al., 2000). CO_2 emissions in Europe are around 5.2 t/person as an average, which is significantly lower than the average for USA and Canada (14.5 t/person). However, this value is higher than the average in Asia, which is 4.6 t/person.

Environmental policies are becoming more and more important and a consequent increase in research is observed (Hawkes, 1995; Rotmans et al., 2000; Song, 2011). Moreover, PM_{10} values could also be reduced by greens areas thanks to the retention of aerial particles by the aerial parts of plants (Little, 1977; Smith and Staskawicz, 1977; Hosker and Lindberg, 1982).

Consequently, all the efforts are addressed to obtain more sustainable cities fulfilling all social, ecological and economic necessities. In that sense, Chiesura (2004) stated urban nature is a key ingredient for city sustainability. Kenworthy (2006) stated eco-cities should consider, include and protect natural areas inside and around cities. Furthermore, Song (2011) claimed that human survival and development have been predatory for lots of years although the situation is changing. In that sense, nature is present in cities with both positive and negatives effect as is presented below.

2.3. POSITIVE INTERACTIONS BETWEEN LIVING ORGANISMS AND STRUCTURES

Unfortunately, the urbanisation process makes difficult to increase nature into cities in terms of parks, gardens and others. Then, an increasing interest on the greenery of structures was observed, which is considered as a desirable interaction. Vegetation is the main group of living organisms traditionally incorporated into buildings and other structures. A general classification of the systems that allows the incorporation of those living organisms is based on the plane on which vegetation is applied. In this sense, different systems were developed for the incorporation of vegetation horizontally, which corresponds to the green roofs, and vertically, the green walls. A brief introduction of both typologies is provided below.

A **green roof** is a conventional roof covered with vegetation, being also known as living roof. It is difficult to establish the origin although some authors consider the Hanging Gardens of Babylon the common origin for green roofs and walls (Dunnett and Kingsbury, 2004). Different examples of green roofs can be found from the Middle Ages (Grant, 2006), which is the case of the gardens of Mont Saint Michele (abbey from the 8th century, where a garden was constructed in the upper part in the 13th century). Another example may be found in Italy, where the Guinigi Tower in Lucca supports some trees from the specie *Quercus ilex* (Figure 2.3 (a), Dunnett and Kingsbury, 2004). A more recent example is located in Austria, installed during the 1950s. The Hundertwasser of Vienna consists of a stepped frontage, where the roof of a house was the garden of the upper house (Figure 2.3 (b), Dunnett and Kingsbury, 2004). More recent structures are the City Hall of Chicago as well as the School of Art, Design and Media at Nanyang Technolgical University in Singapore (Figure 2.3 (c), Earth Pledge Foundation, 2005).

Figure 2.3.- Examples of different green roofs in a) Italy b) Austria and c) Singapore.

The structure of a green roof mainly consists of a drainage layer, a substrate layer and a vegetation layer on top of the roof. The common classification differentiates two different types based on the depth of the substrate layer (Kolb and Schwarz, 1999). First, extensive green roofs are the ones with a substrate layer of maximum 200 mm depth in which plants similar to *Sedum* species are present. Moreover, a maximum inclination of 45° may be applied. Then, intensive green roofs consist of green roofs with substrate layers with more than 200 mm depth. In that case, the slope could not be higher than 10° and a higher diversity of plants may be found. A comparison between both systems is presented in Table 2.2.

Table 2.2.- Main characteristics of extensive and intensive roofs (adapted from Oberndorfer et al., 2007).

Characteristic	Extensive roof	Intensive roof
Purpose	Functional: storm-water management, thermal insulation, fireproofing	Functional and aesthetic: increased living space
Structural requirements	Typically within standard roof weight-bearing parameters; additional 70 to 170 kg/m² (Dunnett and Kingsbury, 2004)	Planning required in design phase or structural improvements necessary; additional 290 to 970 kg/m²
Substrate type	Lightweight: high porosity, low organic matter	Lightweight to heavy: high porosity, low organic matter
Substrate depth	2-20 cm	20 or more cm
Plant communities	Low-growing communities of plants and mosses selected for stress-tolerance qualities (e.g., *Sedum spp., Sempervivum spp.*)	No restrictions other than those imposed by substrate depth, climate, building height and exposure, and irrigation facilities
Irrigation	Most require little or no irrigation	Often require irrigation
Maintenance	Little or no maintenance required; some weeding or mowing as necessary	Same maintenance requirements as similar garden at ground level
Cost (above waterproofing membrane)	70-220 €/m²	Minimum of around 100 €/m²
Accessibility	Generally functional rather than accessible; will need basic accessibility for maintenance	Typically accessible; bylaw considerations

Higher resistance capacity of the building is required when a green roof is constructed. The above is consequence of the higher load due to the sum of the wet substrate, the plants, other materials required in the system and the people and/or machines. For instance, considering a deck of sawn timber covered with tiles of a load between 100 and 150 kg/m², the fact of adding a vegetation substrate of 50 mm with plants of the genus *Sedum* would increase the load with around 70 kg/m². In contrast, the use of alternative materials such as geotextiles may reduce the load by means of reducing the substrate layer thickness. Consequently, the additional load would be ranged from 30 to 40 kg/m². However, an extensive green roof for trees with a substrate layer of around 600 mm thickness may increase the load until 1 t/m² (Grant et al., 2003).

Benefits solely attributed to green roofs are basically the storm-water management and urban habitat provision. In that sense, green roofs store the water from the rain delaying the

runoff and favouring the evapotranspiration of the remaining water. The above depends on the substrate layer depth, the plant community, the slope of the roof and the pattern of the rainfall (Mentens et al., 2005). Then, benefits concerning provision of urban habitats refer to their contribution to local habitat conservation. Different groups of organisms such as insects usually inhabit green roofs, although they are also suitable for nesting birds or the spontaneous colonisation of autochthonous species (Coffman and Davis, 2005; Baumann, 2006; Köhler, 2006). Furthermore, different benefits for humans were also reported going from human health to urban agriculture by using the roofs as agricultural fields, or reductions in local pollution (Hartig et al., 1991; Dunnet and Kingsbury, 2004).

Similarly, **green walls** are defined as "vegetation that grows directly onto a building's façade or to vegetation that is grown on a separate structural system that can be freestanding and adjacent or attached to the wall" (Loh, 2008). Other terms used to refer to green walls are green façades, living walls or vertical gardens. That concept involves a higher number of different systems as the twelve systems compiled by Pérez (2011) or the classification given by Ottelé (2011).

First, a general classification is provided in which green walls are divided into two main groups depending on the rooting location. First group refers to green walls rooted into the ground in which two different types are included. Those two types correspond to the fact growing plants use directly the façade as a guide or they use an auxiliary support system. Then, the second group includes the green walls rooted in artificial substrates and potting soil mixtures. In correspondence with the classification of the first group, in the second group also a distinction is made between systems in which plants use the façade as a support and the systems in which an additional supporting element is required. Some examples of the different systems are provided below.

1. Systems rooted into the ground (Figure 2.4):
    - The use of climbers rooted into the ground, which grow using the wall as a support may be considered as the pioneer systems, since they are also present in the natural environment.
    - The application of plants rooted into the ground similar as in previous system but without an auxiliary element to support the growing plants.

2. Systems rooted in artificial substrates and potting soil mixtures:
    - Climbers rooted in containers, which can grow using directly the wall or the auxiliary support (Cummins Parking Garage, www.greenrooftops.com, Figure 2.5 (a)).
    - Modular systems based on small squared containers giving the opportunity to produce different designs (Can Felipa project, www.buresinnova.com, Figure 2.5 (b)).

- Hydroponic systems: vegetable substrate is not required in those systems and plants grow attached to a felt mesh placed on an auxiliary element (Muharroq Green Gate, www.verticalgardenpatrickblanc.com, Figure 2.5 (c)).

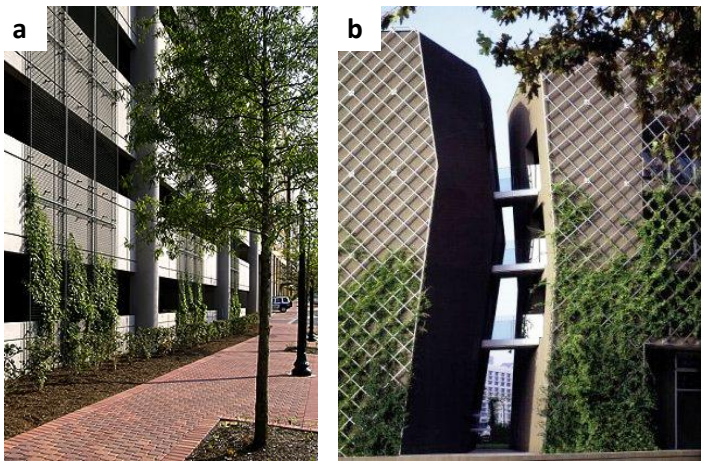

Figure 2.4.- Green walls rooted into the ground: a) Fishers Place at Metro Centro (Rockville, MD. Source: www.greenscreen.com) and b) Offices building (Lambertini et al., 2007).

Figure 2.5.- Examples of green walls systems not rooted into the ground: a) Climbers rooted into containers; b) modular system and c) hydroponic system.

Most of the benefits of green walls are also obtain with to green roofs. Within the common benefits, the improvement of the air quality, protection against driving rain and sun radiation, temperature regulation as well as insulating properties, noise reduction, social impact

and economic savings may be the most interesting to the society (Ottelé, 2011, Pérez, 2011). In general, green plants absorb gaseous pollutants through leaf stomata, which react with water to form other compounds inside the plant.

Furthermore, suspended particles dispersed by the wind may also be intercepted by plants (Bidwell and Fraser 1972; Baldocchi et al., 1987). Consequently, several authors do research on estimating the air pollution mitigation effect by green walls and roofs (Currie and Bass, 2008; Pugh et al., 2012). According to Dunnet and Kingsbury (2004), vegetation may reduce the maximum temperatures of a building thanks to its leaves, which provide shadow to the surface. Therefore, buildings with plant-covered walls are usually cooler than when other solutions are applied such as light coloured surfaces (Hoyano, 1988). Furthermore, plants act as solar filters since they absorb the solar radiation to use it for developing their biological functions (Perini et al., 2011).

The noise attenuation due to vegetation is also well known. Vegetation affects the sound in urban areas by means of sound absorption, sound diffusion and sound level reduction due to the transmission of the sound waves through those organisms (Wong et al., 2010; Van Renterghem and Botteldooren, 2011). Regarding economic saving, it is mainly associated to energy saving since green walls act as passive systems reducing the energy consumption. Moreover, that benefit is closely related to the thermal regulation capacity thanks to the shadow produced by the plants, to the thermal insulation provided not only by the plants but also by the substrate and the evaporative cooling, which is consequence of the evapotranspiration process (Pérez et al., 2011). However, the economic benefits are also linked to the reduction of the air pollution since pollution fees would decrease.

Additionally to all the aforementioned benefits, there is a general positive perception of the incorporation of green elements as already referenced. In that sense, some works reveal the positive psychological effect of nature on people (Anderson et al., 1984; Dinsdale et al., 2006) although quantitative measurements of the wellness effect are difficult to achieve (Pérez, 2011).

Unfortunately, the aforementioned systems for both green walls and green roofs present several disadvantages in terms of integration, costs and/or harmful effects. A general low integration between vegetal elements and the structures is observed for most of the systems. Regarding green roofs, the use of vegetal substrate is a prerequisite to allow the plants, which force the use of different layers. According to Pérez (2011), seven layers between the roof structure and the vegetation compose a standard model. The roof needs to be protected by a waterproofing layer, an insulation layer and a root barrier layer. Furthermore, the protection, drainage and filter layers should be installed before application of the substrate. Consequently, a low level of integration is observed between the natural element and the structure due to all the intermediate layers. Finally, the consequent additional load may reduce the installation of green roofs on new constructions.

According to Oberndorfer et al. (2007), initial costs of installing green roofs are ranged from 70 to 220 €/m². Additionally, costs related to installation of green walls are ranged from 30 €/m² for the simplest systems to 1200 €/m² (Ottelé, 2011). However, maintenance costs should be added and they are similar to the ones corresponding to conventional gardens. Consequently, the economic benefits could not be reported until the coverage of the installation and maintenance costs. The possible harmful effects refer to dampness problems especially for old structures (Lourenço et al., 2006), or acceleration of the deterioration processes (Johnston et al., 2004). However, damage caused by roots intrusion is not frequent and mainly corresponds to an incorrect installation of the vegetal element.

Aforementioned disadvantages drive researchers to think about more integrated solutions, which would consequently reduce associated costs. In this sense, Ottelé et al. (2010) proposed the integration of plant growth in building materials by means of designing a bilayer concrete. One of the layers has the structural function and is produced with self-compacting concrete while the other has a high porosity, which is filled by a soil mixture as shown in Figure 2.6. The integration level of that system is significantly higher than in the aforementioned systems although the necessity of including soil and the fact that plants should be planted show that further research is required.

Figure 2.6.- Green concrete proposed by Ottelé et al. (2010).

2.4. NEGATIVE INTERACTIONS BETWEEN LIVING ORGANISMS AND STRUCTURES

Undesirable biological colonisation is common on diverse cementitious materials such as monuments, historical buildings or simply old constructions. The above is consequence of three interconnected factors, which are the presence of pioneer living organisms in the environment, the environmental conditions and the properties of a material (Figure 2.7). In that sense, first organisms successively colonising surfaces may be bacteria, algae and fungi (Deruelle, 1991; Dubosc et al., 2001; Shirakawa et al., 2002; Escadeillas et al., 2007), which may be dispersed by the wind (Langerberg et al., 1977; Reponen et al., 1997). Environmental conditions have a great influence on the presence of dispersed living organisms. According to Lighthart (2000), the concentration of bacteria-associated atmospheric particles varies temporally and geographically due to factors such as the moment of the day (Lighthart and

Shaffer, 1994), the season (Bovallius et al., 1978) or the location, in terms of urban areas, forests or rural-agricultural areas (Shaffer and Lighthart, 1997).

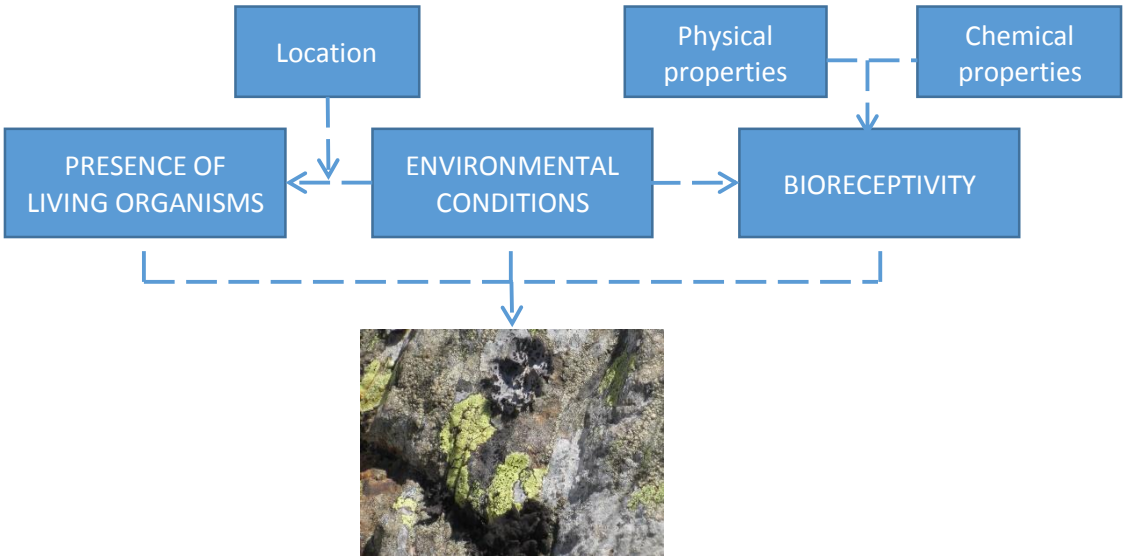

Figure 2.7.- Causes of the colonisation of stone materials.

Related to the aforementioned factors, meteorological conditions as well as topographic conditions also have a great influence on the major or minor presence of microorganisms in the environment (Lighthart, 2000). Considering meteorological conditions, the wind direction, the rain, and the solar radiation may have the major contribution (Lighthart and Shaffer, 1994). Consequently, the environmental conditions have a double function, dispersing the pioneer microorganisms by the air or depositing them on the soil or other surfaces. However, the anchorage and development of the microorganisms will depend not only on the environmental conditions, in which more factors such as temperature, relative humidity and nutrient availability are of importance (Guillitte, 1993), but also on the properties of the material. In that sense, Guillitte (1995) proposed the concept of bioreceptivity, which was adopted from the medical field. Bioreceptivity was defined as "*the aptitude of a material (or any other inanimate object) to be colonised by one or several groups of living organisms without necessarily undergoing any biodeterioration*".

The different levels of bioreceptivity were defined by Guillitte (1995), being the primary, the secondary and the tertiary bioreceptivity. The primary bioreceptivity refers to the initial state of the material, the secondary bioreceptivity refers to the new properties of a material because of the action of microorganisms or other factors and the tertiary bioreceptivity concerns to any posterior human activity affecting the material. Primary receptivity is the one affecting the development of pioneer organisms although the second one may have more influence on the establishment of other groups of organisms such as lichens and mosses.

It is well known that microorganisms are ubiquitous and found widespread. Regarding the current topic, stone materials, both natural and artificial, are common habitats for

microorganisms. In that sense, Figure 2.8 shows an example of a statue from Queluz National Park in Portugal (Miller et al., 2012), a portico of the crypt of Francisco Arrechea in Argentina (Guiamet et al., 2012) and a detail of St. Mark's church in Belfast (McCabe et al., 2011). Several authors studied the presence and effect of microorganisms on natural stone (Mansch and Bock, 1998; Hoppert et al., 2004) as well as on building materials (Crispim et al., 2006; Coutinho et al., 2013). Properties included in the term bioreceptivity are both physical and chemical properties such as surface roughness, open porosity, permeability, chemical composition and pH (Guillitte and Dreesen, 1995; Tiano et al., 1995; Tomaselli et al., 2000; Favero-Longo et al., 2009; Miller et al., 2009; Miller et al., 2010; Manso et al., 2014a, 2014b).

Figure 2.8.- Examples of colonised building materials: a) statue in Portugal, b) portico of a in Argentina and c) detail of a church in Belfast.

The study of the natural colonisation of building materials is commonly studied from a biodeterioration and biodegradation point of view. According to Hueck (1965), biodeterioration is "*any undesirable change in the properties of a material caused by the vital activities of living organisms*". Afterwards, Berthelin (1983) divided that term into two processes depending if the causal mechanisms is soluble or insoluble. Soluble mechanisms comprise acid and basic reactions, complexation and both enzymatic and non-enzymatic processes, while insoluble mechanisms refer to oxidative or reductive reactions. There is an important established research area on biodeterioration, which tries to define the negative effects of biological growth on building materials and mechanisms to prevent the biofouling. Some authors studied the microorganisms colonising building materials due to mainly bacteria and algae (Gaylarde et al., 2003; Fernandes, 2006; Gorbushina, 2007; Gorbusina and Broughton, 2009).

Others focused their research on specific groups of organisms such as Starks and Shubert (1982), who stated the presence of cyanobacteria and algae on a surface enable the disintegration of the material. Afterwards, Saiz-Jimenez (1995) studied the deposition of anthropogenic compounds and the effect on airborne microorganisms. Piervittori et al. (1994, 1996, and 1998) focused their work in the lichens colonisation and the consequent effects on the materials. Then, authors such as Ortega-Calvo et al. (1995) or Tiano et al. (1998) investigated

on the detrimental effects of photosynthetic organisms. Favero-Longo et al. (2004) focused their research on lichens while Macedo et al. (2009) did the same for cyanobacteria and green algae. Others like De Muynck et al. (2009) evaluated different mechanisms for algal biofouling prevention. Finally, Cutler and Viles (2010) studied the effects of eukaryotic microorganisms on stone materials biodeteriortion. The aforementioned authors are some examples although a longer list could be obtained from the literature.

In summary, many authors do research on colonisation of stone materials and biodeterioration to identify the involved organisms and their effects on the materials. Nonetheless, only few of them studied the properties of the materials implicated in that process. Table 2.3 resumes the main properties of the stone materials studied by most of the authors. However, some other authors are less critical and suggest biofouling should not be considered always as a material pathology. For instance, Dukes (1972) and Tiano (1986) stated the only important damage caused by those organisms is aesthetical. Guillitte (1993) also asserted that knowledge in terms of bioreceptivity could be also used to stimulate biofouling in specific cases. Ariño et al. (1995) stated that the biodeterioration and bioprotection are in unstable equilibrium, which can be unbalanced due to environmental conditions, the substratum and the colonising organisms.

Table 2.3.- Materials properties studied by several authors

Properties	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]
Chemical composition	•	•	•	•		•	•	•	•	•	•	
pH		•		•						•		•
Texture	•	•	•				•	•		•		
Roughness		•		•	•	•				•	•	•
Porosity	•	•	•	•	•	•		•		•	•	•
Capillary coefficient				•	•	•				•		
Water content			•	•								
Bulk density				•	•							
Dry density			•									
Grain density			•									
Permeability										•		

[1] Guillitte and Dreesen (1995)
[2] Tiano et al. (1995)
[3] Papida et al. (2000)
[4] Prieto and Silva (2005)
[5] Barberousse et al. (2006)
[6] Escadeillas et al. (2007)
[7] De los Ríos et al. (2009)
[8] Favero-Longo et al. (2009)
[9] Giannantonio et al. (2009)
[10] Miller et al. (2006, 2009, 2010)
[11] Portillo et al. (2011)
[12] Tran et al. (2012)

2.5. CONCLUDING REMARKS

The general positive perception of nature, together with the environmental problems of big cities, means an increased interest in integration of green areas. However, the rise in population makes it difficult to increase the surface of parks and gardens in cities. Consequently, the alternative of covering buildings and other structures with vegetal elements gained more significance. A big number of systems integrating vegetal elements as building envelopes were found, showing the importance of incorporation of nature into cities. Even though in most of the green walls and green roofs the level of integration, the installation and maintenance costs, and the limitations due to extra load, could be improved. Therefore, to make the optimized and efficient design of green elements possible, several advances to the state of the art are still needed.

In order to improve the aforementioned inconvenience, a better understanding of the properties involved in bioreceptivity, which of them could have a greater role, and the ways to produce those materials, is needed. As already mentioned, the negative impact of organisms on building materials should be considered depending mainly on the application.

On the other hand, there are few studies and laboratory results comparing different materials designed for the purpose, and there is a lack of environmental experimental programs. Accelerated laboratory tests give a fast approach for comparisons between the effects of colonisation on different materials by one or few organisms. Nonetheless, the real natural conditions significantly differ from the controlled laboratory conditions and in reality many parameters in combination give different results. Verification of that fact as well as a better understanding of the interactions is also required.

3. DEVELOPMENT OF A LOW-PH CEMENTITIOUS MATERIAL

3.1. INTRODUCTION

Despite of the numerous parameters involved in the design of a cementitious material as a biological substrate, pH was taken as a priority. Two different hydraulic binders were considered in order to develop a low-pH cementitious material: Ordinary Portland Cement (OPC) and an acid-base cement. Regarding OPC, the selection was basically based on the wide use of this material in construction. Then, Magnesium Phosphate Cement (MPC) was chosen from the group of acid-base cements. The decision was based on the slightly acidic pH as well as references in the literature about its use in construction (Li et al., 2004; Qiao et al., 2009a; Formosa et al., 2011).

Naturally, carbonation of OPC is taking place during its service life when this material is exposed to the environment. Consequently, pH decreases progressively reaching pH values around 8. For this reason, one of the ways proposed to diminish the pH of the material was accelerated carbonation. However, this process changes different properties of the material as it was detailed in the literature. In parallel, a trial to decrease the pH from the beginning was carried out by means of acid additions. With this aim, an evaluation of acid additions in pH as well as mechanical properties was conducted.

The experimental program has been carried out in different laboratories: at the Structure Technology Laboratory Luís Agulló (Polytechnical University of Catalonia), Center for Research in NanoEngineering (Polytechnical University of Catalonia), Scientific and

Technological Centers (University of Barcelona) and premises of the company Cementos Portland Valderrivas.

The main goal pursued in this chapter is to develop a low-pH cementitious material in order to improve bioreceptivity of building materials. For that, the following specific objectives are defined:

- Evaluate the effect of acidic compounds added to Ordinary Portland Cement (OPC) on its pH as well as physical-chemical and mechanical properties;
- Characterize different Magnesium Phosphate Cement (MPC) formulations to evaluate their suitability as a building material;
- Define a suitable MPC formulation depending on physical-chemical and mechanical properties.

An experimental program is defined to study and characterize properties of cementitious materials in section 3.2. In particular, both ways to decrease pH of the final material are defined as well as tests to prove their suitability. Subsequently, results for OPC and MPC experimental programs are analysed in sections 3.3 and 3.4 respectively.

3.2. MATERIALS AND METHODS

In the framework of the mentioned objective, the following methodology was developed. On one hand, evaluation of the effect of acidic additions to OPC properties was carried out. On the other hand, a complete characterization of different MPC formulations was developed. Since MPC pH is suitable for the use, an evaluation of the formulations' suitability as a cementitious material was necessary Figure 3.1.

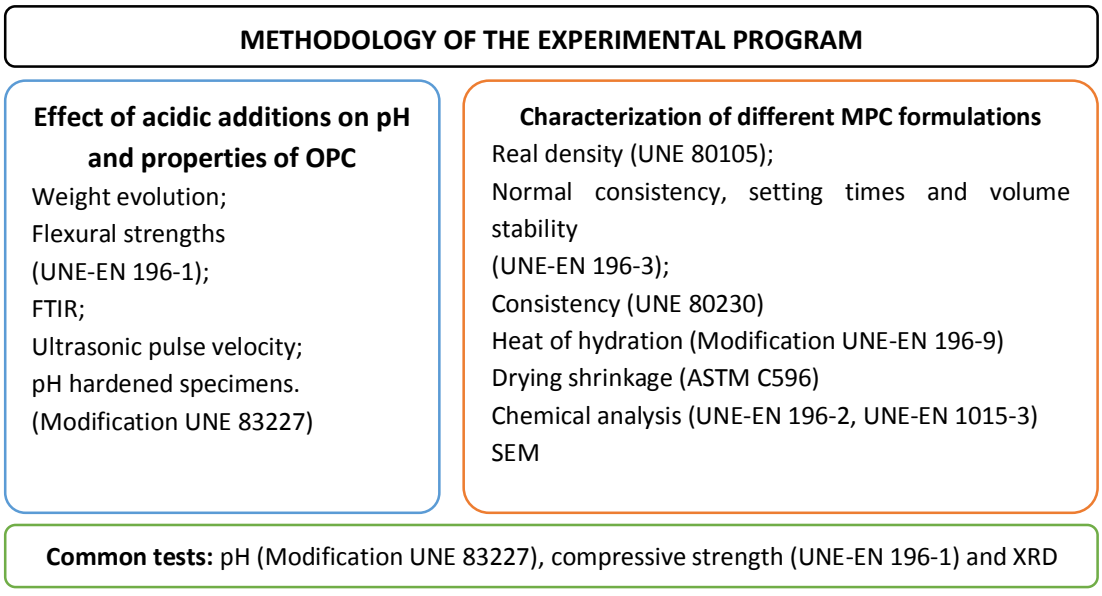

Figure 3.1.- Scheme of the experimental program.

3.2.1. Materials and hydration reactions

Ordinary Portland Cement

Additives used in the fabrication of OPC to decrease its pH were boric acid (H_3BO_3) and oxalic acid ($(COOH)_2 \cdot 2H_2O$) as a weak and a strong acid respectively. Additionally, oxalic acid is a diacid considered as a strong acid in its first hydrolysis reaction. These acids were selected on the basis of previous studies from literature (Demirbaş and Karslioglu, 1995; van Eijk and Brouwers, 2001; Boncukcuoğlu et al., 2002; Elbeyli et al., 2003; Singh et al., 2003).

Equation 3.1 shows the reaction corresponding to dissolution of boric acid in distilled water. Additionally, equation 3.2 shows the reaction in presence of calcium, where calcium diborate is formed.

$$H_3BO_3 + H_2O \xrightleftharpoons{pk_a=9.24} B(OH)_3^- + H_3O^+ \quad [3.1]$$

$$2[B(OH)_4^-] + Ca^{2+} + H_2O \rightleftarrows Ca[B(OH)_4]_2 \cdot 2H_2O \quad [3.2]$$

Van Eijk and Brouwers (2001) modelled and reconfirmed the coating theory and models proposed by Yousuf et al. (1995) and Cocke et al. (1995). According to van Eijk, the initial presence of borates in the mixing water produces an increment of borates. As a result, the equilibrium of the reaction 3.2 is displaced to the right and the hydration product, calcium diborate, precipitates. The above hydration product has a low solubility ($5.4 \cdot 10^{-7}$ mole/l) and this fact avoids the dissolution of anhydrous cement since the precipitation is on the cements particles. Accordingly, no dissolution of cement particles involves no releasing of hydroxyl ions, responsible of the first increase of pH of the mixture. The main consequence is the delay of the hydration process.

Furthermore, Lieber and Richartz (1972) defined previously that depending on the amount of acid added, a higher or lower degree of coverage of the cement particles is achieved. Therefore, the delay is increasing until the inhibition of the hydration process. In this regard, van Eijk and Brouwers (2001) obtained from their model that this inhibition takes place when additions higher than 1 % over cement weight (ocw) are used, due to the total coverage of the cement particles. However, when amount of boric acid added is not enough to stop the hydration process, portlandite is progressively forming. This fact causes an increment in pH inducing redissolution of calcium diborate and the restoration of the hydration process.

Regarding additions of oxalic acid, a different mechanism of action is described. In particular, oxalic acid acts as a set accelerating additive and dissolution reactions without (equation 3.3 and 3.4) and with presence of calcium (equation 3.5) as follows:

$$H_2C_2O_4 + H_2O \xrightleftharpoons{pk_a=1.27} HC_2O_4^- + H_3O^+ \quad [3.3]$$

$$HC_2O_4^- + H_2O \xrightleftharpoons{pk_a=4,27} C_2O_4^{2-} + H_3O^+ \quad [3.4]$$

$$C_2O_4^{2-} + Ca^{2+} + H_2O \rightleftarrows CaC_2O_4 \cdot H_2O\downarrow \quad [3.5]$$

Taylor (1990) described that this acid affects C_3S, at the beginning of the hydration process. Oxalate tends to combine quickly with free calcium ions forming calcium oxalate. This reaction product precipitates maintaining low calcium levels until total reaction of oxalates. Furthermore, this acid is also known for its effect as a consumer of water and consequently, increases in amount of acid additions cause reductions in workability.

Additionally, Ramachandran (1976) suggested that additions higher than 0.1 % cause acceleration of aluminates hydration. Then, silicates hydration is subsequently delayed producing a postponement of the induction period appearance. However, different mechanisms are supposed to contribute, mainly the permeability of the precipitation product and consequently its solubility ($9.5 \cdot 10^{-5}$ mole/l) and consumption of available calcium.

Magnesium Phosphate Cement

Regarding MPC, three compounds were used to produce the specimens. These cementitious materials are mainly produced by mixture of magnesium oxide and a phosphate source. For this research, Magnesium Oxide with low reactivity (MgO), Ammonium Dihydrogen Phosphate ($NH_4H_2PO_4$, ADP) and borax ($Na_2B_4O_7 \cdot 10H_2O$), as a retarder, were used.

According to Yang and Wu (1999), hydration of MPC with addition of borax starts with a fast dissolution of borax and the ADP in the mixing water. Moreover, this fact causes a drop in the pH of the mixture (equations 3.6 and 3.7).

$$Na_2B_4O_7 \bullet 10H_2O \rightarrow 2Na^+ B_4O_7^{2-} + 10H_2O \quad [3.6]$$

$$NH_4H_2PO_4 \rightarrow NH_4^+ + H_2PO_4$$

$$H_2PO_4 \rightarrow HPO_4^- + H^+ \quad [3.7]$$

$$H_2PO_4 \rightarrow PO_4^{2-} + 2H^+$$

$B_4O_7^{2-}$ ions are absorbed on the periclase surface and, subsequently, borate compounds cover them. This fact hinders the contact between NH_4^+ and $H_2PO_4^-$ with the periclase, delaying the hydration process. However, since borax does not act as an inhibitor, NH_4^+ and $H_2PO_4^-$ diffuse through the borates covering the magnesium and formation of an amorphous gel occurs. Then, the gel starts to crystallize destroying the borates of magnesium by volumetric expansion. From this moment on NH_4^+, $H_2PO_4^-$ and Mg^{2+} react and different phosphate hydrates cover all the particles. The main hydration product is struvite ($NH_4MgPO_4 \cdot 6H_2O$) as shown in equation 3.8.

$$MgO + NH_4H_2PO_4 + 4H_2O \rightarrow NH_4MgPO_4 \bullet 6H_2O \quad [3.8]$$

Nevertheless, other compounds with similar composition could be formed. Those compounds include bobierrite ($Mg_3(PO_4)_2 \cdot 4H_2O$), dittmarite ($NH_4MgPO_4 \cdot H_2O$), hannayite ($(NH_4)_2Mg_3(HPO_4)_4 \cdot 8H_2O$), newberyite ($MgHPO_4 \cdot 3H_2O$) or schertelite ($(NH_4)_2Mg(HPO_4)_2 \cdot 4H_2O$). From these compounds, dittmarite and schertelite are the most common compounds after struvite. Furthermore, their presence is related to a lack of water either by not enough mixing water or evaporation due to hydration heat (Hall et al., 1998). However, struvite is quickly formed when more water is available since the other products are less stable.

3.2.2. Dosages and methods

There is little knowledge about variations in pH in OPC due to additions as well as in MPC as a result of changes on components' percentage. Consequently, a first test to evaluate how the pH is affected was carried out. Additionally, it was possible to select different acid concentrations as well as different formulations of MPC to further research.

First, different percentages (% ocw) of both acids were diluted in distilled water, mixed with cement pastes and finally added to mortar to measure their pH per triplicate. pH measurements of fresh mortar were taken 10 minutes after the mixing and duplicate measurements were taken in order to compare results of two different methodologies of addition: previous dilution of the addition in the water of the mixture and without previous dilution. Boric acid concentrations were in the range of 0 and 8 %, while oxalic acid concentrations were between 0 and 13 %. Subsequently, different $NH_4H_2PO_4$:MgO ratios (P:M ratios) with and without addition of a fixed amount of borax (6 % ocw) were also tested. The mortars with selected P:M ratios, which are shown in Table 3.1, were then tested physico-mechanically and chemically in order to compare them with Ordinary Portland Cement (OPC) CEM I 52.5R.

Table 3.1.- Composition of mortar specimens

Boric acid		Oxalic acid	
Specimen	[addition] (% ocw)	**Specimen**	[addition] (% ocw)
AB0	0	**AO0**	0
AB1	1.5	**AO1**	3
AB2	2.5	**AO2**	6
AB3	4	**AO3**	9
AB4	6	**AO4**	11
AB5	8	**AO5**	13

After selection of different percentages of addition, OPC mortars according to pertinent standard UNE-EN 196-1 (CEM I 52.5R, silica aggregates 0/2 mm and aggregates:cement:water ratio of 3:1:0.5) were produced and stored in a chamber at 21 ± 1°C and 50 ± 5 % relative

humidity (RH) until being tested. A high adhesion between MPC and metallic moulds was observed, therefore coverage of the moulds with a plastic wrap was applied. Compositions of these samples are shown in Table 3.1. Afterwards, weight evolution (balance precision of ± 1 g), ultrasonic pulse velocity using direct transmission, pH and compressive and flexural strength were measured until 28 days of age. Moreover, XRD and FTIR spectroscopy techniques on cement paste samples with the maximum amount of addition and without any addition as control were determined. Cement paste samples of 15 g were produced and they were ground and treated with acetone and ethanol to detain the hydration process (Valls and Vàzquez, 2000; Hernández et al., 2011). Powdered samples were placed on a filter located on a vacuum pump, for carrying out first a bath of 45 seconds with acetone and covering afterwards with ethanol.

Ultrasonic pulse velocity using direct transmission was determined by means of a tester with transducers of 37.5 mm at a frequency of 55 kHz. A pH-meter was used to determine the pH by using two different electrodes depending on the nature of the sample (Standard Ag/AgCl with single-junction for dilutions and cement pastes and a double junction one for fresh mortar). Measurements were obtained according to the standard UNE 83227 used also by other authors (Valls, 1999; Iyengar and Al-Tabbaa, 2007) but modifying the solid:liquid ratio. In the current research, samples were crushed and the powder was resuspended in distilled water with a solid:liquid ratio of 1:10. Results were obtained after one hour while stirring on three replicates and three measurements per replicate until 28 days. Flexural and compressive strength were obtained by means of a strength tester and according to the relevant standard UNE-EN 196-1.

Accordingly, after selection of different P:M ratios, tests of the hydraulic binder, cement pastes and mortars were carried out (Figure 3.1). The chemical composition of the MPC ranged from 1:1 to 1:2 with an addition of 6 % of borax (ocw). First, physical-mechanical properties were evaluated. Real density of the hydraulic binder (without borax) was determined by means of a liquid pycnometer according to the standard UNE 80105.

Subsequently, normal consistency, setting times and volume stability of cement paste was carried out in agreement with standard UNE-EN 196-3. Then, consistency of fresh mortar by means of flow tables was also tested as well as heat of hydration under semi-adiabatic conditions conforming to UNE-EN 1015-3 and UNE-EN 196-9 respectively. For the last mentioned, a modification of the container was applied due to the maximum temperature reached in a previous test (Figure 3.2). The above was done according to results of other authors (Hall et al., 1998; Mestres and Ginebra, 2011).

Finally, having the samples already hardened, compressive strengths at 1 hour, 1, 2, 7 and 28 days and drying shrinkage until 200 days were determined according to standards UNE-EN 196-1 and ASTM C596-09 respectively.

Figure 3.2.- General (a) and detailed (b) view of the heat of hydration test.

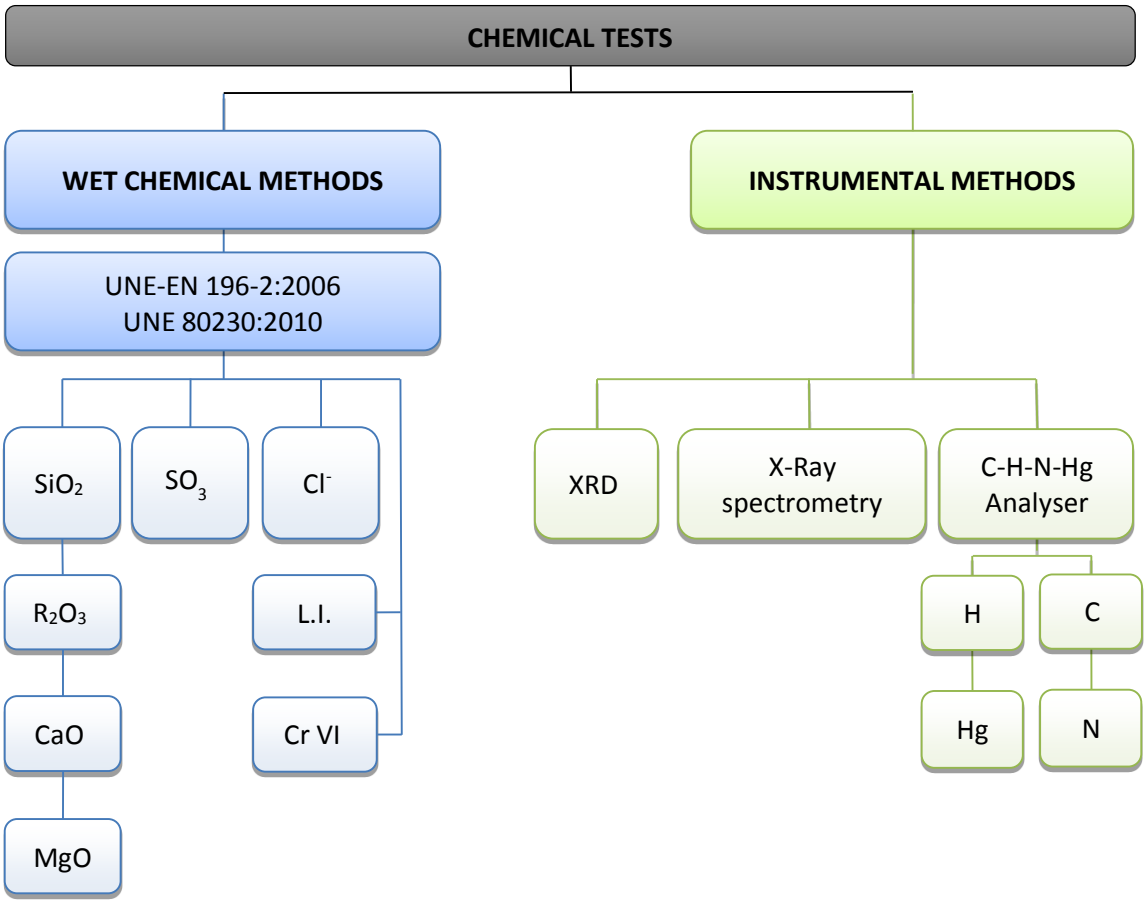

Figure 3.3.- Diagram of the chemical tests carried out.

3.3. RESULTS AND ANALYSIS RELATED TO ACID ADDITIONS IN OPC

This particular section includes all the results obtained in the OPC experimental program. As mentioned before, the purpose was to clarify the effect of boric and oxalic acid additions on OPC properties as was shown in Figure 3.1.

3.3.3. pH

First results of pH determination for both additions are given in Figure 3.4. As a first approximation, a decrease of pH was observed in all the samples, independently of the nature of the sample or addition. However, the behavior of different curves is significantly dissimilar.

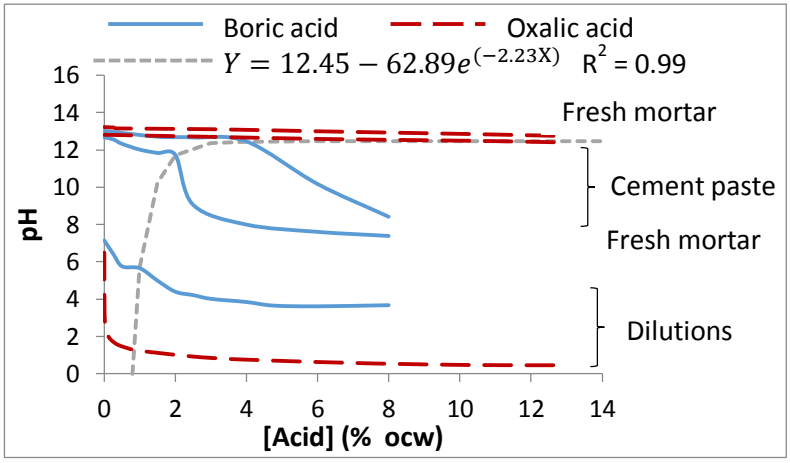

Figure 3.4.- pH determination for specimens with boric and oxalic acid.

pH measurements of acid dilutions in distilled water were carried out. Due to the ease and low cost, a great number of dilutions were measured in order to reduce the number of cement pastes and mortars to test. Boric acid curves show different behaviors, especially between dilutions and cement mixtures. pH of boric acid dilutions in distilled water shows a significant decreasing curve, reaching the equilibrium at a concentration of around 5 % of addition. The cement paste curve shows a first stage with a slight drop for additions lower than 4 %. However, a second stage displays a sharp drop in pH for bigger concentrations, reaching a pH around 8 for additions of 8 %. Regarding fresh mortar, only results of a mixture without diluting the acid in the mixing water are shown. The above is due to non-significantly different results between both mechanisms of addition. Subsequently, curves for fresh mortar show a similar trend despite of a displacement in the inflection point from 4 % of addition in cement pastes to 2 % in fresh mortar. Furthermore, stabilization at pH around 7.5 is noticeable in fresh mortar although this equilibrium is not reached in the cement paste curve.

As mentioned previously, a low effect of small percentages of boric acid in the pH was observed. The following equation is proposed to correlate these different inflection points depending on the nature of the mixture:

$$y = 12.45 + 62.89\, e^{(-2.23x)} \qquad [3.9]$$

where *y* is the pH, *x* is the percentage of boric acid addition and A (12.45), B (62.89) and C (2.23) are parameters determined by chemical and physical factors. In the current situation, A is the maximum pH when there is no addition of acid, and is function of the type of cement, w/c ratio and pH of the mixing water. B describes de relative responsiveness of a mixture, and so is

dependent upon the levels of boric acid in the mixture. Finally, C describes de curvature of the response and is affected mainly by type of matrix and purity of boric acid.

The curve corresponding to dilutions reaches the inflection point sooner than for the other two mixtures and this is reasonable because there are no other components interfering in the chemical reaction. Moreover, this first stage could be related to the weakness character of the acid. However, adding cement and aggregates to the mixture displaces the inflection point, implying a higher needed percentage of addition. For cement paste mixtures, addition of an alkaline compound, Portland cement, might produce a buffer effect. This effect is also observed in fresh mortar although it is achieved earlier than in cement paste mixtures. The above might be caused by addition of aggregates since available water should react with more compounds (wetting aggregates and dissolving anhydrous cement as well as boric acid). This fact may favor dissolution of boric acid instead of Portland cement, reducing the inflection point from 4 to 2 %.

Furthermore, this inflection point could also indicate the cease of the hydration process due to the low permeability of the calcium borate. Depending on the nature of the matrix an increase from 2 to 4 % for cement pastes and fresh mortars respectively was observed. As shown before by Lieber and Richartz (1972) as well as van Eijk and Brouwers (2001), concentrations of boric acid bigger than 4 % provoke the cease of the hydration process. Accordingly, no presence of aggregates could physically present better conditions to cover anhydrous cement with the calcium borate.

In contrast, the oxalic acid dilutions curve shows a similar behavior than the one corresponding to boric acid dilutions. The main difference is the stronger character of the oxalic acid showing a sharp drop in pH. The pH equilibrium was not achieved until around a 9 % of oxalic acid addition and a pH of 0.5. However, both cement pastes and fresh mortar illustrate a slight linear drop in pH of less than 1 point between 0 % and 13 % of addition. Permeability of the precipitates layer could be the cause of this low drop and this fact lets the water pass through this layer, allowing the hydration process to continue. According to Ramachandran (1976), considering calcium oxalate as a permeable compound, this minimum effect in pH could be caused by solubility of calcium oxalate and, consequently, consumption of available Ca^{2+}.

Finally, it is important to remark how different both acids act upon OPC. Boric acid acts as a retarder of hardening, arriving to stop the process with additions up to 2 %. Nonetheless, oxalic acid seems to produce the opposite effect, acting also as a water reducer with the consequent impact on workability. From this test, percentages of addition to produce standardized mortar were chosen (Table 3.1).

3.3.4. Weight evolution and ultrasonic pulse velocity

Figure 3.5 shows the average of the loss of weight for OPC samples with addition of boric acid and oxalic acid between 7 and 28, taking 7 days as the initial weight. The above is consequence of the effect mentioned above: the specimens could only be removed from the

mould after 7 days due to the retarder effect of boric acid. A drop in weight is observed for all the samples, including samples without boric acid. This fact is the consequence of maintaining specimens in the climate chamber. Results for specimens with between 0 and 4 % of boric acid addition showed a clear increase on loss of weight as percentage of addition increases, being between 2.5 and 9.7 % respectively. However, samples with higher percentage of addition showed lower loss than that 9.7 %. The hydration process of these specimens was not finished and this fact caused loss of material during manipulation. By contrast, samples with addition of oxalic acid did not show significant differences, the maximum loss of weight being 2 %.

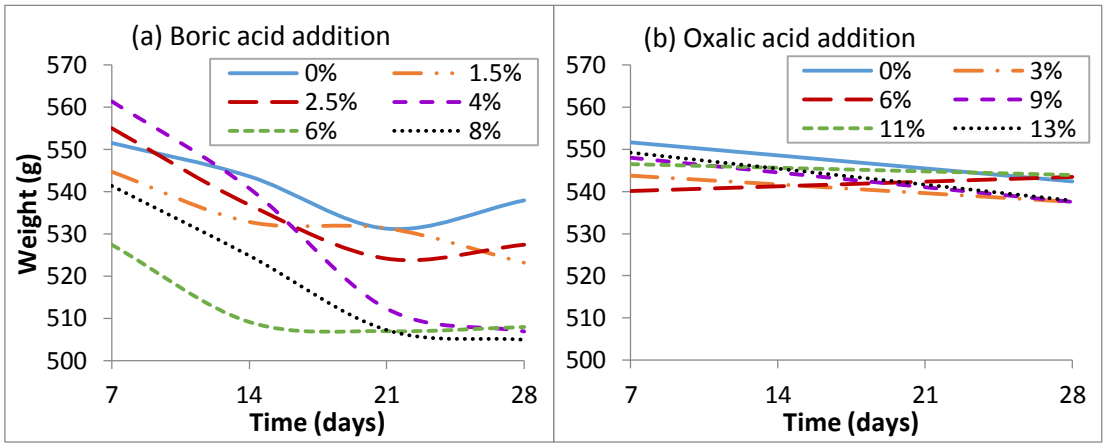

Figure 3.5.- Loss of weight for specimens with addition of (a) boric acid and (b) oxalic acid.

Table 3.2 shows the data relating to ultrasonic pulse velocity. Acceptable range for conventional concrete is between 3500 and 4800 m/s (Henry, 2003) although it is slightly lower for mortars, which values between 3000 and 3500 m/s (Rebolledo, 2010). Consequently, mortar values will be taken as a reference.

Table 3.2.- Ultrasonic pulse velocity (m/s)

	Addition (% ocw)	7 days	14 days	21 days	28 days
Boric acid	0	3891.6 ± 124.7	3934.4 ± 85.5	4018.7 ± 39.0	3988.1 ± 80.8
	1.5	3344.6 ± 170.9	3534.1 ± 58.1	3373.8 ± 205.5	3566.6 ± 88.9
	2.5	2439.5 ± 383.9	3521.7 ± 76.5	3236.4 ± 83.4	
	4	837.5 ± 18.3	3218.5 ± 100.2	1438.4 ± 348.1	1073.9 ± 34.6
	8	796.1 ± 5.8	510.3 ± 20.8	541.6 ± 27.3	552.8 ± 9.5
Oxalic acid	0	3935.7 ± 78.6			4066.5 ± 45.9
	3	3896.6 ± 49.5			3959.2 ± 46.3
	6	3868.5 ± 27.0			3913.7 ± 20.4
	9	3816.1 ± 46.8			3838.6 ± 20.5
	11	3807.0 ± 45.4			3748.9 ± 40.8
	13	3773.7 ± 25.8			3747.1 ± 13.6

3.3.5. Compressive strength

Table 3.3 displays compressive strengths of samples with both additions. In accordance to previous tests, samples with additions from 4 % on of boric acid showed an important affectation of compressive strengths, having maximum compressive strengths below 60 % of the final value. Specially, additions lower than 2.5 % induce the slowdown of the hydration process due to precipitation of calcium diborate. Consequently, this product could affect the matrix.

Table 3.3.- Compressive strength until 28 days (MPa)

	[Acid] (% ocw)	7 days	14 days	21 days	28 days
Boric acid	0	27.12 ± 2.61	38.78 ± 0.59	40.95 ± 2.12	47.90 ± 2.12
	1.5	11.12 ± 1.02	23.96 ± 1.06	24.73 ± 1.92	25.37 ± 0.39
	2.5	10.43 ± 0.55	22.55 ± 1.88	19.82 ± 0.94	21.78 ± 0.83
	4	6.23 ± 0.45	13.5 ± 0.66	1.15 ± 0.25	0.62 ± 0.06
	6	2.29 ± 0.01	0.78 ± 0.01	0.2 ± 0.02	0.29 ± 0.02
	8	0.18 ± 0.03	0.43 ± 0.39	0.1 ± 0.00	0.12 ± 0.00
Oxalic acid	0	44.16 ± 0.05			51.38 ± 0.28
	3	44.98 ± 1.51			48.71 ± 0.82
	6	42.06 ± 0.74			47.72 ± 0.51
	9	42.99 ± 1.33			46.87 ± 1.06
	11	39.30 ± 2.24			45.50 ± 2.01
	13	39.72 ± 0.17			42.96 ± 0.28

Nevertheless, bigger additions than 4 % resulted in little compressive strength, almost null because of detention of the hydration process. Otherwise, samples with addition of oxalic acid show an increase of compressive strengths until 28 days, independent of the amount of addition. However, compressive strengths decreased as oxalic acid addition increased.

3.3.6. pH of hardened specimens

Figure 3.6 shows results of pH evolution of hardened specimens. Concerning specimens with boric acid addition, a decrease of pH as boric acid concentration rose. The above was detected for all test times. However, reductions lower than a 7 % of pH at 28 days were recorded between specimens with the maximum boric acid concentration (8 %) and specimens without boric acid addition.

Furthermore, constant pH values for specimens with higher amounts of boric acid were expected due to the detention of the hydration process. However, the methodology to prepare the sample for pH determination could affect the results. As mentioned before, pieces of the specimens were crushed and resuspended in distilled water. This process could favor the contact between anhydrous particles and distilled water and consequently the reaction would distort

pH values. On the other hand, no significant differences were observed for samples with addition of oxalic acid.

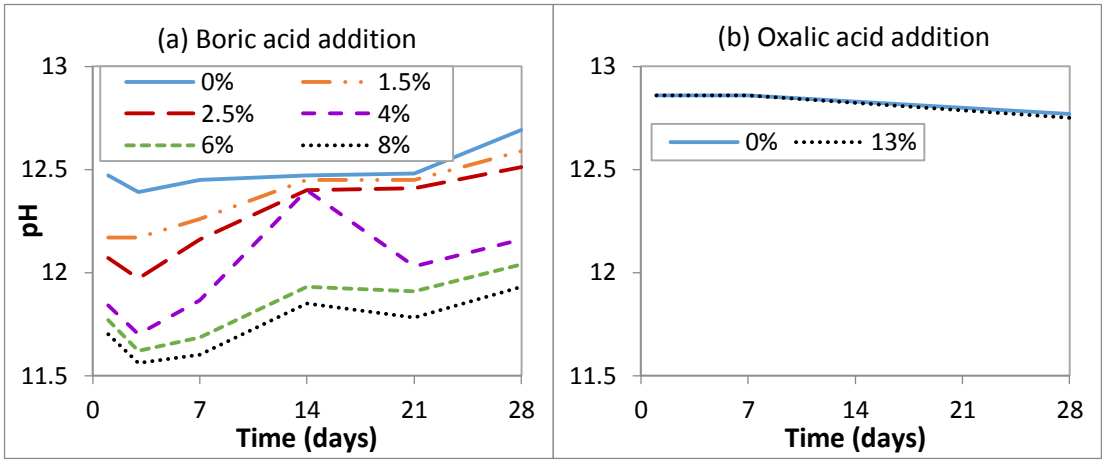

Figure 3.6.- Evolution of pH until 28 days for specimens with (a) boric acid and (b) oxalic acid.

3.3.7. XRD and FTIR

Figure 3.7 shows XRD results for control specimens (0 % of addition), OPC with addition of boric acid (8 %) and with addition of oxalic acid (13 %). The curve of OPC with boric acid showed presence of anhydrous compounds as well as gypsum even though no presence of portlandite or ettringite was detected. Consequently, the hypothesis of no hydration of specimens with 8 % of addition is accepted although no presence of hydrated calcium diborate (2θ: 11.4 and 26.6) was found probably due to a low peak intensity.

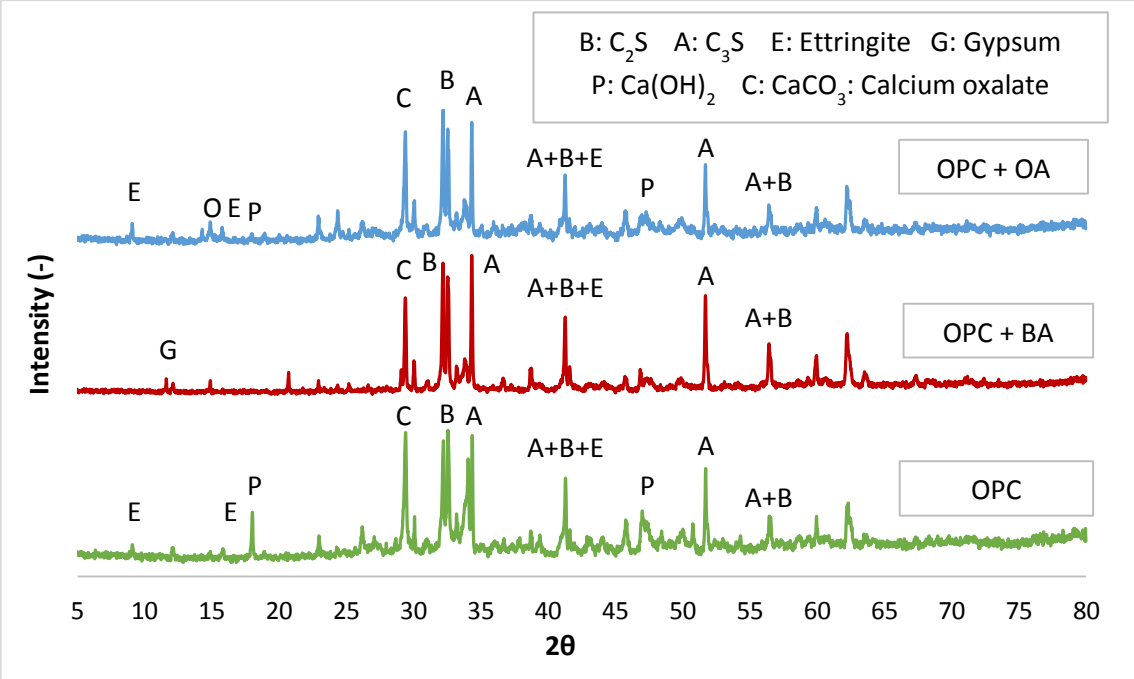

Figure 3.7.- RXD for control specimens (OPC), with addition of boric acid (OPC + BA) and oxalic acid (OPC + OA).

On the other hand, specimens with addition of oxalic acid showed presence of calcium oxalate, as well as a high intensity of ettringite and lower of portlandite in comparison with samples without additives. As mentioned before, calcium oxalate is the precipitation product due to oxalic acid addition. Moreover, Figure 3.8 shows the comparison between samples with 13 % of oxalic acid addition at 1, 7 and 28 days in order to justify variations on peak intensity for ettringite and portlandite.

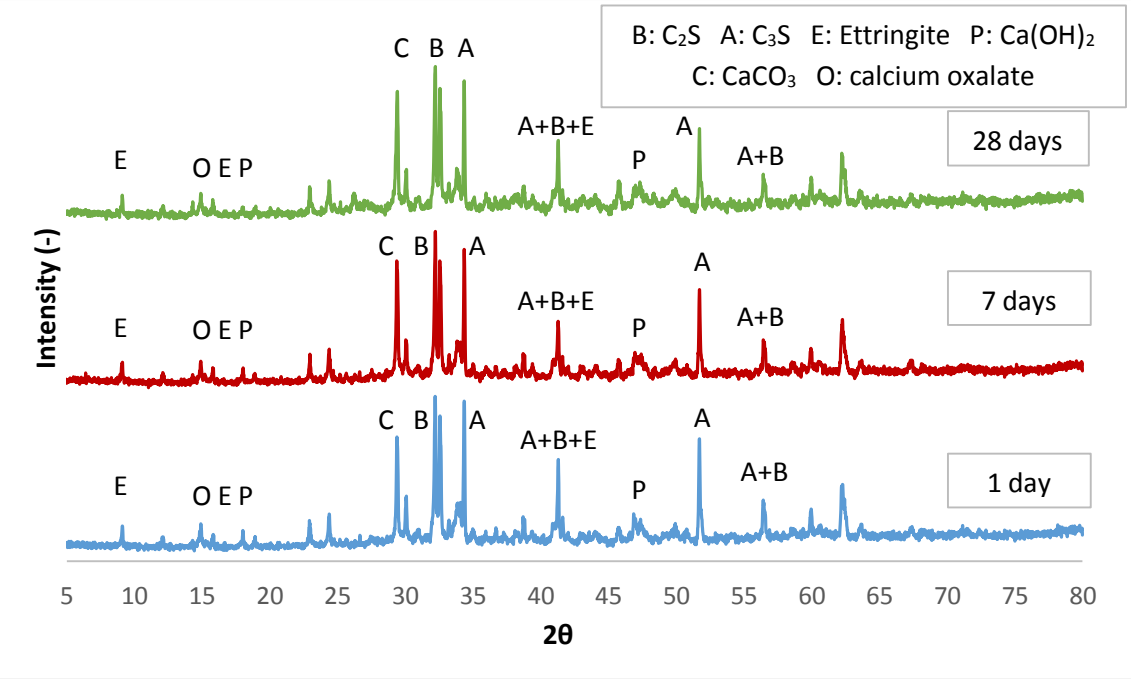

Figure 3.8.- RXD for specimens with addition of oxalic acid at 1, 7 and 28 days.

Analysing first reductions on peak intensity of portlandite on Figure 3.7 and observing evolution in time in Figure 3.8, a progressive reduction in amount of Portlandite is supposed. According to that, formation of portlandite would occur although afterwards it would be consumed.

Moreover, consumption of portlandite could be caused by reaction with oxalate or by reaction with silicates in case dissolution of oxalic acid facilitates this reaction. Finally, ettringite seems to remain constant between 1 and 28 days although peak intensity is slightly higher in comparison to the reference pattern. In fact, the above could be caused by an initial acceleration of hydration of aluminates (Ramachandran, 1976).

Lastly, Figure 3.9 shows FTIR curves comparing anhydrous OPC, hydrated OPC, samples with 8 % of boric acid addition and samples with 13 % of oxalic acid addition. In order to clarify results, two areas were drawn grouping curves with similar pattern. At the top the curves corresponding to hydrated OPC and samples with 13 % of oxalic acid addition are represented while at the bottom the anhydrous cement and OPC with addition of 8 % of boric acid curves are represented. Similarity between samples is clearly evident, concluding that there is no

hydration of samples with higher amounts of boric acid. Furthermore, curves at the top of Figure 3.9 show a similar pattern except for a peak of water at 1623 cm^{-1}, which could correspond to the water contained in the structure probably due to the presence of calcium oxalate. Subsequently, the peak at 1315 cm^{-1} could correspond to calcium oxalate.

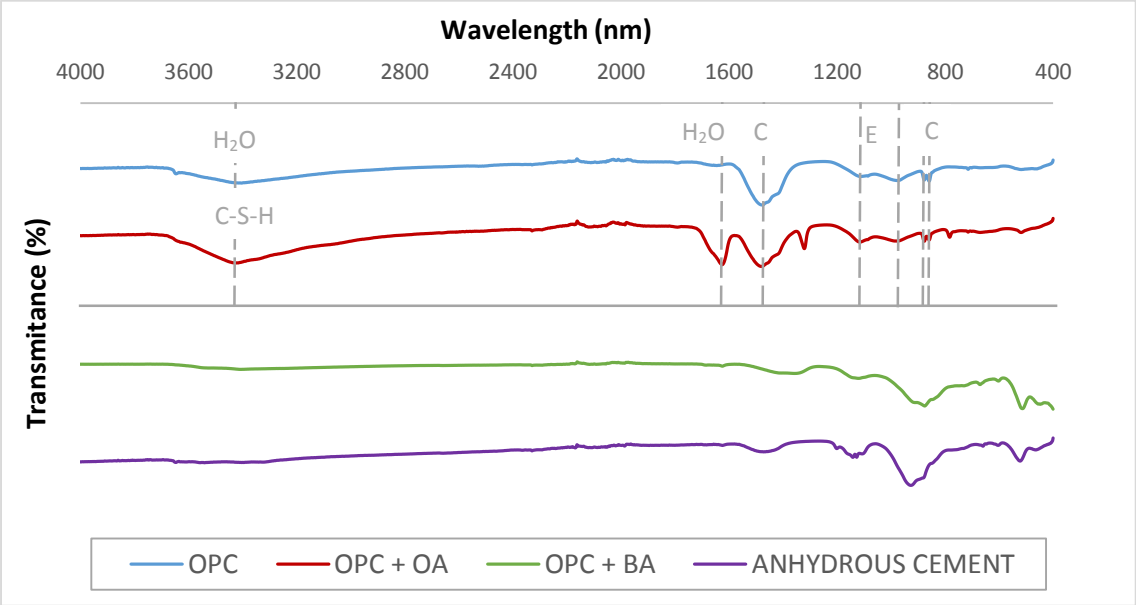

Figure 3.9.- FTIR for specimens control (OPC), with addition of oxalic acid (OPC + OA), with addition of boric acid (OPC + BA) and anhydrous cement.

3.4. RESULTS AND ANALYSIS RELATED TO CHARACTERIZATION OF DIFFERENT MPC FORMULATIONS

As mentioned before, MPC can be formed by different compounds and also ratios between them can vary. According to this, a characterization of five different P:M ratios was carried out. As it was shown in Figure 3.1, physico-chemical and mechanical properties were evaluated in comparison with Portland cement.

3.4.1. pH and real density

pH evolution until 28 days for samples with and without 6 % of borax is shown in Figure 3.10. This hydraulic binder has an evident slightly acidic character, getting pH between 5.5 and 7 for all samples. Moreover, both graphs show that pH decreases as P:M ratio increases and it is probably due to initial pH of each compound. Considering that the trend is maintained in both graphs, with and without borax addition, changes in pH might be consequences of P:M ratio. However, increases in borax additions could also affect the pH of the mixture.

Comparing both graphs, different behaviours between samples with highest and lowest P:M ratios were observed. This fact could be attributable to formation of different crystalline structure depending on P:M ratio and some masking effect of borax addition.

Based on the results, five different P:M ratios were selected (1:1, 1:1.25, 1:1.5, 1:1.75 and 1:2). Addition of 6 % of borax was chosen in order to improve production conditions, mainly regarding setting times. Moreover, selection of the mentioned P:M ratios was based on production, where it was evident that not all $NH_4H_2PO_4$ was diluted for mixtures with higher P:M ratio. The above was observed after mixing, at the moment of filling the moulds. Due to the different densities of different compounds forming the mixture, non-diluted crystals of $NH_4H_2PO_4$ tend to float, forming clearly two different layers per specimen.

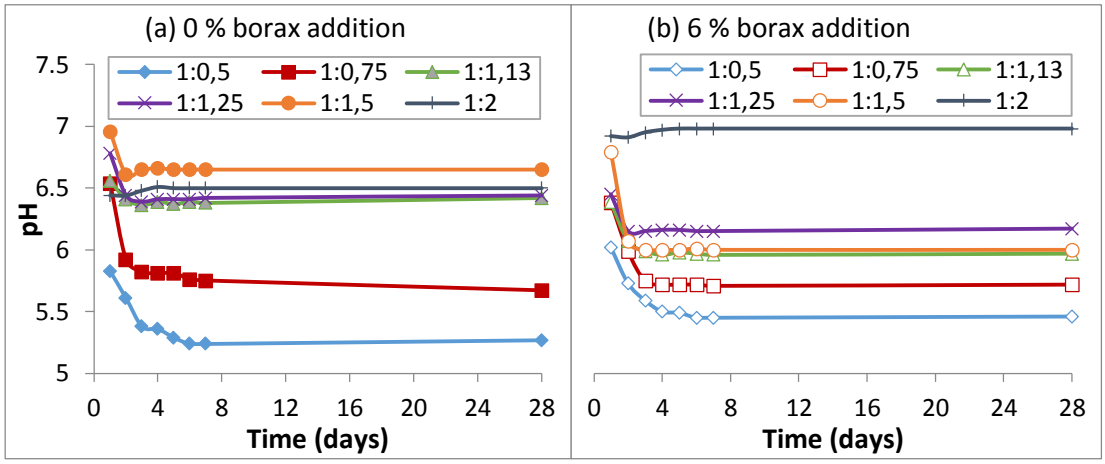

Figure 3.10.- Evolution on time of pH for specimens (a) without borax and (b) with addition of 6 % in borax.

Concerning to MPC real densities, different results were obtained depending on P:M ratios. Higher real density was obtained for the lower P:M ratio (2.69 g/cm³) although this value is still lower than the OPC one.

3.4.2. Normal consistency, setting times and volume stability

Table 3.4 shows results for normal consistency, setting times and volume stability of cement pastes. Normal consistency presents a difference between samples no higher than 1.7 ± 0.5 %. Moreover, values observed for MPC mortars were visibly lower than the reference value for CEM I 52.5R. Differences observed could be consequence of the fluxing effect of borax as well as a lower stoichiometric amount of water required for the hydration process (Sarkar, 1990; Hall et al., 1998; Yang et al., 2000; Hall et al., 2001). Furthermore, it was observed that small increases in water content caused the loss of normal consistency.

Subsequently, setting times were determined. MPC samples presented markedly shorter setting times than OPC, final setting times being shorter than 30 minutes. Moreover, differences observed between initial and final setting times were always less than 5 minutes.

Factors influencing setting time are mainly reactivity of magnesia, fineness of MgO, w/c ratio, amount of retarder and P:M ratio (Popovics and Rajendran, 1987; Hall et al., 1998; Yang and Wu, 1999; Qiao et al., 2009b). Finally, results obtained for volume stability showed no

significant differences between samples of different P:M ratios. In all cases, volume stability values were close to zero, 10 mm being an acceptable value for CEM I 52.5R cement paste.

Table 3.4.- Results of normal consistency, setting times and volume stability for MPC samples and OPC (CEM I 52.5R) as reference values.

P:M	Borax (% ocw)	Normal consistency (%)	Initial setting time (min)	Final setting time (min)	Volume stability (mm)
1:1	6	6.4 ± 0.5	16	20	0.5
1:1.25		6.0 ± 0.5	20	24	
1:1.5		6.4 ± 0.5	21	24	
1:1.75		7.4 ± 0.5	16	18	
1:2		6.8± 0.5	16	19	
OPC		25-35 ± 0.5	110 (min. 45)	170 (max. 720)	Max. 10

3.4.3. Consistency of fresh mortar and hydration heat

Previous works already mentioned w/c ratios used for OPC mortars and concretes were high for MPC production. However, those previous researches were carried out for different MPC compositions. Then, a w/c ratio of 0.5 according to the standard UNE-EN 196-1 was used to produce standardized mortar in order to produce standardized mortar specimens. Segregation was evident during the filling of the mould as well as after demoulding (Figure 3.11). Afterwards, an optimal w/c ratio for each P:M ratio was measured. Although there was a considerably difference between them, a w/c ratio from the lower P:M ratios was selected. The above decision was based not only on economic reasons ($NH_4H_2PO_4$ is more expensive than MgO) but also for a better workability of the mixture. Due to this fact, a w/c ratio of 0.28 was adopted.

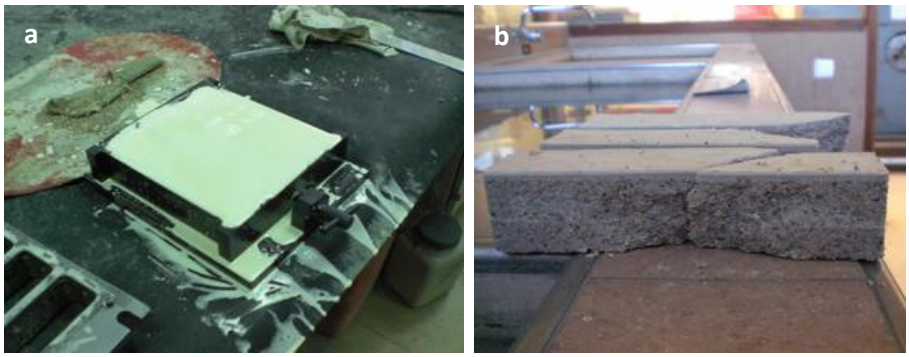

Figure 3.11.- Segregation in standardize mortar production.

Accordingly, new mortar mixtures were produced using the w/c ratio of 0.28 and consistency of fresh mortar was determined. Considering a consistency of 45 % for standardized OPC mortar, it was observed that only MPC mortars with P:M ratios between 1:1.25 and 1:2 obtained a similar consistency.

Figure 3.12 shows the evolution of hydration heat of two MPC samples (P:M ratio of 1:1.25 and 1:2) and the reference sample of OPC. This test was carried out for P:M ratios of 1:1.25, 1:1.5, 1:1.75 and 1:2, but differences between MPC samples were not significant. Because of this, only samples with the extreme ratios are represented in Figure 3.12. Graph (a) shows the curves up to 50 hours while (b) shows only a detail of the first 15 hours.

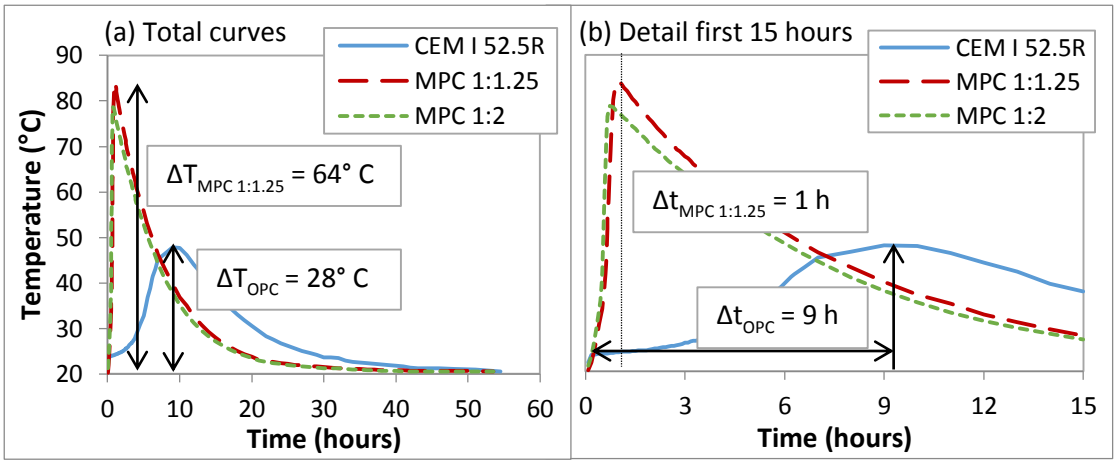

Figure 3.12.- Evolution of heat hydration for reference specimen and MPC with lower and higher P:M ratios.

The hydration heat test showed higher thermal peaks for MPC samples as well as an earlier maximum for the temperature. Maximum temperatures registered for 1:1.25 and CEM I 52.5R represent a difference of 36° C, being 48° C and 84° C respectively. Furthermore, the maximum was observed in the first hour for the MPC sample (1:1.25) and after 9 hours for the reference.

3.4.4. Compressive strength and drying shrinkage

Figure 3.13 presents compressive strength results for MPC samples in comparison with the reference. Reference values show a growing trend reaching the maximum value of around 60 MPa at 28 days. Regarding MPC samples, compressive strength at 1 hour is in a range between 2 and 8.3 MPa for samples with higher and lower P:M ratios respectively. Furthermore, the evolution of compressive strength is faster for these samples, reaching 70 % of the final compressive strength at 1 day. This fact could be related to the fast set, since this behaviour is also observed in other fast-setting cements. Moreover, it is know that an excess of MgO is needed and important in order to guarantee the strength development (Soudée and Péra, 2000).

The last physical test carried out was drying shrinkage of samples with P:M ratio of 1:1.25 and 1:2. Results are shown in Figure 3.14, with graph (a) representing the expansion due to air curing and (b) the expansion as a result of water curing. Generally, OPC samples show shrinkage with air curing and a slight expansion with water curing. MPC samples showed less

shrinkage and expansion than the reference except samples with a P:M ratio of 1:1.25. These samples presented a higher expansion than the reference in water curing until 200 days.

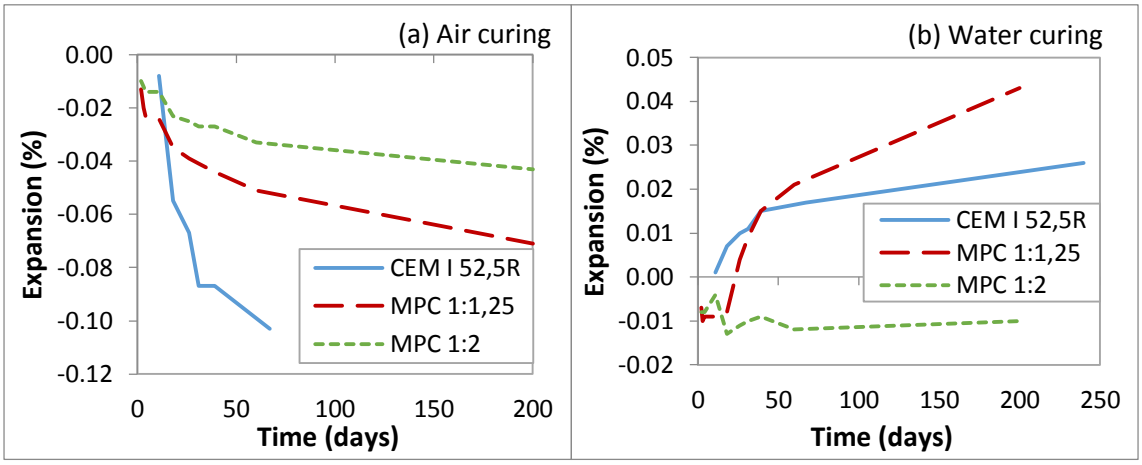

Figure 3.13.- Compressive strength for MPC specimens and reference OPC values.

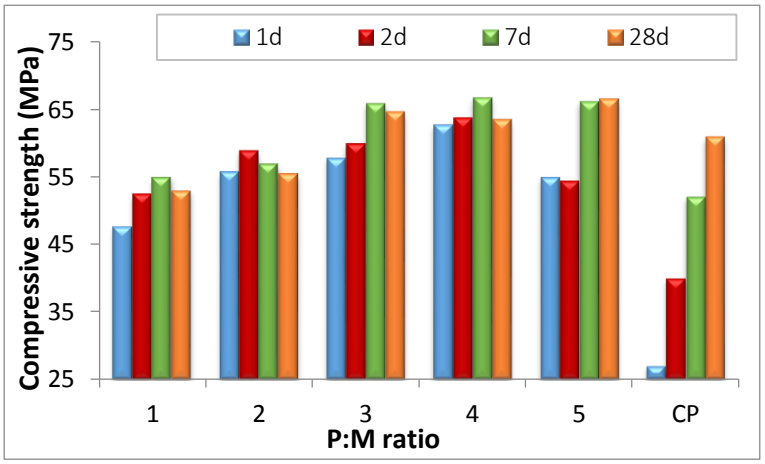

Figure 3.14.- Expansion of specimens after (a) air curing and (b) water curing.

Figure 3.14 might reflect a better volume stability of samples as MgO concentration is increasing. The behaviour of the reference after 67 days being unknown, it is not possible to determine if MPC with 1:1.25 P:M ratio will achieve similar shrinkage values. However, results at 200 days showed lower shrinkage than the reference at 67 days, this fact being a positive achievement.

Moreover, the graph on the right shows a slight shrinkage for 1:2 MPC samples while 1:1.25 samples exceed the expansion of the reference. These samples presented an expansion value at 60 days equivalent to 150 days value for the reference. Furthermore, it is thought that shrinkage in MPC samples could be caused by drying or autogenous shrinkage. Finally, expansion in MPC samples may be consequence of hydration of anhydrous cement compounds as well as formation of intermediary compounds by water incorporation.

3.4.5. Scanning electron microscopy and chemical analysis

Figure 3.15 shows scanning electron microscopy (SEM) images corresponding with different fragments of MPC samples at 1 day. Images a, b, c and d correspond with fragments of samples 1:1, 1:1.25, 1:1.75 and 1:2. First two images show a glassy appearance and no presence of struvite crystals was detected. As mentioned before, samples with higher P:M ratios showed a drier workability indicating the necessity of a higher amount of water. Furthermore, knowing that struvite has 6 molecules of water in its structure, this appearance and absence of struvite may be due to the lack of water for consumption. On the other hand, images corresponding to samples with lower P:M ratios show a completely different appearance, where the presence of struvite crystals is manifest.

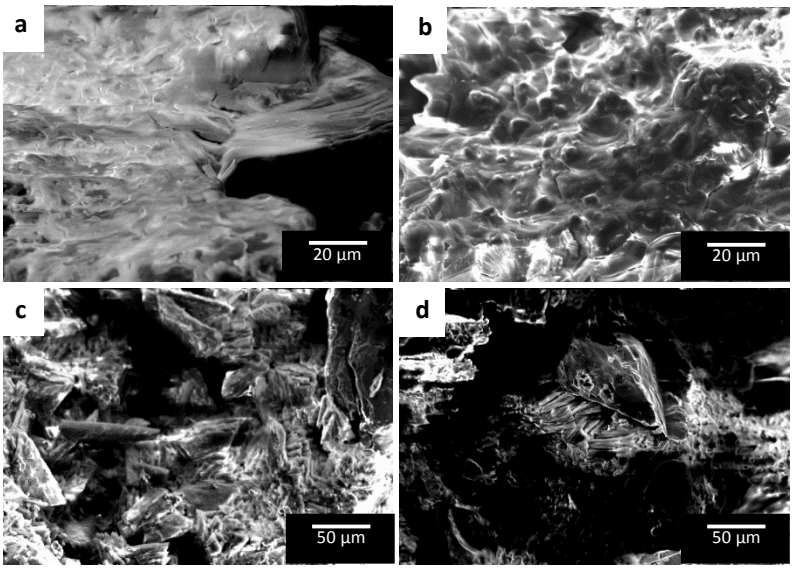

Figure 3.15.- SEM images of specimens with a (a) P:M ratio of 1:1, (b) P:M ratio of 1:1.25, (c) P:M ratio of 1:1.75 and (d) P:M ratio of 1:2 at one day.

Table 3.5 shows the comparison between the chemical composition that is usual and acceptable for OPC (CEM I 52.5R) and experimental results for MPC samples. Nevertheless, determination of carbon, hydrogen, nitrogen and mercury was only carried out on MPC samples. Taking into account the different nature between both types of cement, results should be interpreted as informative. However, SO_3, chlorides, loss on ignition and insoluble residue should be considered as exceptions due to standards for cementitious materials.

All MPC samples exceeded the amount of MgO, free CaO and loss on ignition defined in the standards although this should not be a problem for its use. In the specific case of MgO, traces of MgO in OPC and low-reactivity MgO used for production of MPC are significantly different. Similarly, free CaO has low reactivity avoiding chemical incompatibilities between both of them. Moreover, free CaO detected comes from impurities of the product.

Finally, loss on ignition for OPC is regulated due to its cause. Fundamentally, it is caused by presence of water, organic matter and other non-contributory impurities. However, the cause of loss of ignition for MPC is mainly the degradation of ADP releasing ammonium and water.

Table 3.5.- Chemical composition of MPC samples and OPC (CEM I 52.5R) as the reference value (LI: Loss on ignition; IR: Insoluble residue; R_2O_3: Al_2O_3 + Fe_2O_3)

Compound	1:1	1:1.25	1:1.5	1:1.75	1:2	OPC
Free CaO (%)	0.2	0.1	0.5	0.1	0.3	< 0.1
SiO_2 (%)	0.1	0.1	0.1	0.1	0.1	18-26
R_2O_3 (%)	0.4	0.4	0.4	0.3	0.3	4-14
MgO (%)	48.3	53.8	59.8	62.5	77.0	0-5
SO_3 (g)	0.01	0.01	0.02	0.02	0.02	< 4
Cl- (%)	0	0	0	0	0	< 0.1
LI (%)	18.8	16.7	19.5	16.1	18.4	< 5
IR (%)	0.1	0.1	0.1	0.1	0.1	< 5
C	0.4	0.4	0.4	0.4	0.4	-
H	2.88	2.47	2.43	2.26	1.41	-
N	6.56	5.50	5.34	4.92	2.90	-
Hg	0.03	0.03	0.02	0.02	0.02	-

3.4.6. XRD

Figure 3.16 shows the comparison between different MPC formulations. Three compounds were undoubtedly detected: struvite, dittmarite and periclase. However, other compounds could be also present and not detected due to coincidence on peaks position. Presence of periclase reveals an excess of this product and the other two compounds are the most common hydration products.

Comparing different formulations, a significant decrease of peak intensity for dittmarite seems to be related to a decrease of P:M ratio. In order to justify these results, stoichiometric water necessary for the hydration process was estimated. The main reaction is as follows:

$$MgO + NH_4H_2PO_4\ 5H_2O \rightarrow NH_4MgPO_4 \bullet 6H_2O \qquad [3.10]$$

From equation 3.10, it is clear that the stoichiometric molar ratio between MgO, ADP and water is 1:1:5. However, in order to estimate if a w/c ratio of 0.28 introduced an excess or deficit of water, only the limiting factor, ADP, was considered. Furthermore, water coming from borax was also estimated, which represented extremely small amounts due to the percentage of addition.

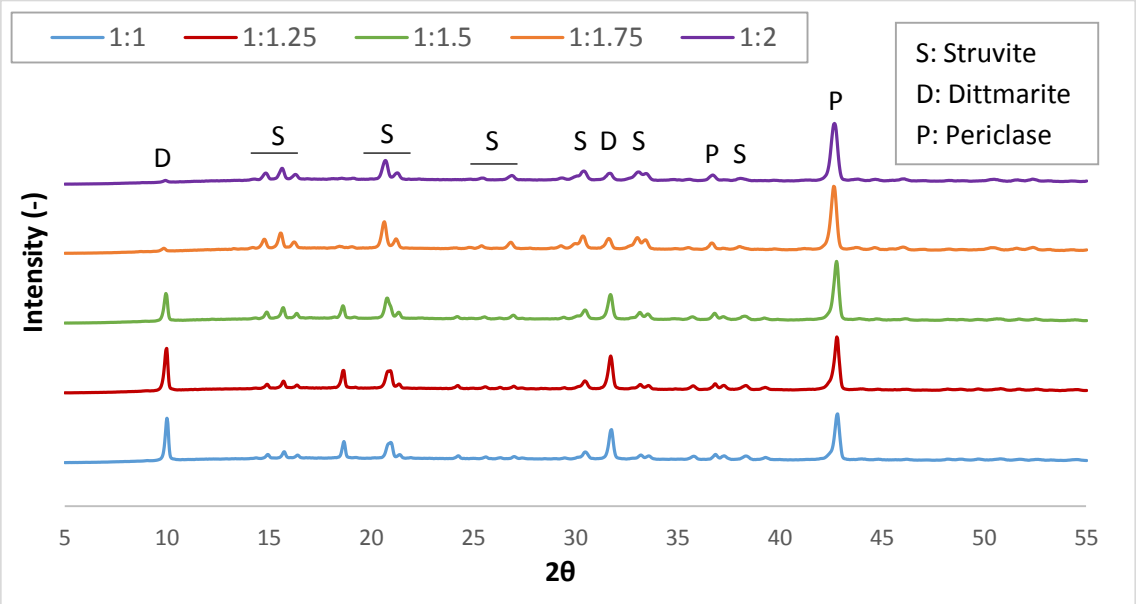

Figure 3.16.- XRD of different MPC formulations at 7 days.

Related to results observed in the drying shrinkage test and taking into account the dosage used, the molar mass of available water was estimated. Subsequently, both quantities were compared and results showed a deficit in water for mixtures 1:1, 1:1.25 and 1:1.5, concurring with higher signal strength of dittmarite. Mixtures with P:M ratios of 1:1.75 and 1:2 had a slight excess of water and the intensity of the dittmarite peak is significantly lower. Nevertheless, presence of dittmarite could be associated to some minimal loss of water. Moreover, results shown regarding the heat of hydration test reveal maximum temperatures lower than 100° C. However, no adiabatic conditions were achieved; therefore, there is no certainty of the real extreme temperature. Assuming some water evaporation due to hydration heat, intermediate products as dittmarite would not be able to incorporate water to form struvite.

Furthermore, this hypothesis is more consistent taking into account results from the drying shrinkage test. According to Wagh (2004), the final hydration product, struvite, is more stable than dittmarite. Conforming to this, air-curing conditions could be causing the loss of water, whether from pore solution, adsorbed water or molecules of water from intermediate products not detectable in XRD. Moreover, problems to detect them in RXD could be caused by two reasons; because of their amorphous nature or low signal strength. Likewise, expansion in water curing conditions could be consequence of struvite formation. Dittmarite could incorporate five molecules of water becoming struvite and this transformation would involve a volume expansion due to different molar volumes of both crystals. Considering that dittmarite has a molar volume of 71.02 cm^3 compared to 127.78 cm^3 for struvite, this modification results in a volume increment of 80 %.

Figure 3.16 also shows that samples with lower P:M ratios are more stable due to predominance of struvite as a crystalline phase. According to Sarkar (1990), temperatures up to

50° C are necessary to cause struvite loss of water. However, higher P:M ratios showed a bigger signal strength for dittmarite, suggesting higher possibilities of transformations and consequently volumetric changes.

3.5. CONCLUSIONS

The present research has studied different ways in order to develop a low-pH cementitious material. Trials to decrease pH of OPC and characterization of MPC to evaluate its suitability for construction and industrial applications were carried out.

Concentrations lower than 2 % of boric acid slowdown the setting and also affect significantly physico-chemical properties of the material, although its pH is still too alkaline. Furthermore, concentrations up to 2 % had the effect of stopping the hydration process. On the other hand, oxalic acid presented a totally different behaviour, not introducing any significant decrease in pH.

According to results and analysis of trials to reduce the pH of OPC based samples, use of OPC with acid additions was discarded. The final pH is not low enough to be used as a biological support and affectation of specimens' properties, mostly mechanical properties, were the main reasons for discarding them.

MPC characterization showed different interesting properties of this material. First, pH range is acceptable for the intended application (5.8 and 7) and comparatively to OPC (CEM I 52.5R), MPC requires lower amounts of water for hydration. Consequently, this fact is positive in order to reduce the amounts of water needed in industrial production. However, a slightly bigger water demand was observed for lower P:M ratios than for higher ones. Therefore, low setting times were obtained, being still lower for lower P:M ratios. Furthermore, good results for volume stability were obtained. Results were constant independently of P:M ratio and significantly lower than the maximum limit for OPC.

A w/c ratio of 0.28 was fixed thinking on an industrial application. Formulations with lower P:M ratios were the most interesting ones mainly for economic reasons as well as for better workability so the w/c ratio to obtain similar OPC consistency was selected. Consequently, better results were obtained in all characterization tests for formulations with lower P:M ratios. Furthermore, chemical analysis gave an idea about different compounds present in the sample, including percentage, and crystalline products after the hydration process. No presence of dangerous compounds for human health (during production) or structural durability were observed.

To sum up, in order to produce a low-pH cementitious material in compliance with requirements of use, MPC mortar is the most suitable one. Its pH is closer to neutrality independently of the P:M ratio within the range used. Furthermore, it is important to remark that lower P:M ratios are more interesting due to better physico-mechanical properties as well

as from an economic point of view. Accordingly, the P:M ratio 1:1.75 with a 6 % borax addition was selected to continue the research. P:M ratio of 1:2 was discarded since results between these two compositions were quite similar and a better workability was observed for P:M ratios of 1:1.75.

4. BIORECEPTIVITY MODIFICATIONS OF CEMENTITIOUS MATERIALS

4.1. INTRODUCTION

The study of Chapter 3 showed the possibility of using MPC mortars as a low-pH cementitious material. Results obtained in the previous chapter showed that higher P:M ratios could be appropriate for the production of non-structural elements. More specifically, the formulation based on a P:M ratio of 1:1.75 and the addition of 6 % of borax was chosen and it was based on economics as well as properties and workability of the mortar. However, as it was already mentioned in Chapter 2, not only chemical composition and pH define the bioreceptivity of a material. In this sense, the combination of both chemical and physical properties is important to define that term.

The bioreceptivity of cementitious materials has been usually studied to get a better understanding of the microorganisms' colonisation behaviour, as well as for the effect of microorganisms on cementitious materials appearance and properties. Texture, mineralogy, open porosity, roughness of the surface, permeability, and bulk density, between others, are the most studied physical parameters of cementitious materials relating to bioreceptivity (Guillitte and Dreesen, 1995; Tiano et al., 1995; Tomaselli et al., 2000; Miller et al., 2010). However, most of the experts in bioreceptivity repeatedly studied only few of those properties.

In the light of the exposed, the objective of this chapter is to develop materials with different degrees of bioreceptivity to stimulate natural colonisation of living organisms. The

above was carried out by means of changes in porosity and roughness of the hardened material. To achieve this goal, the following specific objectives are defined:

- Define a methodology of dosage to produce mortar specimens made by carbonated OPC and MPC with different roughness and porosity;
- Characterize the different mixtures in terms of porosity and roughness;
- Select the concrete types to be exposed to biological colonisation.

The experimental program was carried out in two different laboratories: the Structures Technology Laboratory Luis Agulló (Polytechnical University of Catalonia) and the Magnel Laboratory for Concrete Research (University of Ghent).

4.2. MATERIALS AND METHODS

4.2.1. Methodology for bioreceptivity modification

First stage of natural colonisation is based on the presence of living organisms in the surrounding environment. Then, contact between them and material's surface is necessary, where roughness of the material plays an important role. Roughness of the material is also important for nutrients accumulation (for instance dust transported by the wind or drops of water). Once the contact is achieved, development of living organisms depends on climate conditions. As already mentioned, wind is the carrier for dust and other nutrients from the environment, which would be retained on the material's surface thanks to the aforementioned roughness. Furthermore, water is also necessary for organisms' development and this mainly depends on the porosity of the material, which will determine the humidity of the material based on the water retention. Consequently, porosity and roughness are two of the most important and studied physical properties of a material. Table 4.1 shows some of the authors who tested one or both parameter in their research.

Table 4.1.- List of authors who studied porosity and roughness related to bioreceptivity

References	Porosity	Roughness
Guillitte and Dreesen, 1995	•	
Tiano et al., 1995	•	•
Tomaselli et al., 2000	•	•
Prieto and Silva, 2005	•	
Miller et al., 2006, 2009, 2010	•	•
Cámara et al., 2008	•	
Giannantonio et al., 2008	•	•
Tran et al., 2012, 2013	•	•

Modifications of the dosage of the different samples were carried out in order to produce specimens with different porosity and roughness. Two different groups of pores

depending on their size can be defined, being microporosity and macroporosity. The microporosity could be modified by means of the use of different w/c ratios while macroporosity can be changed by varying aggregates size and the cement paste content. Accordingly, roughness can be modified mainly by the cement paste content and secondly by the aggregates size.

The tool used to select cement paste contents was the model proposed in the PhD thesis of Klein (2012). Klein studied the physical role of water when covering and wetting the surfaces of different particles of the mixture. Moreover, the method can be extrapolated to the case of cement pastes with the aim of obtaining the minimum amount of cement paste to join all the aggregate particles to produce the mortar. Figure 4.1 shows a scheme of the procedure followed to estimate the different amounts of cement paste.

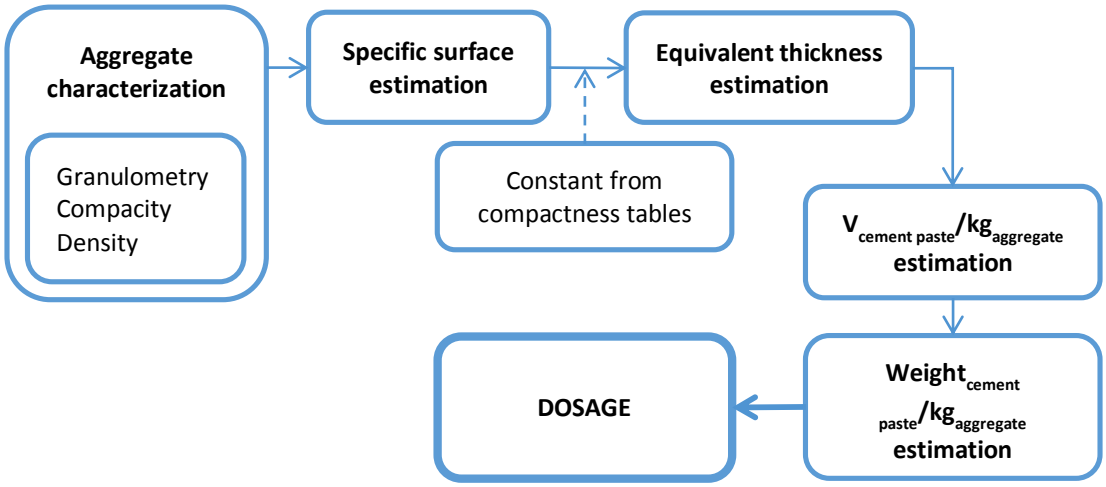

Figure 4.1.- Flow chart of the dosage methodology.

Different parameters should be previously estimated to obtain the value. Klein developed a model considering particles of the same size and consequently the average radius of the aggregates is one of the parameters to estimate. This parameter could be obtained from the granulometry of the aggregates, considering all the particles as spheres. Then, the area of the aggregates surface (m²), SA_{Agg}, is estimated based on the percentage of aggregate retained in a mesh (%), $Agg_{\%}$, and considering the value of the mesh n (M_n), which corresponds to the maximum particle size not retained in the mesh. Subsequently, the total area (m³), Ta, and the average radius (mm), r_a are calculated by the summation of all SA_{Agg} obtained as follows:

$$SA_{Agg} = \frac{M_{n+1} + M_n}{2} \left(Agg_{\%\,(n+1)} - Agg_{\%\,(n)}\right) \quad [4.1]$$

$$Ta = \sum SA_{Agg} \quad [4.2]$$

$$r_a = \frac{\sum SA_{Agg}}{2\,(Agg_{100\,\%} - Agg_{\max\,\%})} \quad [4.3]$$

where $Agg_{100\,\%}$ is 100 % of aggregates (%) and $Agg_{max\,\%}$ is the percentage of aggregate retained in the mesh of bigger diameter considered (%). From this parameter, r_a, area (m²), A_{Agg}, volume (m³), V_{Agg}, and mass (kg), m_{Agg}, of each aggregate particle are estimated to finally obtain the specific surface area (m²/kg), *SSA*, of all of them.

$$A_w = 4\,\pi\,r_a^2 \quad [4.4]$$

$$V_{\Phi_\varepsilon} = \frac{4}{3}\,\pi\,r_a^3 \quad [4.5]$$

$$m_{Agg} = V_{Agg}\,\rho_{Agg} \quad [4.6]$$

$$SSA = \frac{A_{Agg}}{m_{Agg}} \quad [4.7]$$

where ρ_{Agg} is the aggregate density, which corresponds to 2600 kg/m³ for the siliceous aggregate used. Then, the weight of cement paste per kilogram of aggregate is determined. With this purpose, the equivalent thickness (m), t_e, of the cement paste is estimated according to equation 4.8 considering a uniformly volume distribution of the cement paste (m³), V_{cp}, on the surface area (m²/kg), *SA* of 1 kg of aggregates.

$$t_e = \frac{V_{cp}}{SA_{Agg}} \quad [4.8]$$

However, this equivalent thickness depends on the average radius, the compactness, and the spatial organization of the aggregates. Klein presented all the possible values obtained from a model and determining that the equivalent thickness equals to a constant (*k*), which depends on the compactness, multiplied by the average radius as follows:

$$t_e = \mathrm{k}\,r_a \quad [4.9]$$

Consequently, from equations 4.8 and 4.9 and considering that surface area, SA_{Agg}, equals to specific surface area, *SSA*, multiplied by the amount of aggregate (*Agg*), the final equation to estimate the volume of cement paste per kilogram of the aggregate used, is:

$$\frac{V_{cp}}{SA_{Agg}} = k \cdot SSA \cdot r_a \quad [4.10]$$

In this context, the average radius was obtained from the granulometry of both aggregates. Figure 4.2 shows both granulometric curves and the average radius obtained for aggregate 0/2 mm and 2/4 mm, which were 0.58 mm and 2.90 mm respectively. Afterwards, compactness was determined according to the relevant standard (UNE 7088), obtaining a compactness of 0.67 and 0.62 for aggregates of 0/2 mm and 2/4 mm respectively. Finally, the last parameter required from the aggregates' characterization was the density, which corresponds to 2630 kg/m³.

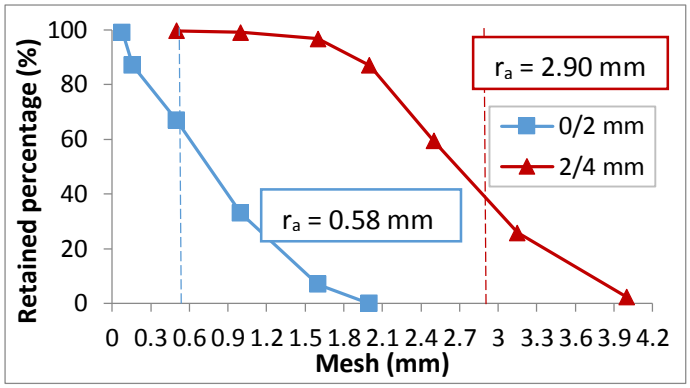

Figure 4.2.- Granulometry of the silica aggregates used (0/2 mm and 2/4 mm).

Subsequently, considering the compactness of each aggregate, *k* was obtained from table 4.3 of Klein's thesis, with a value of 0.115. However, the value of *k* used was slightly higher, 0.169, to obtain a high porous material but having more cement paste to improve material's properties.

According to equation 4.10, the final volume of cement paste per kilogram of aggregate to join all the aggregate particles was 1.93 x 10^4 m^3/kg corresponding to 0.299 kilograms of cement paste per kilogram of aggregate. No differences were observed in this value for each aggregate sample, since both samples had similar specific surfaces and compactness. The value obtained from equation 4.10 was considered as a reference value.

Regarding the roughness of the specimens' surface, previous hypothesis pointed out that specimens with lowest cement paste content and highest aggregate size will obtain higher values. Accordingly, higher porosity is expected for OPC and MPC specimens with lowest cement paste content and 2/4 mm aggregates.

4.2.2. Materials and dosages

Related to materials, CEM I 52.5R was the hydraulic binder for OPC specimens' production. MPC specimens were produced with Ammonium Dihydrogen Phosphate (ADP, $NH_4H_2PO_4$) and Magnesium oxide (MgO) maintaining an P:M ratio of 1:1.75 as concluded at the end of the previous chapter. Moreover, a 6% of borax ($Na_2B_4O_7{\cdot}10H_2O$) was added to the mix as a retarder. Additionally to the hydraulic binders, two different particle sizes of siliceous aggregates were used (0/2 mm and 2/4 mm), as well as distilled water.

Three different considerations to compose both kinds of mortar specimens (OPC and MPC specimens) were taken into account. First, two different aggregate sizes were used in order to modify macroporosity of the specimens. Then, three different w/c ratios for each kind of cement were defined. Regarding OPC, three different values in the range between 0.3 and 0.6 were selected: 0.3, 0.4, and 0.6. In contrast, w/c ratios used for MPC production were significantly lower. As mention in the previous chapter, a w/c ratio of 0.28 was fixed and small

increments of this ratio produced loss of consistency and segregation. Therefore, w/c ratios lower than or equal to 0.28 were selected: 0.15, 0.20, and 0.28. By these different w/c ratios, modifications of microporosity of the specimens were achieved.

Finally, different cement paste contents were used. Regarding the OPC samples, the reference value obtain from equation 4.10 was contemplated as the minimum cement paste content needed to bring together the aggregate particles. That minimum cement paste content theoretically corresponds to the maximum value of porosity for a given sample of that material. Afterwards, increments of 25 %, 50 %, and 75 % were considered to obtain a variety of porosities for this material; increasing the amount of cement paste will be related with reductions in the porosity of the material.

In contrast, for the MPC samples, first trials of specimens' production showed that the value obtained from equation 4.10 produced quite dense specimens with low macroporosity. A possible explanation is the use of borax as a retarder, which also acts as a fluidizer and modifies the viscosity of the cement paste. Therefore, the value of cement paste content was considered as the amount of cement past needed to obtain a dense specimen; using again that value as a reference, reductions of 25 % and 50 % were considered to obtain high-porosity specimens. Table 4.2 and Table 4.3 present the combinations of aggregate sizes, w/c ratios and cement paste contents for each hydraulic binder. "P" and "M" designates the hydraulic binder used corresponding to OPC and MPC respectively.

Table 4.2.- Dosage for OPC specimens production

Specimen	Type of cement	Aggregate size (mm)	w/c ratio	Cement paste (%)
Pa40-1C	Ordinary Portland Cement (OPC)	0/2	0.4	Reference
Pa40-1.25C				Increment of 25 %
Pa40-1.5C				Increment of 50 %
Pa40-1.75C				Increment of 75 %
Pa60-1C			0.6	Reference
Pa60-1.25C				Increment of 25 %
Pa60-1.5C				Increment of 50 %
Pa60-1.75C				Increment of 75 %
PA30-1C		2/4	0.3	Reference
PA30-1.25C				Increment of 25 %
PA30-1.5C				Increment of 50 %
PA30-1.75C				Increment of 75 %
PA40-1C			0.4	Reference

Table 4.3.- Dosage for MPC specimens production

Specimen	Type of cement	Aggregate size (mm)	w/c ratio	Cement paste (%)
Ma20-0.75C	Magnesium Phosphate Cement (MPC)	0/2	0.2	Reduction of 25 %
Ma20-1C				Reference
Ma28-0.5C			0.28	Reduction of 50 %
Ma28-0.75C				Reduction of 25 %
Ma28-1C				Reference
MA20-0.5C		2/4	0.2	Reduction of 50 %
MA20-0.75C				Reduction of 25 %
MA20-1C				Reference
MA15-0.5C			0.15	Reduction of 50 %
MA15-1C				Reference

Furthermore, the letter “a” or “A” was used to differentiate both aggregate sizes, corresponding to 0/2 mm and 2/4 mm respectively. Then, the two-digit-number refers to the w/c ratio, using for instance 40 for the w/c ratio of 0.4. Finally, “C” designates the reference value of cement paste content multiplied by a factor to indicate the different cement paste contents. For instance, a specimen designed as Ma28-0.5C means a MPC specimen with 0/2 mm aggregate size and with the 50 % of the reference cement paste content.

4.2.3. Specimens production and tests methodology

40 x 40 x 160 mm³ OPC specimens according to the standard UNE-EN 196-1 were produced. 40 x 40 x 160 mm³ MPC specimens were also produced specifically for determination of flexural and compressive strength as the relative standard specifies. However, metallic moulds were covered with plastic to avoid direct contact between MPC mortars and the moulds due to the high adhesion of this material. For this reason, MPC specimens for other tests followed a non-standardized procedure. The fabrication of the other MPC specimens was made in polyurethane elastomer moulds, property of Escofet 1886 S.A. and two different specimens sizes could be produced (80 x 20 x 80 mm³ and 135 x 30 x 285 mm³). Difficulties due to the fast setting time and the amount of specimens required led to the selection of the biggest one to afterwards cut the specimens into smaller ones.

Moreover, times and procedure for mortar production according to the standard UNE-EN 196-1 were not appropriate for MPC. Accordingly, the mixing procedure followed was: mixture of the dry components (ADP + MgO + borax + aggregates) during 30 seconds at low speed (28.5 Hz). Then, distilled water was added to the mix and the mortar was mixed during 60 more seconds at the same speed (28.5 Hz). After those 90 seconds, the Maxine was stopped for the next 45 seconds (removal of the mixture from the mixing container during the first 30

seconds). Finally, another 60 seconds of high speed mixing (52 Hz) were accomplished to guarantee a proper homogeneity of the sample.

Last particularity of the specimens' production was the compactness method used for both OPC and MPC specimens. This fact was consequence of segregation problems observed in specimens with 2/4 mm aggregates. In consequence, a modified method to halve the compaction energy was carried out. First, the compaction energy (kg·cm/cm³), ε_c, was estimated by following the standard, as follows:

$$\varepsilon_c = \frac{(N \cdot n \cdot W \cdot h)}{V} \quad [4.11]$$

where *N* corresponds to the number of the piston stroke, *n* the number of compacting layers, *W* is the piston weight (kg), *h* the height (m) and *V* the volume of the mould (m³).

To avoid the segregation of the samples it was considered to half the compaction energy and the corresponding apparatus was designed (Figure 4.3). The apparatus consisted on two parts: first a metallic cylinder, which fixed the position and the height and second a metallic plate joint to a tube, which to favoured the movement. Finally, OPC and MPC specimens were demoulded after 24 hours and 1 hour respectively and placed in the humid chamber (20 ± 2° C and 98 ± 2 % RH) until a maximum of 28 days.

Figure 4.3.- Apparatus used to compact.

Regarding test methodology, measurements of **flexural and compressive strengths** as additional information were obtained. In the previous chapter, flexural and compressive strengths were obtained although for a different dosage, which will lead to different results. Moreover, information about those parameters for specimens older than 28 days for standardized specimens was considered interesting. MPC specimens were produced to be tested at 1, 7, 28, 90 and 180 days per triplicate and OPC specimens per triplicate, were tested at 80 days.

During production of 135 x 30 x 285 mm³ specimens, **heat of hydration** of MPC specimens was recorded. Previous chapter showed high temperature development during hydration in semi-adiabatic conditions compared to standardized OPC samples. Therefore, the maximum temperatures reached in real production conditions were quantified. The equipment used to record time-temperature profiles was a Squirrel Data Logger with sensors introduced in the centre of the specimen. Measurements were saved every 5 seconds until demoulding.

After demoulding, MPC specimens were cut as mentioned before to obtain smaller specimens with a final size of 135 x 30 x 150 mm³. Then, **porosity** of all specimens was determined by determining the percentage of voids according to the ASTM C642-13. The saturated dry surface weight is one of the parameters required to estimate the percentage of voids. However, specimens with higher porosity, generally the ones with 2/4 mm aggregates and lower cement paste amount, presented problems in water retention. The above made it impossible to obtain a reliable weight for the saturated specimen with dry surface. Consequently, Archimedes' principle was applied in order to avoid loss of water during the weighting process. That principle states that a body immersed in a fluid is buoyed up with a force *F* equal to the weight of the displaced fluid. Accordingly, this force can be estimated as follows:

$$F = V_s \cdot \rho_f \cdot g \quad [4.12]$$

$$F = W_{ds} - W_h \quad [4.13]$$

where V_s is the volume of the specimen (m³), ρ_f the density of the fluid (kg/m³), g the gravity (m/s²), W_{ds} the weight of the dry specimen (kg) and W_h the hydrostatic weight (kg). Combining these two equations (4.12 and 4.13) results in equation 4.14:

$$V_s = \frac{W_{ds} - W_h}{\rho_f \cdot G} \quad [4.14]$$

Additionally, the volume of voids can be estimated by subtracting the volume of the specimen from the geometrical volume. The results of both methodologies (ASTM C642-13 and Archimedes) are compared in order to show differences between those methods. Afterwards, Mercury Intrusion Porosimetry (MIP) was carried out to obtain more information about the specimens with the most different visual aspect in terms of open porosity and roughness. This method allows characterization of microporosity and gives information about pore distribution, giving information about porosity of the cement paste.

Due to the non-wetting property of mercury, as well as no capacity of spontaneous penetration in pores by capillary action, it must be forced into the pores by the application of external pressure and that pressure is inversely proportional to the size of the pores. The subsequent analysis allows obtaining the pores volume and sizing distributions from the pressure versus the intrusion data by means of the use of the Washburn equation. The aforementioned equation defines the applied pressure (Pa), *P*, to introduce the mercury into the pores as follows:

$$P = \frac{-4\,\gamma\,\cos\theta}{D} \quad [4.15]$$

where γ is the surface tension of mercury (N/m), ϑ is the contact angle between mercury and the solid surface, and D the pore diameter (m). By measuring the volume of mercury intruded into the material at each pressure step, the volume of pores in the corresponding size class can be determined.

Different classifications for pores size in cementitious materials are present in the scientific and technical literature. The one proposed by Mindess et al. (2002) classifies pores in concrete in six groups by their pore diameter (Ø):

- Interlayer micropores: Ø < 0.5 nm
- Micropores: 0.5 < Ø < 2.5 nm
- Small capillary pores (gel): 2.5 < Ø < 10 nm
- Medium capillary pores: 10 < Ø < 50 nm
- Big capillary pores: 50 nm < Ø < 10 µm
- Trapped air: 0.1 < Ø < 1mm

The **roughness** of specimens' surface was determined for the same dosages as MIP by means of a high precision laser beam mounted on an automated laser measurement table developed at Ghent University (De Belie et al., 2004), which is presented in Figure 4.4. From the measurements of the surface profile, the centre-line roughness (mm), R_a, can be calculated according to the standard BS 1134 (Figure 4.4). Additionally, the root mean square roughness (mm), R_q, is also presented. For the determination of the roughness, 72 surface profiles were obtained from three replicates for each of the six compositions (four profiles per specimen with profile length 8 cm and 1.5 cm between each profile). For each profile, after correction for the slope, three R_a-values were measured over separate parts of the profile (reference length: 40 mm). The R_a-values were calculated as the average of the 12 R_a-values obtained per specimen type.

Finally, **differential thermal analysis and thermogravimetry (DTA-TG)** was carried out to characterize the hydrated compounds of the matrix. In contrast, obtaining the same results by means of XRD presents the problem of the high intensity peak corresponding to presence of silica aggregates if samples are not previously sifted. DTA gives information about thermal changes occurring in the sample during heating by comparison to a reference sample. TG measures the change in mass of the sample as a function of time over a temperature range using a predetermined heating rate. Samples were heated until 1100° C with a heating rate of 10° C/min, under a N_2 flux of 100 mL/min.

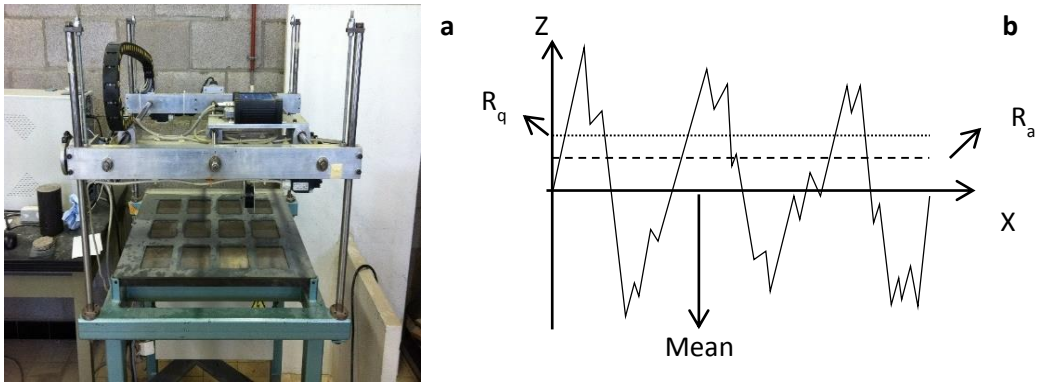

Figure 4.4.- Image of the high precision laser beam (a) and graphical explanation of roughness parameters (R_a and R_q values) (b).

Combination of the results of DTA and TG allows the characterization of the hydrated compounds of a sample. Those results are also used to quantify the loss of weight corresponding to dehydration, dehydroxylation, and/or decarboxylation. For OPC samples, *dehydration* takes place bellow 400° C, the loss of free water occurring bellow 100° C. Then, the loss of weight from 100° C to 400° C is related to the CSH gel and AFt and AFm phases although it is difficult to differentiate in mortars which loss of weight corresponds to each phase.

Next, *dehydroxylation* takes place between 400° C and 500° C corresponding to portlandite decomposition. Next, a characteristic peak related to the *α-β SiO_2 transformation* of siliceous aggregates at around 575° C is present in DTA curves. Finally, *decarboxylation* occurs between 600° C and 800° C corresponding to calcite, dolomite and magnesite decomposition. In contrast, loss of weight observed for MPC samples corresponds mainly to dehydration, which may be due to the chemical composition of the predominant hydrated compounds as struvite ($NH_4MgPO_4 \cdot 6H_2O$).

Quantification of the loss of weight without aggregate's contribution requires a calibration of the method and this is obtained thanks to the presence of silica aggregates. The amount of aggregate (SiO_2) present in the sample is determined from different DTA curves corresponding to different known amounts of aggregate. Then, by measuring the area under the curve of the peak around 570° C, which corresponds to the α-β transition of the silica aggregates, and given the amount of SiO_2 of the DTA-TG samples, it is possible to determine the amount of cement paste, subtracting the amount of SiO_2 from the initial amount of sample as follows:

$$W_{cp} = W_i - (60.0843 \cdot M_{SiO2}) \quad [4.16]$$

where W_{cp} is the weight of cement paste (g), W_i the initial weight (g) and M_{SiO2} the amount of SiO_2 in moles. The loss of weight obtained by means of thermogravimetry corresponds to each temperature interval fixed previously, which is finally expressed in moles of each component (1 mole of H_2O is 18 g/mole and 1 mole of CO_2 is 44.01 g/mole). Finally, it is possible to convert moles into milligrams.

4.3. RESULTS AND ANALYSIS

This subsection presents all the results as well as the analysis of all the data obtained. Discussion of the results will be presented for each test separately. For every one a constant methodology will be considered as follows: a) the influence of the amount of cement paste, b) the influence of w/c ratio variations, c) the influence of aggregates sizes and, finally, the influence of the hydraulic binder used if necessary. Subsequently, comparisons between results of different tests will be analysed.

4.3.1. Heat of hydration

Previous chapter showed results for heat of hydration in semi-adiabatic conditions. However, only results for samples of 1:1.25 and 1:2 P:M ratios were shown. Figure 4.5 shows the results for the first 2 hours of the test, were the maximum temperature was already reached. The curve shows a fast increase of temperature during the first 50 minutes approximately before reaching the maximum temperature of 80.7° C.

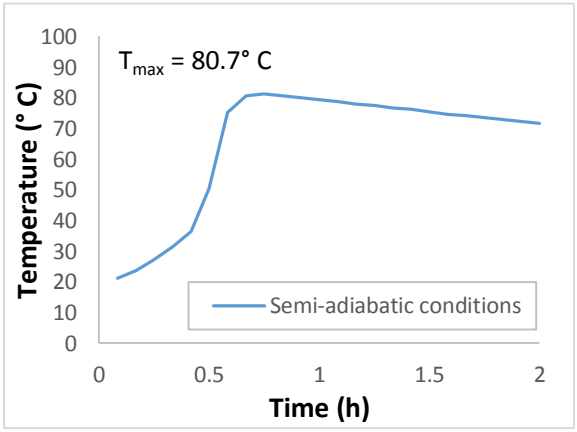

Figure 4.5.- Heat of hydration under semi-adiabatic condition for MPC mortar (aggregate:MgO:ADP:borax:water = 8.3 : 1.8 : 1 : 0.2 : 0.8).

The information extracted from heat of hydration curves is mainly: the maximum temperature reached and the time of that maximum. In the current experimental program, three variables were changed to produce different dosages. Considering the variables (size of the aggregates, w/c ratio and cement paste content), cement paste content will be the parameter that most affects the heat of hydration. Maximum cement paste contents are expected to show the highest maximum temperature, which will be reached earlier. Then, w/c ratio is also expected to influence the moment in which the maximum temperature is reached. In contrast, silica aggregates are not expected to influence heat of hydration.

Figure 4.6 shows heat of hydration of all dosages classified by the cement paste content in order to show the influence of this parameter. First, Figure 4.6 (a) shows curves corresponding to dosages with half of the reference cement paste. Then Figure 4.6 (b) shows curves of dosages with 75 % of the reference cement paste content and, finally, Figure 4.6 (c) shows curves

corresponding to dosages with the reference cement paste content. Moreover, the average curve of each cement paste content is also shown in each graph. Maximum temperatures observed for each curve show an increasing trend as cement paste contents rise.

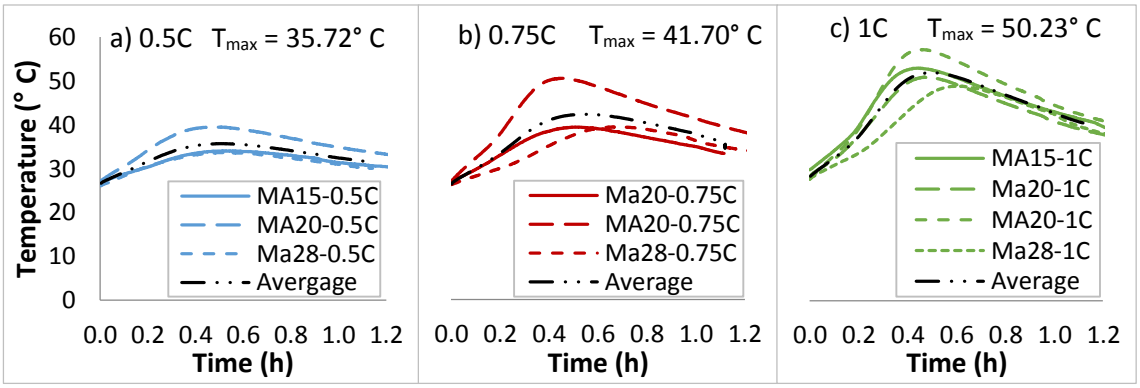

Figure 4.6.- Influence of cement paste content on heat of hydration.

Exothermal reactions during hydration are direct consequence of the amount of hydraulic binder. Consequently, higher cement paste contents provoke a highest maximum temperature and thus, by increasing the cement paste content higher maximum temperatures shall be seen. Cement paste:aggregate ratio was maintained for each cement paste content, just modifying w/c ratios between dosages with the same amount of cement paste. Therefore, there are slight differences in cement:aggregate ratio (considering cement as the addition of MgO, ADP and borax), which may be inducing those minimal differences. Furthermore, the above may be also consequence of difficulties to fix the position of the sensors. The thickness of these specimens was 3 cm and consequently, small differences on the distance between the sensor and the surface could favour the heat dissipation.

According to Hall et al. (1998), increments of w/c ratio led to an increase on hydration heat and to evaporation of excess water. Moreover, the above also increased setting times, slowing the heat evolution, and increasing the maximum temperature. Figure 4.7 shows the influence of w/c ratios on heat of hydration. Figure 4.7 (a) presents the curves regarding w/c ratio of 0.15, Figure 4.7 (b) the ones corresponding to w/c ratio of 0.2 and Figure 4.7 (c) curves of 0.28 w/c ratio.

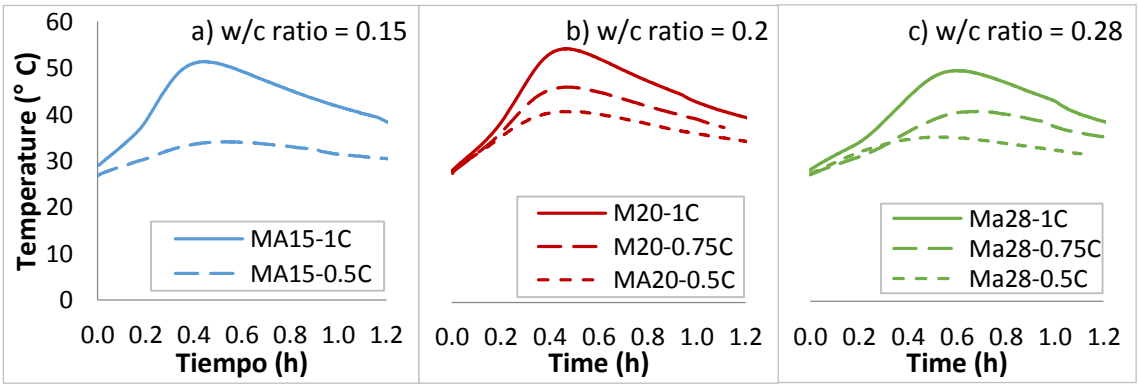

Figure 4.7.- Influence of w/c ratio on heat of hydration.

All three graphs show the same trend, where dosages with higher cement paste contents present higher temperatures. However, no increment in the maximum temperature was observed when the w/c ratio increased and the slowing of the process is not clear from these results. In this sense, more replicates would be necessary in order to get more information about the process in MPC mortars due to the fact that it replies to a complex mixture of parameters.

Comparison between Figure 4.5 and Figure 4.6 shows two clear aspects. First, the maximum temperature in real production will never reach the maximum obtained under semi-adiabatic conditions. Secondly, due to the contact between the mortar and the environment, room temperature was achieved much earlier than in semi-adiabatic conditions. Those facts are positive aspects and especially due to the highest temperature reached in the current experimental program, which was around 50° C.

4.3.2. Flexural and compressive strength

Figure 4.8 shows results of compressive and flexural strengths at 80 days of all OPC specimens. The X axis shows the factor multiplying the reference cement paste content value in order to show the trend of compressive and flexural strengths while increments of cement paste content are applied. These results not only give information about differences due to dosages, but also give reference values for the analysis of MPC compressive and flexural strengths. In general, different amounts of cement paste for the same dosage were tested and they correspond to the curves represented. However, just one cement paste content was tested for the dosage with 2/4 mm aggregate size and a w/c ratio of 0.4. That dosage is represented by a point, corresponding to the lowest amount of cement paste used for the production of OPC specimens.

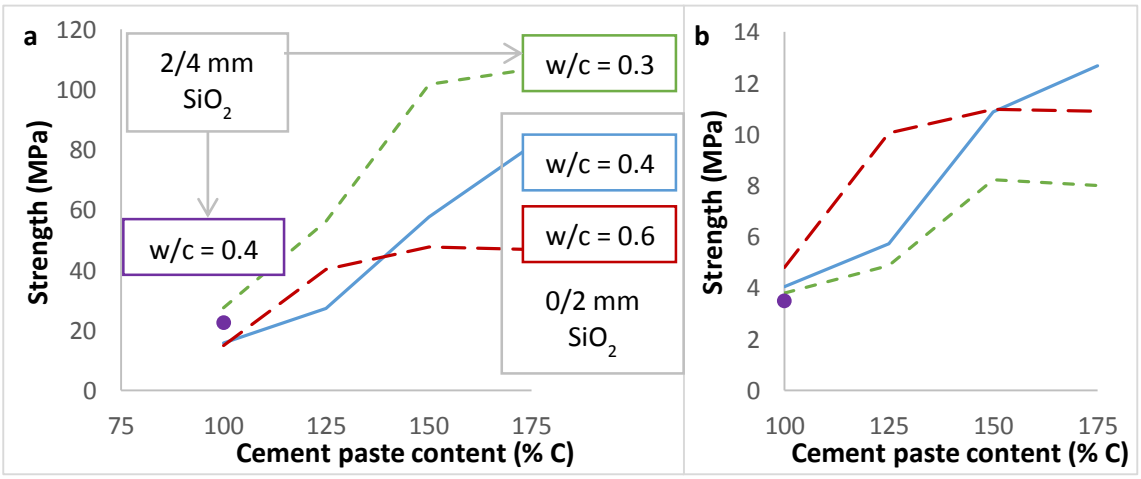

Figure 4.8.- Influence of the cement paste content on compressive (a) and flexural (b) strength of OPC specimens at 80 days.

Both graphs show the same trend, increments of cement paste content imply increments in compressive as well as in flexural strengths until arriving to a saturation point. That point means that higher cement paste contents will not provoke an increment of compressive and/or flexural strengths. Considering the three curves, two of them seem to reach that point while the strength of the dosage corresponding to the 0/2 mm aggregates size and w/c ratio of 0.4 seems to continue growing.

Furthermore, w/c ratios seem to have more influence on compressive and flexural strengths for higher cement paste contents. The above is visible by comparing differences in results of different dosages with the lowest cement paste content, and comparing the differences between the results of specimens with the highest amount of cement paste. For the first case, difference between the lowest and the maximum compressive strength corresponds to an increment of around 85 %, when for the highest cement paste content, the difference is around 130 %. In accordance, the same behaviour is observed for flexural strength results, with increments of around 37 % and 59 % for lower and higher cement paste contents respectively.

Regarding differences due to the aggregate size used, compressive strength results may be higher for lower w/c ratios, corresponding to a less porous matrix of the material. However, results corresponding to dosage Pa40-1.25C seem to be anomalous due to the low result obtained. Moreover, the aforementioned trend is not accomplished for flexural strength results. In that case, curves corresponding to dosages with the smaller aggregate size obtained the highest values.

Regarding MPC specimens, the comparison between compressive strengths at 28 and 180 days did not show significant differences. Consequently, same analysis is presented in Figure 4.9 regarding the influence of the cement paste content on the final compressive and flexural strengths studied at 180 days. Regarding the influence of cement paste content on compressive and flexural strengths, Figure 4.9 shows the same trends as for OPC specimens. All dosages recorded higher strengths when higher cement paste amount was used. However, the saturation point was not reached with any of the dosages and no correlation was observed between w/c ratio or aggregate size and compressive nor flexural strengths. Consequently, more specimens should be tested in order to get more results and a better understanding of their behaviour.

Finally, compressive and flexural strengths of the dosage with a P:M ratio of 1:1.75 presented in the previous chapter were 63.5 MPa and 8.05 MPa respectively. Those results correspond to a dosage with 0/2 mm aggregate size, a w/c ratio of 0.28 and a cement paste content of 135 % of the reference value. Results of the dosage with the smaller amount of cement paste and the w/c ratio of 0.28 presented in Figure 4.9 concern to dosages with a maximum cement paste content equal to the reference value. That maximum presented results of 27.82 MPa for compressive strength and 5.05 MPa for flexural strength that considerably differ from the previously mentioned.

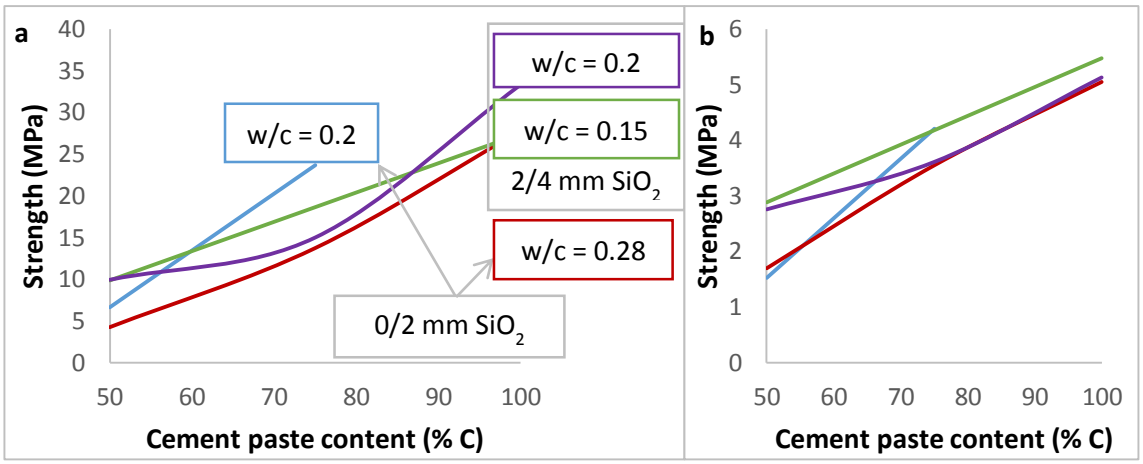

Figure 4.9.- Influence of the cement paste content on compressive (a) and flexural (b) strength of MPC specimens at 180 days.

4.3.3. Porosity

Table 4.4 shows the values corresponding to the dry weight (g), W_{ds}, the immersed weight (g), W_{is} and the weight of the saturated specimen with dry surface (g), W_{sds}, obtained accordingly to ASTM C642-13 standard.

Percentage of voids was then calculated from the values presented above. Figure 4.10 presents the variation of the percentage of voids with the cement paste content. Percentages up to 100 % show results corresponding to MPC specimens and values above 100 % represent OPC specimens. A general trend was observed for all dosages, in which increments of cement paste content imply, as it was expected, a drop in the percentage of voids. Furthermore, it was evident that the reference value of the amount of cement paste, 100 % of C, produces specimens with lower percentage of voids for MPC mortars than for OPC mortars. In addition, MPC specimens with the highest amount of cement paste showed lower percentage of voids than most of the OPC specimens with a cement paste content of 175 % of the reference value.

Specimens with highest aggregate size showed a higher percentage of voids for the same cement paste content, probably due to the biggest diameter of open porosity. Regarding the influence of the w/c ratio on this parameter, a general trend may show that higher w/c ratios produce specimens with higher percentage of voids. In accordance, results obtained for OPC specimens with the highest amounts of cement paste followed that trend. However, specimens with lower amounts of cement paste presented contradictory results for specimens Pa40-1C and Pa60-1C. The above could be consequence of an overestimation of percentage of voids due to the loss of aggregates. The amount of cement paste was too low in those specimens, especially the dosage with the w/c ratio of 0.4, and consequently loss of non-joint aggregates during the immersion period was observed. In contrast, results obtained for MPC specimens did not show the aforementioned trend, being less evident for higher cement paste contents of MPC specimens due to the high reduction of the percentage of voids.

Table 4.4.- Weight of specimens under different conditions

Specimen	W_{ds} (g)	W_{is} (g)	W_{sds} (g)
Pa40-1C	449.8	260.0	506.4
Pa40-1.25C	490.6	278.9	527.8
Pa40-1.5C	572.1	331.0	593.2
Pa40-1.75C	590.6	344.9	609.2
Pa60-1C	462.3	260.0	505.9
Pa60-1.25C	535.2	307.7	564.0
Pa60-1.5C	534.5	307.4	565.9
Pa60-1.75C	534.6	310.9	565.9
PA30-1C	513.9	302.1	539.0
PA30-1.25C	568.2	334.0	587.6
PA30-1.5C	619.6	368.7	633.0
PA30-1.75C	622.0	369.7	635.1
PA40-1C	494.6	287.5	522.4
Ma20-0.75C	1234.6	698.9	1353.8
Ma20-1C	1353.1	749.4	1373.1
Ma28-0.5C	1002.5	569.6	1141.5
Ma28-0.75C	1054.0	606.2	1198.5
Ma28-1C	1345.4	732.4	1360.9
MA20-0.5C	1072.5	624.7	1142.6
MA20-0.75C	1375.4	780.8	1410.1
MA20-1C	1385.0	796.1	1403.1
MA15-0.5C	1366.2	782.5	1383.1
MA15-1C	1098.2	642.5	1167.2

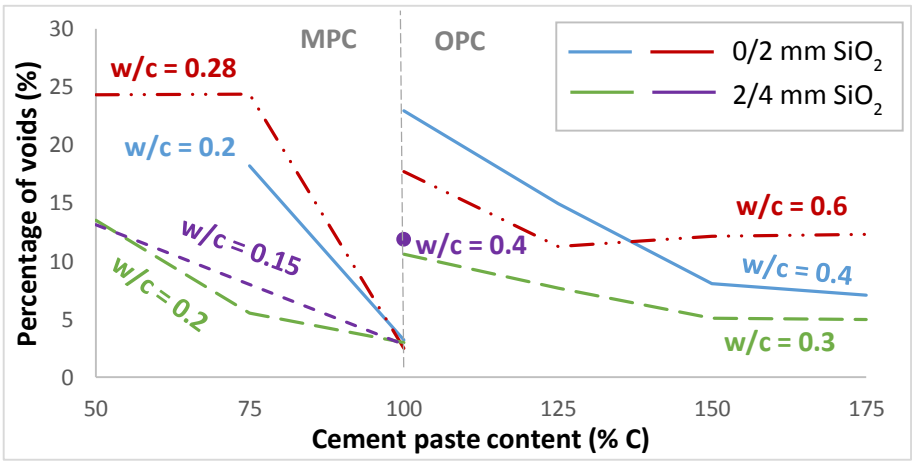

Figure 4.10.- Influence of cement paste content in percentage of voids for MPC and OPC specimens estimated according to the standard ASTM C642-13.

Regarding specimens with higher open porosity, results should be considered with caution. Loss of water was detected in the manipulation process, resulting in an underestimation of the percentage of voids. Consequently, estimation of porosity by means of application of Archimedes' principle was considered. For this purpose, Figure 4.11 shows the percentage of voids obtained according to the Archimedes' principle.

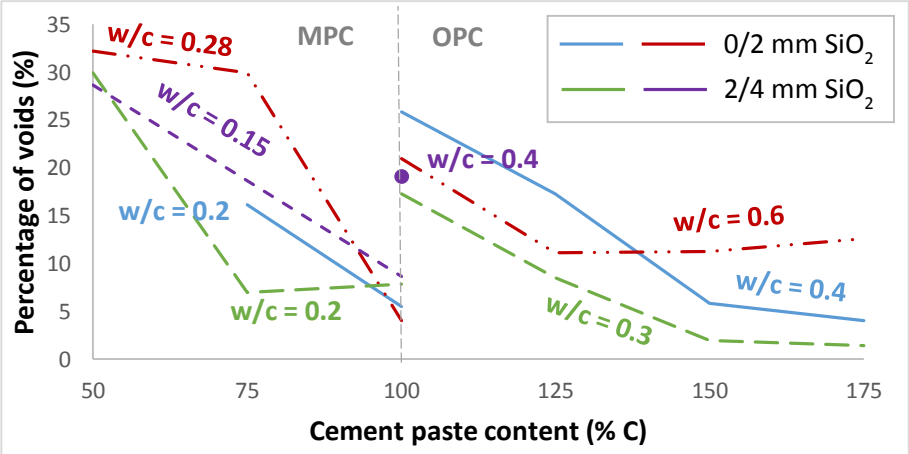

Figure 4.11.- Influence of cement paste content in percentage of voids for MPC and OPC specimens estimated according to Archimedes' principle.

Same general trends were observed for results obtained according to Archimedes' principle. However, some remarkable differences between Figure 4.10 and Figure 4.11 are evident by comparing them. First, higher percentage of voids in MPC specimens was observed independent of the amount of cement paste. Moreover, the objective of determining percentage of voids by means of application of Archimedes' principle was accomplished due to the big increment of percentage of voids observed for specimens with 2/4 mm aggregates size. As mentioned previously, the loss of water during manipulation of specimens and their high open porosity was avoided by mean of this method. Regarding OPC specimens, slight differences were observed by comparing both methodologies.

Regarding the MIP test, Figure 4.12 shows the results of the percentage of porosity as well as the pore size distribution of each sample. Capillary pores are the most interesting due to the purpose of stimulating colonisation of living organisms. Water contained in those pores will be available for the living organisms on the materials' surface. Then, Figure 4.12a shows the porosity estimated by means of MIP and Figure 4.12b shows the percentage of pores classified in each group, the big capillary pores (green) being the most interesting.

In Figure 4.12a, OPC specimens made with 0/2 mm aggregates (Pa40-1C and Pa60-1.75C) showed a percentage of porosity around 15 %, although all the other specimens did not reach the 10 % value. Between the specimens with the lower porosity, Ma28-C showed the highest value of porosity. Differences in the percentage of porosity between OPC specimens mainly correspond to the cement paste content. Due to the higher specific surface of the 0/2 mm

aggregate, more cement paste is required to join all the particles. Consequently, the lowest porosity was recorded for specimens with the lowest cement paste content (PA30-1C). However, higher differences between porosity of specimens Pa40-1C and Pa60-1.75C were expected due to the difference in cement paste content as well as the w/c ratio used. In contrast, MPC specimens provide the expected results in terms of trend. Porosity of specimens increases along with both w/c ratio and cement paste content.

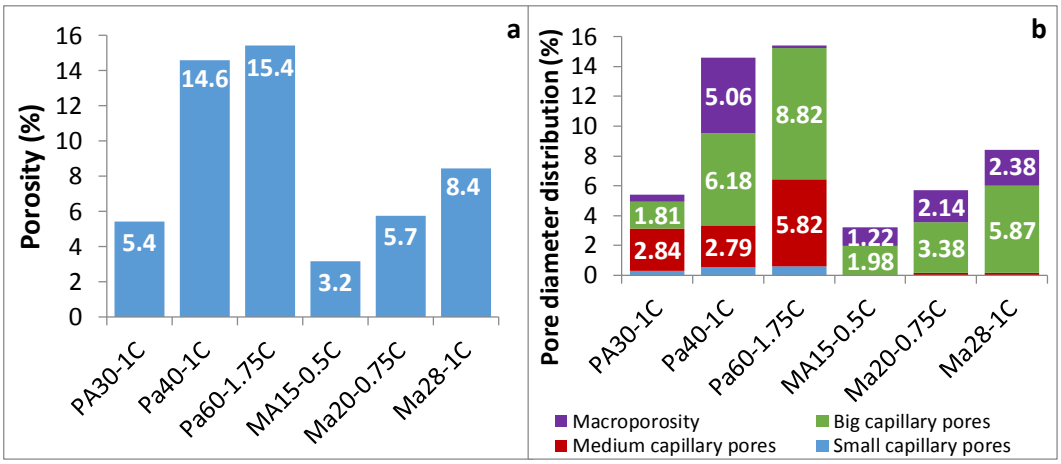

Figure 4.12.- Porosity (a) and percentage-based distribution of pore diameter (b) obtained from mercury intrusion porosimetry analysis.

From the total porosity obtained by MIP, knowledge of the pore size distribution provides information about the most representative group of pores in terms of their diameter (Figure 4.12b). As mentioned before, capillary pores correspond to the most interesting pore size group. Pa60-1.75C shows the highest percentage of big capillary pores although the small percentage of macropores may be a handicap. Water absorption is easily carried out through macropores and the extremely low percentage obtained may indicate possible problems in this sense. Therefore, Pa40-1C and Ma28-1C have a similar percentage of big capillary pores although Ma28-1C has a significantly lower total porosity. Differences between OPC and MPC specimens due to a higher variety of pore size distribution for OPC specimens are shown. In contrast, majority of MPC pores are higher than 50 µm and the highest percentage of big capillary pores was observed in Ma28-1C.

Additionally, Figure 4.13 shows separate curves for each dosage corresponding to the relationship between the intruded mercury with the pore size. Critical diameter for each dosage was determined, which corresponds to the maximum value of the differential intrusion logarithm (Log DI). Significant differences were observed for different hydraulic binders as well as for different dosages. Critical diameters differ between dosages although similar values were obtained for PA30-1C and Pa60-1.75C.

The main conclusion extracted from those results, which were presented in both Figure 4.12 and Figure 4.13, is that MPC specimens presented higher values comparing specimens with similar properties, which may indicate MPC cement paste matrix is more compact than OPC.

Regarding peaks distribution, it is interesting to remark particularities of the curves. Samples did not follow any particular or similar trend. Some specimens presented a predominant peak followed by minor ones (PA30-1C, Pa60-1.75C), others two regions clearly different (Pa40-1C and Ma20-0.75C) and finally, others with more irregular pattern (MA15-0.5C and Ma28-1C).

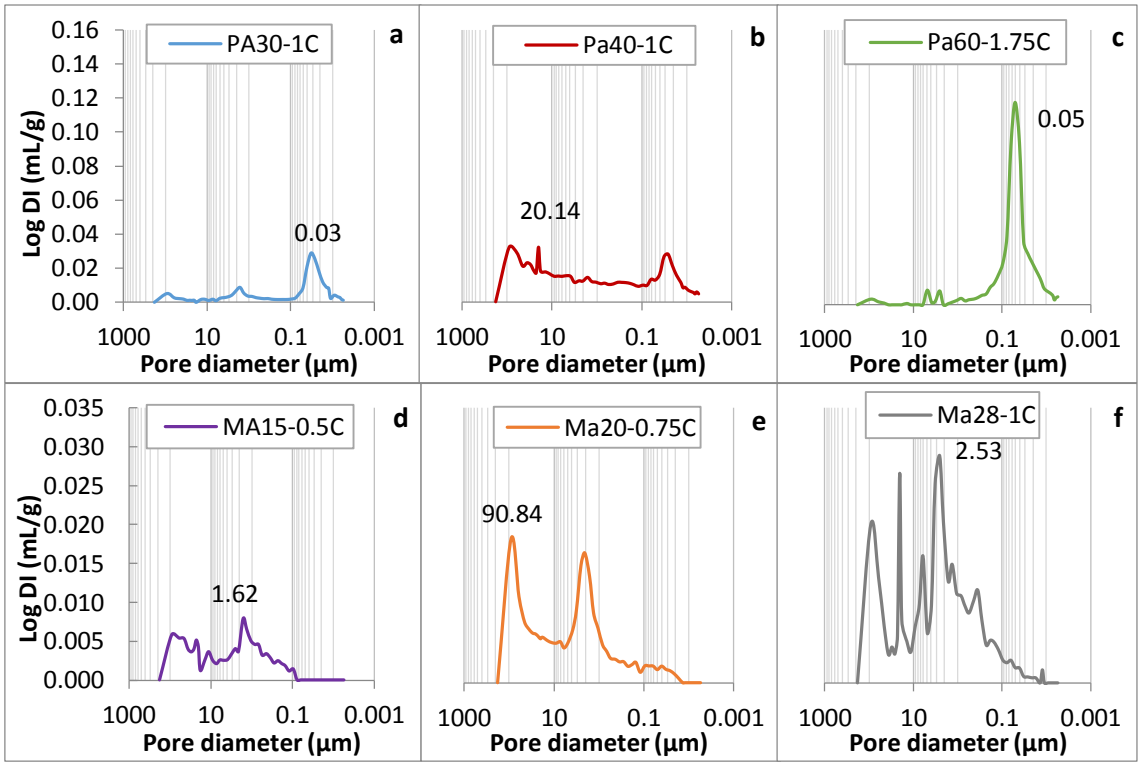

Figure 4.13.- Influence of dosage on critical diameter.

The aforementioned results give an idea about the most interesting dosage to produce mortar with high bioreceptivity. Extreme porosities understood as materials with pores of small diameters as the predominant group, as well as materials mainly composed by macropores, would present problems in terms of water absorption and water retention. The most interesting network of interconnected pores would be formed by a composition of different pore sizes. It is thought that the most interesting relationship should be established between macropores and capillary pores. First group would favour water absorption and the second one would better retain the water inside the material. Accordingly, MPC dosages may be better than OPC in terms of water retention. Figure 4.12 showed less diversity of pores although a better equilibrium between macroporosity and big capillary pores was observed. Figure 4.13 supports this hypothesis due to the most irregular pattern observed in MPC samples.

Additionally, Figure 4.14 shows images of the appearance of those specimens. In that figure higher porous diameters were observed for PA30-1C and MA15-0.5C specimens. Pa60-1.75C specimens showed low amount of internal pores as well as the lowest diameter.

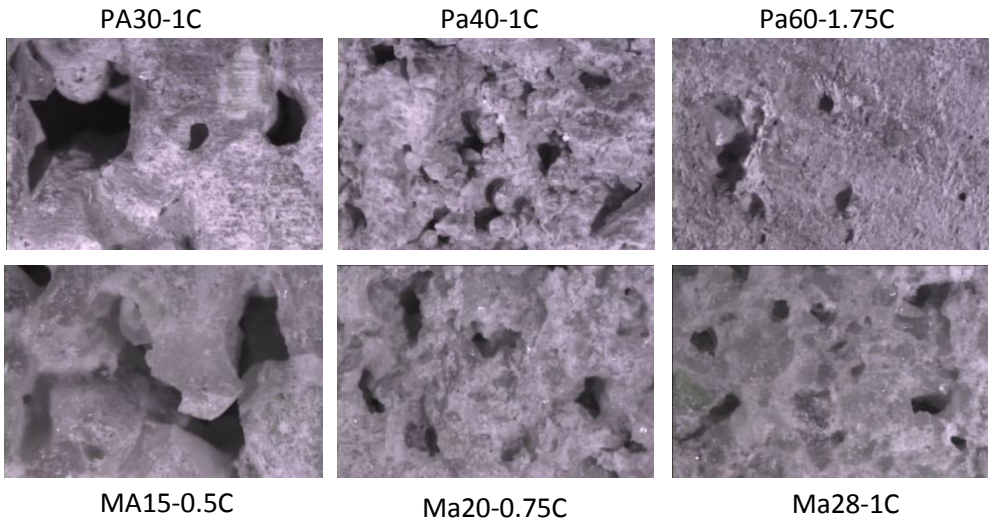

Figure 4.14.- Images obtained by means of a binocular loupe.

4.3.4. Roughness

The previous section showed images of the specimens obtained with a binocular loupe, which gives information about the appearance. Furthermore, visual appearance of the tested dosages is presented in Figure 4.15. Previous hypothesis would suggest specimens with 2/4 mm aggregates would show higher R_a and R_q-values. Then, appearance that is more similar was observed in Pa40-1C and Ma20-0.75C specimens followed by the Ma28-1C dosage and finally, Pa60-1.75C with the lowest roughness results. However, air voids formed in specimens of the last dosage should be also taken under consideration. Although the dosage should provide the specimens with lower roughness, the high cement paste content combined with the modification of the compactation process led to that appearance.

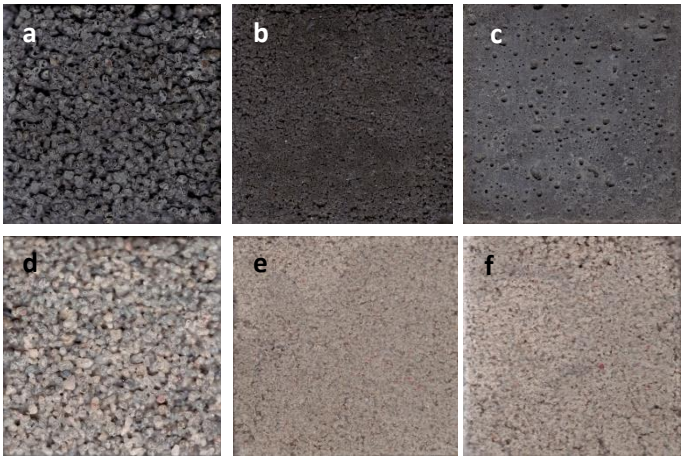

Figure 4.15.- Appearance of specimens surface of (a) PA30-1C, (b) Pa40-1C, (c) Pa60-1.75C, (d) MA15-0.5C, (e) Ma20-0.75C and (f) Ma28-1C.

Previously to presentation of R_a and R_q-values, one roughness profile per dosage is presented in Figure 4.16 to illustrate differences between them. However, the average profile

of the twelve profiles per dosage would slightly differ from the one presented. Therefore, analysis of the results will be done after presentation of the average R_a and R_q-values.

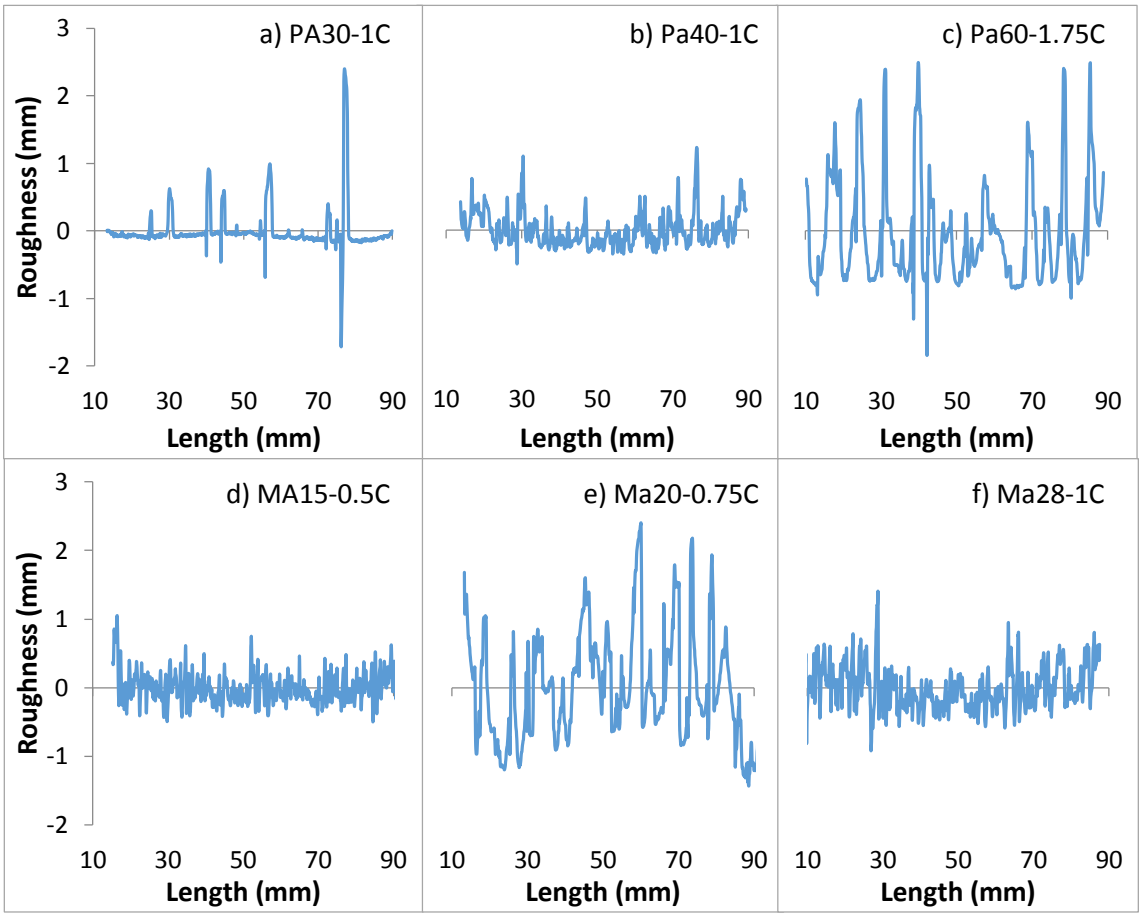

Figure 4.16.- An example of a roughness profile of dosages (a) PA30-1C, (b) Pa40-1C, (c) Pa60-1.75C, (d) MA15-0.5C, (e) Ma20-0.75C and (f) Ma28-1C.

Table 4.5 shows R_a and R_q-values for these dosages where two main groups of samples with similar R_a value can be distinguished. First, specimens with 2/4 mm aggregates showed the maximum surface roughness, OPC specimens being slightly rougher. Then, the second group, corresponds to other dosages. In that last group, the same roughness was obtained for OPC specimens although having completely different surface patterns. Furthermore, Ma28-1C presented a higher roughness than Ma20-0.75C specimens, which also differ from the previous hypothesis.

Regarding the OPC specimens with 0/2 mm aggregate size, it is thought the results correspond to the fact air voids were formed in Pa60-1.75C specimens, modifying the general smooth aspect of the surface. Consequently, previous hypothesis was partially erroneous mainly when talking about both OPC and MPC specimens with 0/2 mm aggregate size.

Table 4.5.- R_a and R_q values obtained.

	Specimens	R_a (mm)	R_q (mm)		Specimens	R_a (mm)	R_q (mm)
OPC	PA30-1C	0.16	0.61	MPC	MA15-0.5C	0.15	0.48
	Pa40-1C	0.03	0.18		Ma20-0.75C	0.04	0.13
	Pa60-1.75C	0.03	0.18		Ma28-1C	0.06	0.18

4.3.5. DTA-TG

As mentioned in the methodology subsection, calibration of the method was carried out first. Five different amounts of aggregate (SiO_2) were tested to measure the area under the curve of the peak around 570° C, which corresponds to the α-β transition of the silica aggregates. Then, the area under the curve directly corresponds to the amount of aggregate tested. Figure 4.17 shows the DTA curves of the different samples used to obtain the calibration curve. After calculating the area from each curve, calibration curves for 0/2 mm and 2/4 mm aggregates were obtained to estimate the amount of SiO_2 in the experimental samples (Figure 4.18).

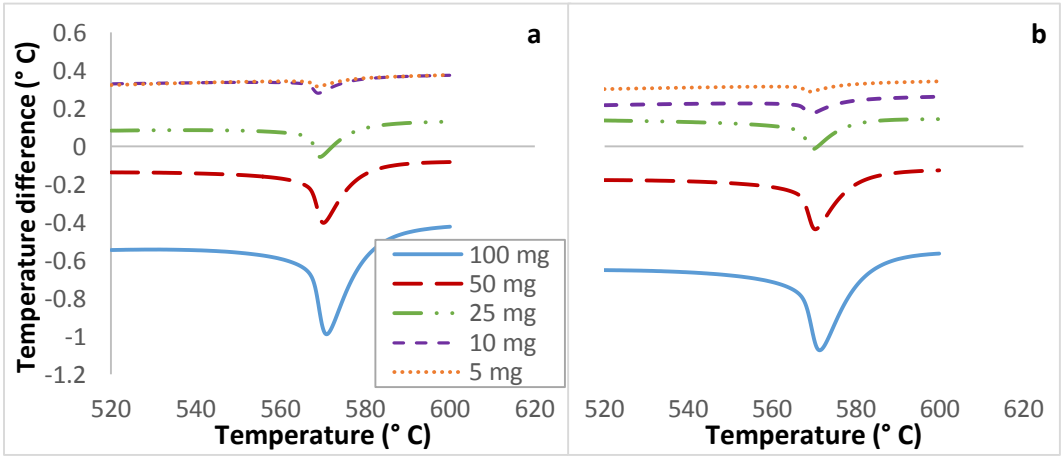

Figure 4.17.- DTA curves for the calibration of the silica aggregates 0/2 mm (a) and 2/4 mm (b).

DTA-TG curves for OPC and MPC specimens differ considerably. Figure 4.19 shows the curves corresponding to DTA-TG of OPC specimens and Figure 4.20 the curves corresponding to MPC specimens. Curves corresponding to OPC specimens were quite similar with the exception of Pa40-1C. In general, a dehydration was observed, which takes place between ambient temperature and 440° C. First range of temperatures until approximately 100° C, corresponds to the loss of free water. Then, a peak around 400° C was recorded corresponding to dehydroxylation mainly of the portlandite. The next one was recorded around 570° C and this is characteristic of the α-β transformation of the SiO_2. Subsequently, an endothermic peak due to decarboxylation was recorded around 680° C. Finally an exothermic peak was detected around 900° C corresponding to an isomorphic transformation which not causes a loss of weight.

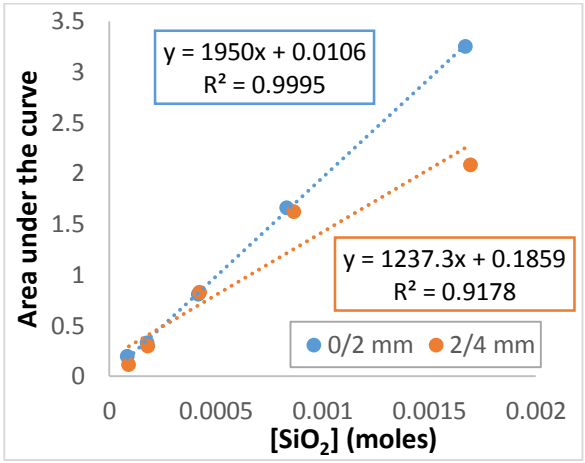

Figure 4.18.- Correlation between the area under the curve and the amount of silica aggregate per granulometry.

The area under the curve of the endothermic peak for the silica aggregates was estimated and the amount of aggregates for each of those three samples was determined. Subsequently, loss of weight due to dehydration, dehydroxylation and decarboxylation of the cement paste of each sample was determined. Results corresponding to that data is shown in Table 4.6.

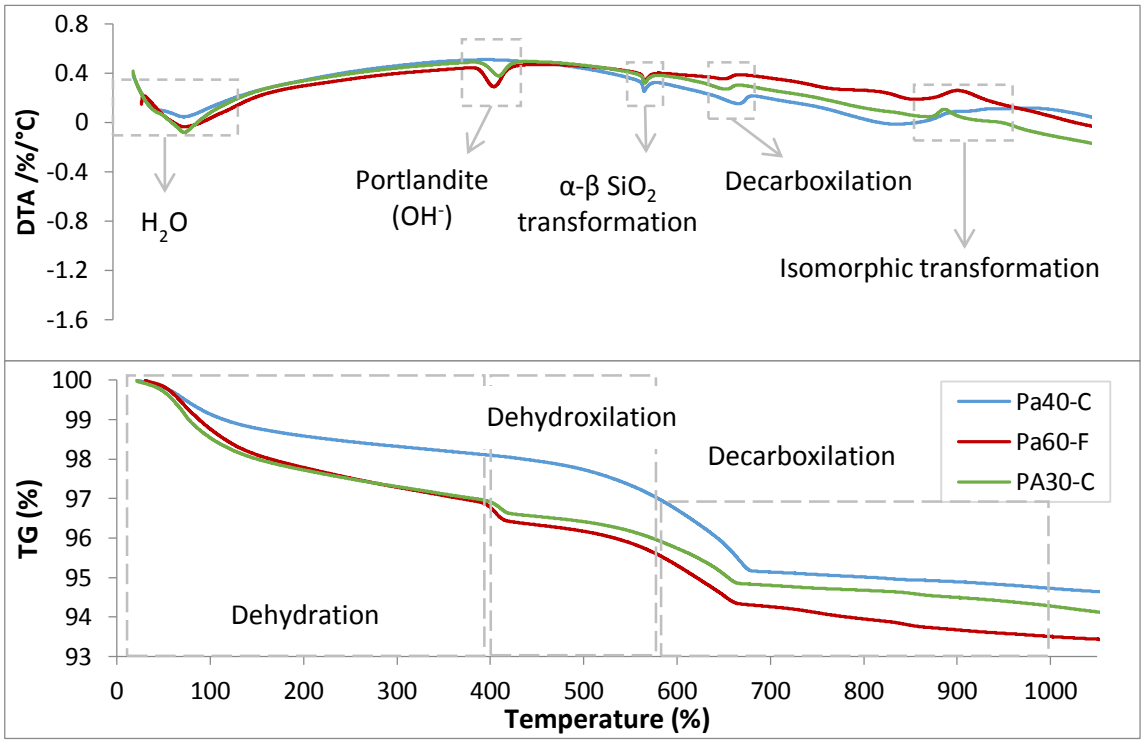

Figure 4.19.- DTA-TG curves corresponding to PA30-1C, Pa40-1C and Pa60-1.75C samples.

The amount of cement paste for Pa-1C, Pa60-1.75C and PA30-1C samples correspond to the 19.9 %, 18 % and 23.1 %, respectively. According to results shown in Table 4.6, the highest loss of weight for all samples corresponds to decarboxylation. Loss due to dehydroxylation is

similar for all the samples and the highest difference was recorded for the loss due to dehydration. In this case, the loss of weight corresponding to Pa40-C sample was significantly lower than for the other samples. It is important to mention that loss of free water (until around 100° C) was not considered for this estimation.

Table 4.6.- DTA-TG quantification for OPC samples

	Pa40-1C	Pa60-1.75C	PA30-1C
Initial weight (mg)	15.865	15.907	15.837
Aggregates weight (mg)	12.714	13.044	12.182
Cement paste weight (mg)	3.151	2.863	3.655
Loss due to dehydration (%)	0.98	1.89	1.56
Loss due to dehydroxylation (%)	1.11	1.22	0.98
Loss due to decarboxylation (%)	2.28	2.06	1.67

Figure 4.20 shows curves obtained for the MPC dosages. Comparing those results with the ones corresponding to OPC specimens, DTA curves showed a bigger endothermic peak at around 90° C corresponding with the maximum loss of weight. More specifically, DTA curves show a smaller peak around 50° C, which does not correspond to an important loss of weight. First peak in the curves may be consequence of the loss of free water while the second one may be consequence of the loss of water from the struvite. The next endothermic peak observed was detected around 570° C due to the α-β SiO_2 transformation, as it was shown in the previous graph. Finally, the last peak recorded was an exothermal peak around 690° C and it may be consequence of the formation of magnesium pyrophosphate.

Table 4.7 presents results of the loss of weight. Almost the totality of the loss of weight of MPC samples was due to dehydration. In the case of OPC samples, quantification of dehydration was considered between 100 and 400° C to avoid overestimation due to loss of free water. The case of MPC samples should be analysed differently mainly due to the chemical composition of the hydrated compounds like struvite, which is a hexahydrate. Consequently, loss because of dehydration is considered between ambient temperature and 400° C. Moreover, loss of weight will be considered because of struvite degradation.

In contrast with what happened with OPC samples, in this case the loss of weight was associated to the amount of cement paste or the w/c ratio. Both parameters decrease while percentage of loss of weight decreases although the w/c ratio may have more influence since there is more water available and consequently more struvite.

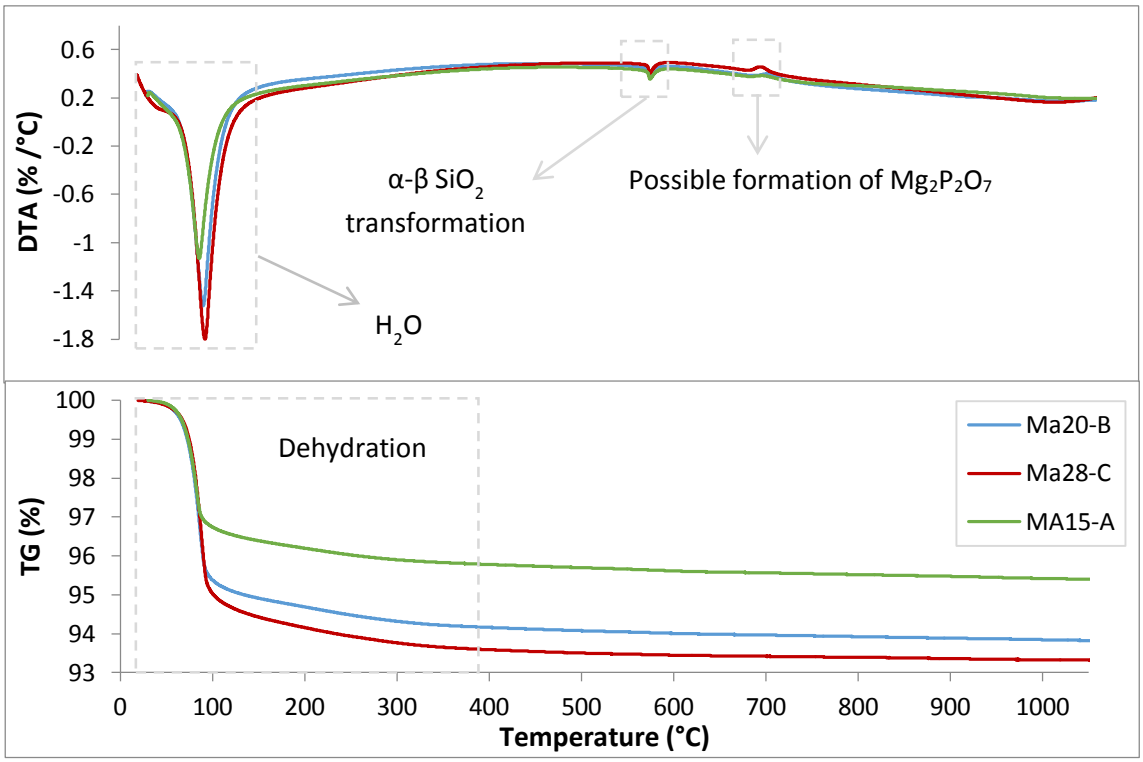

Figure 4.20.- DTA-TG curves corresponding to MA15-0.5C, Ma20-0.75C and Ma28-1C samples.

Table 4.7.- DTA-TG quantification for MPC samples

	Ma20-B	Ma28-C	MA15-A
Initial weight (mg)	15.274	15.457	15.174
Aggregates weight (mg)	13.223	13.279	11.692
Cement paste weight (mg)	2.051	2.178	3.482
Loss due to dehydration (%)	5.79	6.42	4.21

4.4. CONCLUSIONS

Regarding the dosage, the importance of this parameter to obtain mortar or concrete with the desirable configuration is already known. An objective-oriented design was used in the present chapter to obtain different roughness and especially porosity and a confirmation of the fact that it is possible has been shown. However, dosage models may differ between binders or more probably when using additives. The model used worked properly for OPC mortars although was not appropriate for the MPC specimens. The above may be consequence of the use of borax as a retarder due to the fluidizer effect.

Concerning to the mixing procedure, MPC mixtures presented problems of mixing times. In this case, not only shorter mixing times were used, but also a first stage of mixing the dry components was carried out. Additionally, segregation problems because of the compaction process for both binders were overcome by halving the compaction energy.

After solving production particularities, characterization tests were executed. Porosity estimation for cementitious materials is usually determined according to the ASTM C-642-13 standard. However, porous materials presented the problem of the estimation of the saturated weight with dry surface due to the high open porosity. Consequently, Archimedes' principle as an alternative method was implemented.

Subsequently, the main conclusions obtained in this chapter were an improvement of thermal peaks during the hydration process of MPC specimens. Furthermore, it was observed that control of parameters such as porosity was easier for OPC specimens. The above may be connected to the problem of dosage method suitability.

Interesting differences were also obtained in MIP test related to different porosity distribution between OPC and MPC specimens. OPC specimens showed a more continuous porosity covering all different ranges while MPC specimens presented almost negligible percentages for pore diameters lower than 50 nm. The above indicates different water absorption as well as water retention patterns for the different dosages and especially between both binders. Consequently, those different behaviours may promote different colonisation pattern. Regarding compressive strengths, high early strengths may indicate a drop of long-term strength but no significant effects were obtained on the compressive strength evolution after 28 days. More specimens are necessary to be tested in order to determine the trend.

Accordingly, dosages selected for further research and evaluation of their suitability to be colonised by living organisms were Pa40-1C, Pa60-1.75C, PA30-1C, Ma20-0.5C, Ma28-1C and MA15-0.5C. The criterion used to select them was to choose extreme dosages in order to have a wide range of material properties.

5. BIORECEPTIVITY EVALUATION UNDER LABORATORY CONDITIONS

5.1. INTRODUCTION

The study of Chapter 4 showed the possibility of controlling physical properties of cementitious materials by means of dosage modifications. Six different dosages were chosen from the initial twenty-three: PA30-1C, Pa40-1C, Pa60-1.75C, MA15-0.5C, Ma20-0.75C and Ma28-1C. Then, an accelerated laboratory test was carried out to evaluate the behavior of those specimens when they are exposed to colonisation. It is known that living organisms in the environment do not respond in the same way than in laboratory conditions mainly due to the climatic parameters and competition between species. However, these tests are useful for comparison between specimens in the same condition, obtaining results in short times.

Numerous research groups have been investigating biofouling on building materials, and consequently, proposing different methodologies of evaluation. In this study, the modular accelerated algal fouling test developed by De Muynck et al. (2009) was implemented. Contrary to setups like the types proposed by other authors (Guillitte and Dreesen, 1995; Dubosc et al.,2001; Barberousse et al., 2006; Escadeillas et al., 2007), the modular setup proposed by De Muynck et al. (2009) allowed the simultaneous evaluation of different concrete mixtures. Qualitative and quantitative methods described in literature to evaluate bioreceptivity of mortar specimens include colorimetric measurements, image analysis and biomass quantification. Changes in the surface colour of a cementitious material could be easily detected by means of colorimetric measurements and these results can be complemented with image analysis (De Muynck et al., 2009; Ferri et al., 2011; Tran et al., 2012). In addition, a non-destructive method

for biomass quantification according to Eggert et al. (2006) was also used in this study. These methods allow the quantification of biomass without the need to extract chlorophyll a.

Accordingly, the objective of this chapter is to evaluate the colonisation of different degrees of bioreceptivity based on changes in chemical and physical properties of the material. To achieve this purpose, the following specific objectives are defined:

- Determine the suitability of the selected dosages for colonisation of pioneer organisms such as *Chlorella vulgaris*;
- Assess the aptness of the different methodologies of evaluation for the current purpose;
- Define the most suitable mixture or mixtures for pioneers' colonisation in terms of time, amount and homogeneity of the growth.

5.2. MATERIALS AND METHODS

5.2.1. Mortar specimens

Table 5.1 shows the compositions and the main characteristics of the specimens. The data presented is extracted from the results obtained in the experimental program analysed in Chapter 4.

Table 5.1.- Compositions and main characteristics of the specimens

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

[1]CEM I 52.5R; [2] Cement made by $NH_4H_2PO_4$, MgO and borax. $NH_4H_2PO_4$:MgO ratio = 1:1.75 and the addition of borax amounted to 6 % by weight of the sum of $NH_4H_2PO_4$ and MgO weights; a:c:w is the ratio aggregates:cement:water.

With that composition, OPC and MPC specimens were cast into 80 x 80 x 20 mm^3 polyurethane moulds (Figure 5.1). MPC specimens were demoulded after one hour while OPC specimens were demoulded after 24 hours. Both types of specimens were allowed to cure at

22 ± 2° C and 95 ± 5% relative humidity for 28 days even though this curing process is not required for MPC. As done for specimens characterisation, OPC specimens were subjected to accelerated carbonation by storing them for 3 weeks in a container with 100 % CO_2 (65 ± 5 % RH and 1 atm).

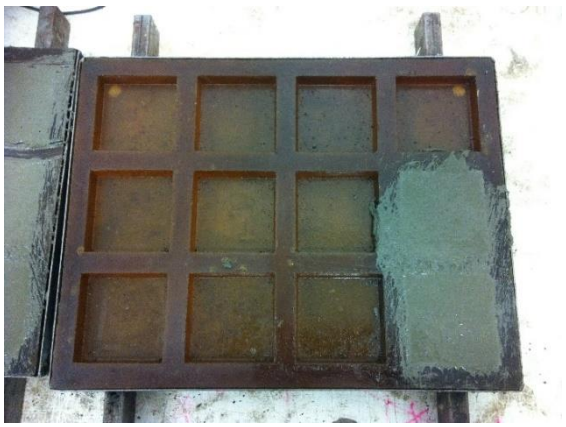

Figure 5.1.- 80 x 80 x 20 mm³ polyurethane moulds.

5.2.2. Accelerated algae fouling test

The algae specie used in this study was *Chlorella vulgaris var. viridis Chodat*. The strain was obtained from the culture collection of algae and protozoa (CCAP) from the Dunstaffnage Marine Laboratory (Scotland; accession number CCAP 211/12). Batch cultures of these algae were grown under sterile conditions in erlenmeyers containing 1 L of Walne medium, with the addition of 0.2 mg thiamin chlorhydrate and 0.01 mg vitamin B12 (http://www.ccap.ac.uk/media/documents/Walnes.pdf). The erlenmeyers were continuously exposed to light by means of Sylvania Grolux 30W lamps on a KS 501 rotary shaker (IkaWerke, Germany) at 100 rpm.

Air was provided by means of an Air plus 3 air pump. For the preparation of the medium, 2 mL of sterile concentrated Walne and 0.2 mL of vitamin solution were added to 1 L of autoclaved mineral water (Cristaline, natural spring water, Merignies, France). Each week, new batch cultures were grown by transferring 200 mL of the one week old culture to 1 L of fresh medium (Figure 5.2 (a)). The remaining culture solution was used to inoculate the water in the PET bottles used in the accelerated run-off test (De Muynck et al., 2009). The amount of cells per mL was determined by means of a Zeiss Axioskop II plus light microscope (Zeiss, Germany) and a counting chamber (Figure 5.2 (b)).

The accelerated algal fouling test was carried out by means of a water run-off test developed at the Magnel Laboratory for Concrete Research of Ghent University (De Muynck et al., 2009), which is shown in Figure 5.3. The run-off period was set to start every 12 hours and ran for 90 minutes. Furthermore, the setup was submitted to a 12 hours day and night regime, which started simultaneously with the run-off periods (De Muynck et al., 2009). During the day regime, light was provided by means of Sylvania Grolux 30 W lamps. The temperature and

relative humidity ranged between 22° C (night) - 25° C (day) and 82 % (day) – 90 % (night), respectively.

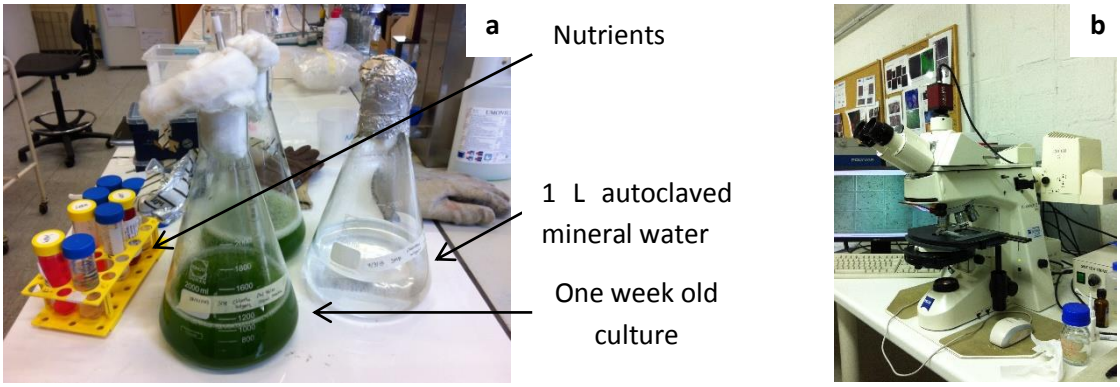

Figure 5.2.- Batch culture and its components (a) and cells count by means of the light microscope (b).

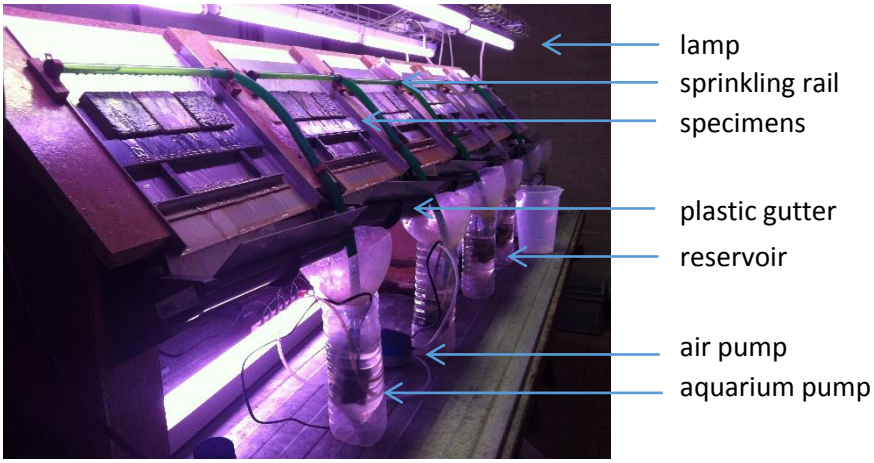

Figure 5.3.- Accelerated algal fouling test setup.

To prevent settling of the algae in the reservoirs and to provide O_2 and CO_2, the algal suspensions were continuously aerated by means of Air plus 3 air pumps. The mineral drinking water contained in the PET bottles was used for the preparation of the culture medium. The contents of each 2 L bottle was used to fill two PET bottles used as algal reservoirs (1 L per reservoir). Subsequently, 2 mL of concentrated Walne medium and 0.2 mL vitamin solution were added to each reservoir. Algae from one week old cultures were inoculated in the reservoir at final concentrations of about 6.5 x 10^8 cells L^{-1}, corresponding to about 2.5 mg dry weight per litre. Every week, the contents of the reservoirs were replaced by new algal cultures, after cleaning of the reservoirs. Additionally, every two weeks, the reservoirs were replaced by new ones.

5.2.3. Evaluation and quantification of biofouling

Different tests were carried out in order to evaluate the visual aspect and degree of bioreceptivity of the samples. Specimens were studied weekly and in triplicate, immediately

after the run-off period in order to have specimens in the same humidity condition. Furthermore, excess water on specimens' surface was removed with a paper towel.

First, **colorimetric measurements** were performed by means of a X-Rite SP60 colorimeter (X-Rite, USA) with an 8 mm aperture (Figure 5.4). Colorimetric methods are currently accepted to monitor biofouling surfaces. The CIELab system defines the colour of an object based on two chromaticity coordinates, a* (green-red component) and b* (blue-yellow component), and lightness factor (L*, black-white component). This method was chosen for being easy and for providing good colour quantification as perceived by the human eye. Figure 5.4 also shows a representation of the L*, a* and b* colour space, where X refers to the baseline colour, Y to the specimen colour and ΔE* the difference between X and Y. Determination of L*, a* and b* parameters as well as reflectance (%) for visible wavelengths (data every 10 nm) were obtained.

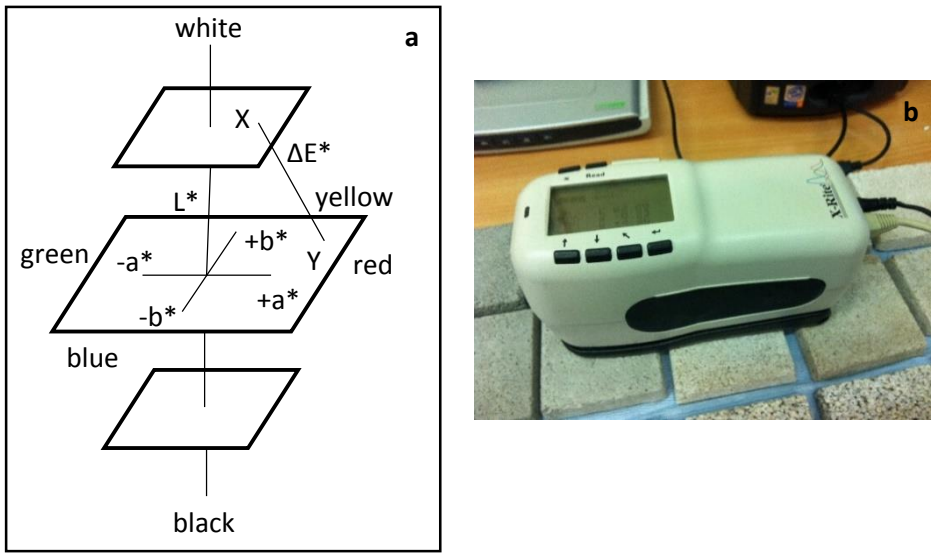

Figure 5.4.- Representation of the CIELab system theory (adapted from Pitts et al., 1998) (a) and image of the colorimeter used (b).

Characteristic pigments of *Chlorella vulgaris* are chlorophyll a, b and carotenoids. Any substance with the capability of absorbing light is considered as pigment and the colour is related to the reflected wavelength. Consequently, each pigment has associated a characteristic spectrum. Chlorophyll a is the most characteristic green pigment of photosynthetic cells. Additionally, chlorophyll b and carotenoids are accessory pigments which absorb the light, while chlorophyll a do not absorb, taking into account that just visible light is absorbed and used for photosynthesis (wavelength range from 400 nm to 720 nm).

Maximum absorbance peaks for each pigment are localised at different wavelengths, localized around 430 nm and 670 nm for Chlorophyll a, around 450 and 640 nm for chlorophyll b and around 460 nm for carotenoids as shown in Figure 5.5 (Babichenko et al., 2001; De Muynck

et al., 2009). Therefore, drops in reflectance should be observed at these specific wavelengths when biofouling is present.

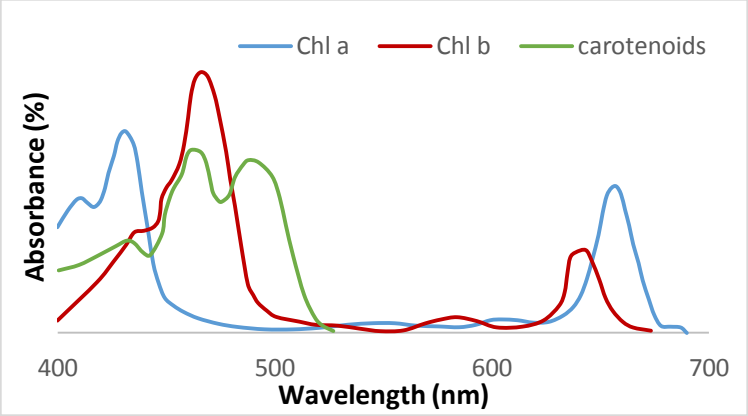

Figure 5.5.- Characteristic absorbance spectrum for photosynthetic pigments.

Four measurements per specimen at fixed positions (on the four corners at 1 cm from the borders) were carried out. Before starting the accelerated algal fouling test, colorimetric measurements were taken at different humidity conditions of the specimens (from wet to dry) to show the influence of humidity on the fouling evaluation parameters. The results obtained in that process are shown in Figure 5.6.

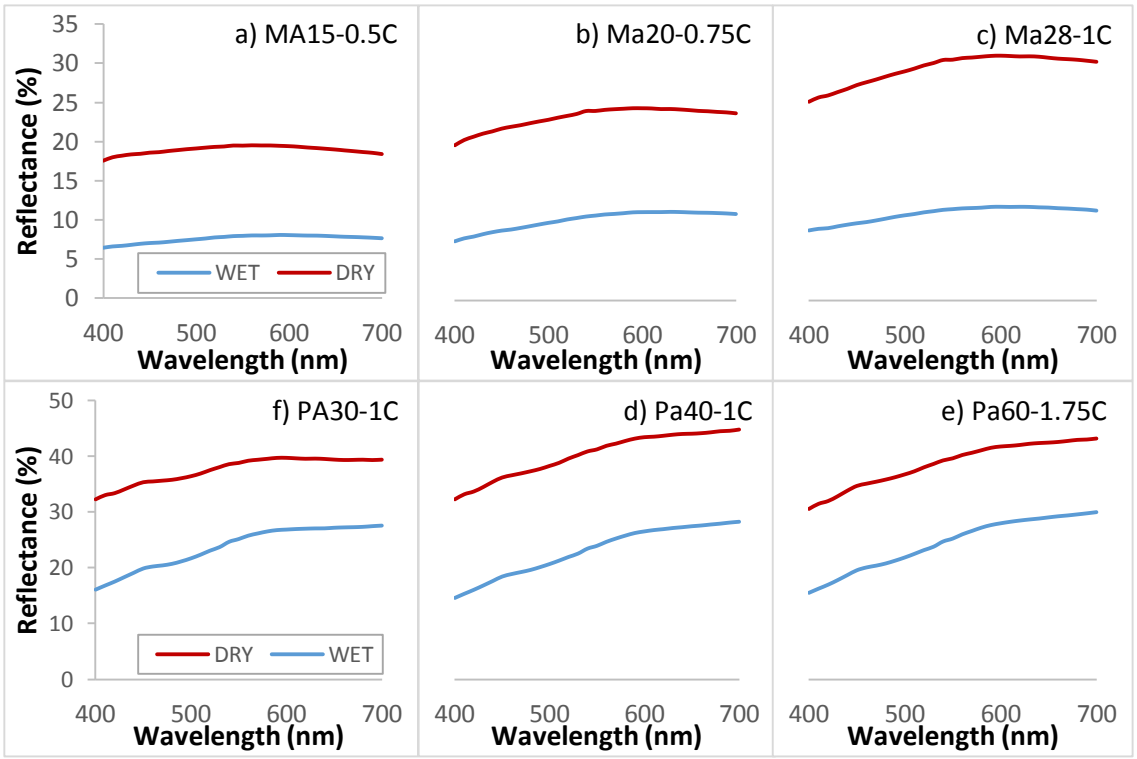

Figure 5.6.- Influence of the humidity on the reflectance spectra of clean (a) PA30-1C, (b) Pa40-1C, (c) Pa60-1.75C, (d) MA15-0.5C, (e) Ma20-0.75C and (f) Ma28-1C specimens.

From colorimetric measurements data, ΔL*, Δa*, Δb*, total colour difference (ΔE*), chromatic variations (ΔC*), changes in hue (ΔH*) and fouling intensity (FI, %) were calculated using the following equations (De Muynck et al., 2009, Ferri et al., 2011):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta a^*)^2} \quad [5.1]$$

$$\Delta C^* = \sqrt{(a_t^*)^2 + (b_t^*)^2} - \sqrt{(a_0^*)^2 + (b_0^*)^2} \quad [5.2]$$

$$\Delta H^* = \sqrt{(\Delta E^*)^2 - (\Delta L^*)^2 - (\Delta C^*)^2} \quad [5.3]$$

$$FI\ (\%) = (R_{700\ nm} - R_{670\ nm})_{t\ weeks,wet} - (R_{700\ nm} - R_{670\ nm})_{0\ weeks,wet} \quad [5.4]$$

where R_{700} and R_{670} are the reflectance of the sample (%) at a wavelength of 700 nm and 670 nm and t is the time after the beginning of the test. Subsequently, photographs of the specimens were obtained with a Canon Scan 3000F scanner; ImageJ 1.38x software was used for the image analysis and to process the images obtained. De Muynck et al. (De Muynck et al., 2009) proposed a quantification of the area covered by algae by means of a threshold analysis on the a* and b* coordinates of the CIELab colour space. Consequently, pixels with a* or b* values higher than the selected threshold were considered as unfouled, and the total amount of black and white pixels was then calculated by means of the Analyze, Histogram function. This is illustrated in Figure 5.7, where images after threshold analysis show the biological growth in black colour and the non-covered area in white.

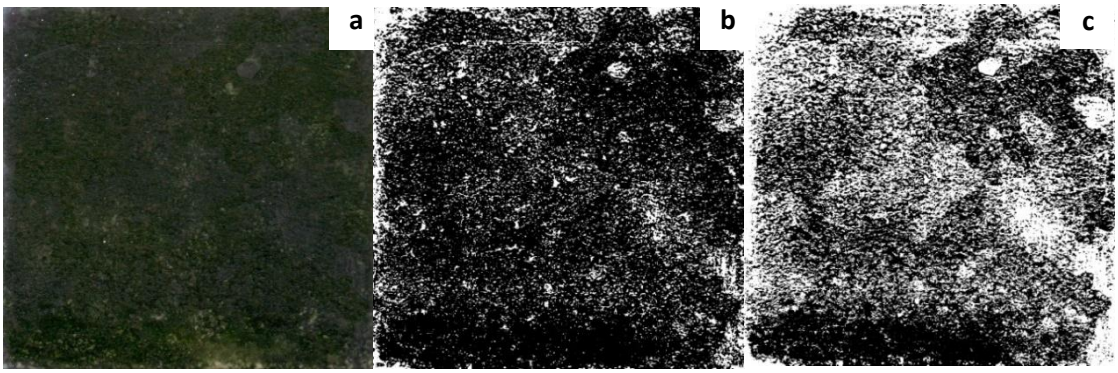

Figure 5.7.- Better correlation between photograph (a) and threshold analysis on the a coordinate (b) tan between the first one and the threshold analysis in the b* coordinates (c).*

Visual comparison between photographs and both threshold analyses on the a* and b* coordinates, as well as histograms show a better correlation using the threshold on a* coordinate. Consequently, pixels with a* values higher than 120 (0-255) were considered as unfouled spots. Subsequently, the percentage of area colonized by the algae could be calculated.

Finally, a non-destructive method for **biomass quantification** of algal biofilms was carried out. This method comprises the measurement of chlorophyll fluorescence by PAM-fluorometry (Eggert et al., 2006). Light energy is absorbed by pigment molecules present in the photosynthetic antenna molecules and the energy can undergo three fates: used for

photosynthesis, dissipated as heat or re-emitted as light-chlorophyll fluorescence (Böger, 1964; Lichtenthaler, 1987). These processes occur in competition so measuring chlorophyll fluorescence is a very useful technique to obtain information on photosynthesis (Figure 5.8 (a)).

The light energy is absorbed by the antennas (light harvesting antenna, LHC) and distributed to the reaction centres of photosystems I and II (PS I and PS II) for photosynthesis. More specifically, most of the Chl a fluorescence is originated in PS II. When samples are kept in dark conditions, the electron acceptor of PS II is in the oxidised state and there is no electron flow in the photosynthetic electron transport chain. In this situation, the reaction centre of PS II remains open and the fluorescence intensity is minimal (F_0). This minimum fluorescence is measured by a weak modulated light beam. Subsequently, a saturating light pulse will raise the fluorescence until its maximum (F_m) giving information about the difference between the maximum and minimum fluorescence (F_v). Moreover, from these parameters it is possible to determine the maximum quantum efficiency of PS II (F_v/F_m), which consequently gives information about photosynthesis efficiency. Figure 5.8 (b) shows a scheme of the dark-adapted chlorophyll fluorescence pattern.

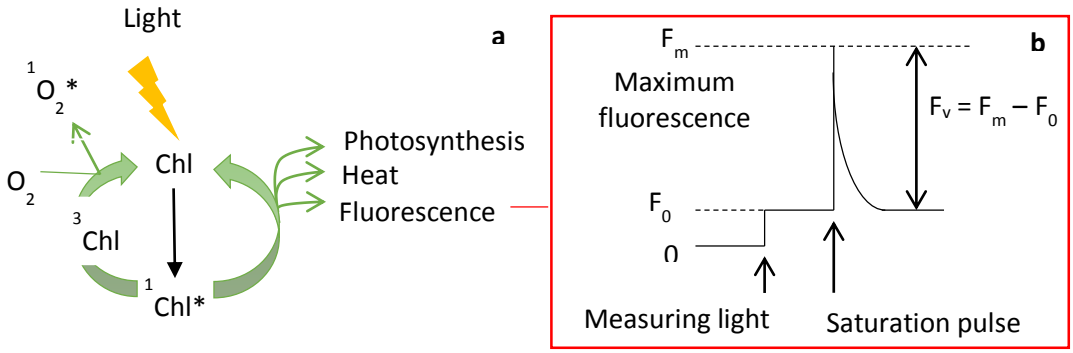

Figure 5.8.- Scheme of possible energy fates (a) and a chlorophyll fluorescence measurement by the saturation pulse method in dark conditions (b).

First, a calibration of the method was realized by means of correlating classic procedures (quantification of Chl a and dry weight) to minimum fluorescence of dark-adapted algae (F_0). Concerning the extraction of Chl a, two different protocols were tested using acetone 90 % at room temperature, and heating at 65° C (Maxwell and Johnson, 2000; Misra et al., 2009). Unfortunately, uncompleted extraction was observed for both protocols, having as a result a completely green pellet. Consequently, calibration of the method was carried out by means of correlating dry weight to initial fluorescence.

Dilutions of the original culture were carried out per quadruplicate. Twelve different algal concentrations were tested in order to obtain a standardized curve of correlation. Two of the replicates were used to determine dry weight and the other two to measure F_0. Filters used to the dry weight measurement were dried before being used. Subsequently, 20 ml per replicate and dilution were filtrated by means of a vacuum pump and corresponding millipore filters

(0.45 µm pore diameter). Afterwards, filters with the algae were dried at 105° C until constant mass. Equation 5.5 shows the formula used to estimate the dry weight of algae:

$$DW = DFA - DF \quad [5.5]$$

where DW is dry weight expressed per litre of the liquid medium (mg/L), DF is the weight of the dried filter (mg) and DFA is the weight of the dried filter with the filtrated algae after being dried at 105° C until constant mass (mg).

Furthermore, another two replicates with the algae were used to estimate F_0. In vivo Chl a fluorescence was determined with a pulse amplitude modulated fluorometer (PAM-2000, Heinz Walz GmbH, Germany) with a 6 mm aperture. After maintaining samples in dark conditions for 15 minutes, F_0 was determined placing the sample at a 90° angle and 7 mm from the filter (Figure 5.9). More specifically, 5 measurements in 30 seconds were taken with 600 Hz pulsed red measuring light (650 nm), measuring light intensity (ML) of 7 and gain (G) of 3. Afterwards, correlation between dry weight and F_0 was determined.

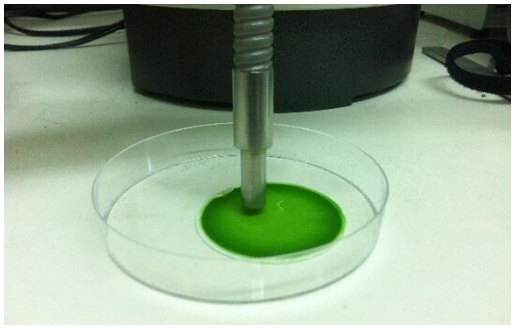

Figure 5.9.- Chlorophyll a determination for calibration of the method (determined in dark conditions).

Samples placed in the accelerated algal fouling test were studied weekly for fluorometric parameters. Five measuring points were chosen on each mortar sample, one in the centre and one positioned at the border of each quadrant (Figure 5.10). First, 5 F_0 measurements in 30 seconds were taken and then, maximum quantum yield of Photosystem II, PSII (F_v/F_m), was determined for each point. This parameter gives information about the steady-state of quantum yield (Y) of PSII and consequently about the physiological status of the algae. Acceptable values of F_v/F_m are considered up to 0.5 according to Maxwell et al. (1994). As a way to monitor the loss of viable algae due to hydric stress, completely fouled MPC samples (10 weeks of fouling) were maintained in the same conditions of light, RH (%) and temperature without provision of water/algal culture, with F_0 of the surface being recorded every week.

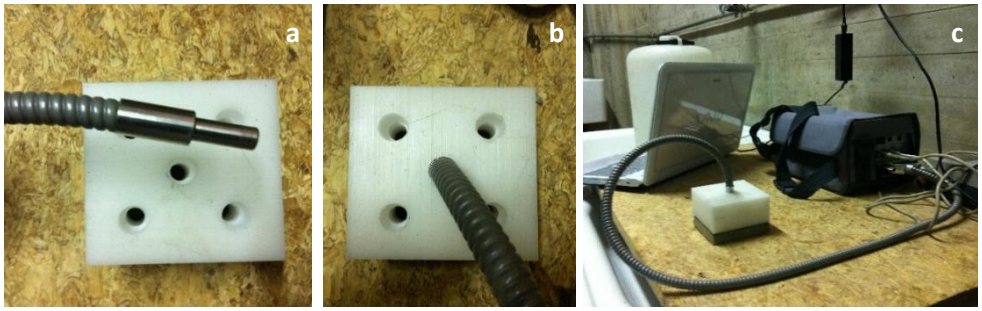

Figure 5.10.- Setup to standardize angle and distance between the simple and the sensor.

5.3. RESULTS AND ANALYSIS

5.3.1. Colorimetric measurements and analysis

Figure 5.11 shows a comparison of changes in reflectance between the six different degrees of bioreceptivity tested along the time. As mentioned before, measurements were obtained weekly although not all the results are presented in order to provide a clear distinction between the curves. Moreover, the scales of reflectance for MPC and OPC graphs are different in order to facilitate interpretation of OPC graphs.

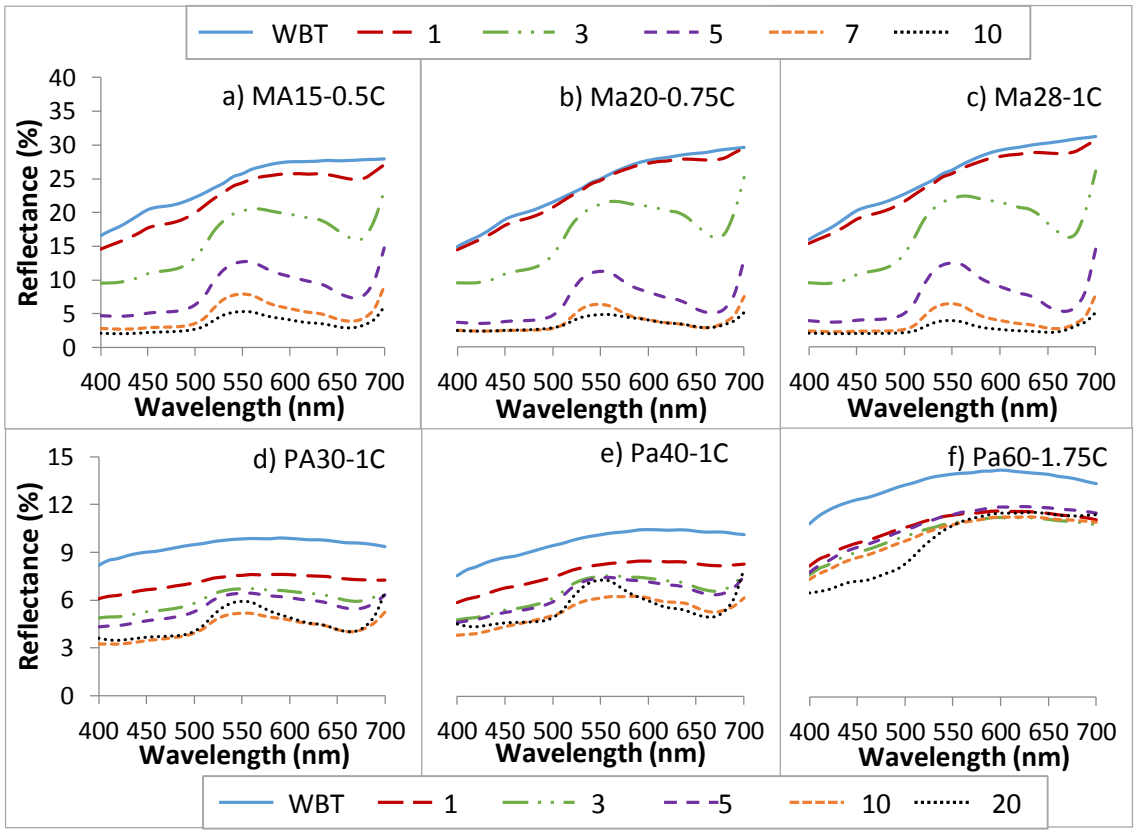

Figure 5.11.- Change in reflectance for different specimens in function of the number of weeks subjected to accelerated fouling WBT: wet before test, a) MA15-0.5C, b) Ma20-0.75C, c) Ma28-1C, d) PA30-1C, e) Pa40-1C and Pa60-1.75C.

Completely dissimilar results were obtained for OPC and MPC mortars. Reflectance curves for the different MPC specimens were quite similar, recording presence of *Chlorella vulgaris* after 1 week of exposure. Furthermore, the test was finished after 10 weeks of exposure for MPC samples. By this time, specimens were completely fouled and they were removed from the setup to study the response in dry periods. Visual inspections showed a completely fouled surface of some of the samples already after 4 weeks.

It is important to mention that the initial reflectance of MPC samples was higher than for OPC ones due to the colour of the specimens' surface. Consequently, not only the initial reflectance was higher for MPC specimens, but also the darkness of the surface due to algal biofouling was it. Figure 5.12 shows the evolution of the visual aspect of MPC surface samples in comparison with the more constant aspect of Pa40-C specimens. They had an initial light brown colour while OPC samples were grey and darker. It was also thanks to this initial light colour of MPC specimens that it was possible to detect colour changes already in the first week, when the specimens' surface become slightly darker.

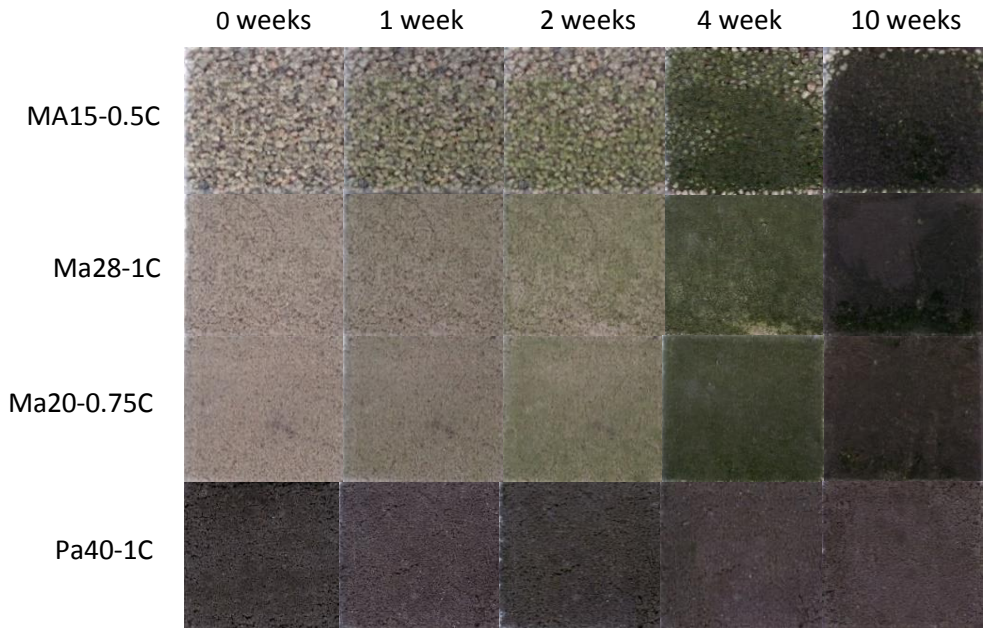

Figure 5.12.- Evolution of the visual appearance of MPC specimens subjected to accelerated fouling and difference in initial colour between MPC and OPC specimens.

Furthermore, from the fourth week until the end of the test accumulation of algae was observed, with a characteristic progressive darkening of the samples (Figure 5.13). Due to this change in lightness, an evident drop in reflectance was observed. Figure 5.13 shows the same results presented in although the same reflectance scale is shown. In that case, differences on initial reflectance is more evident but also it is also shown that difference between initial and final reflectance, taking into account that final reflectance was considered after 10 weeks for MPC specimens and after 20 weeks for OPC.

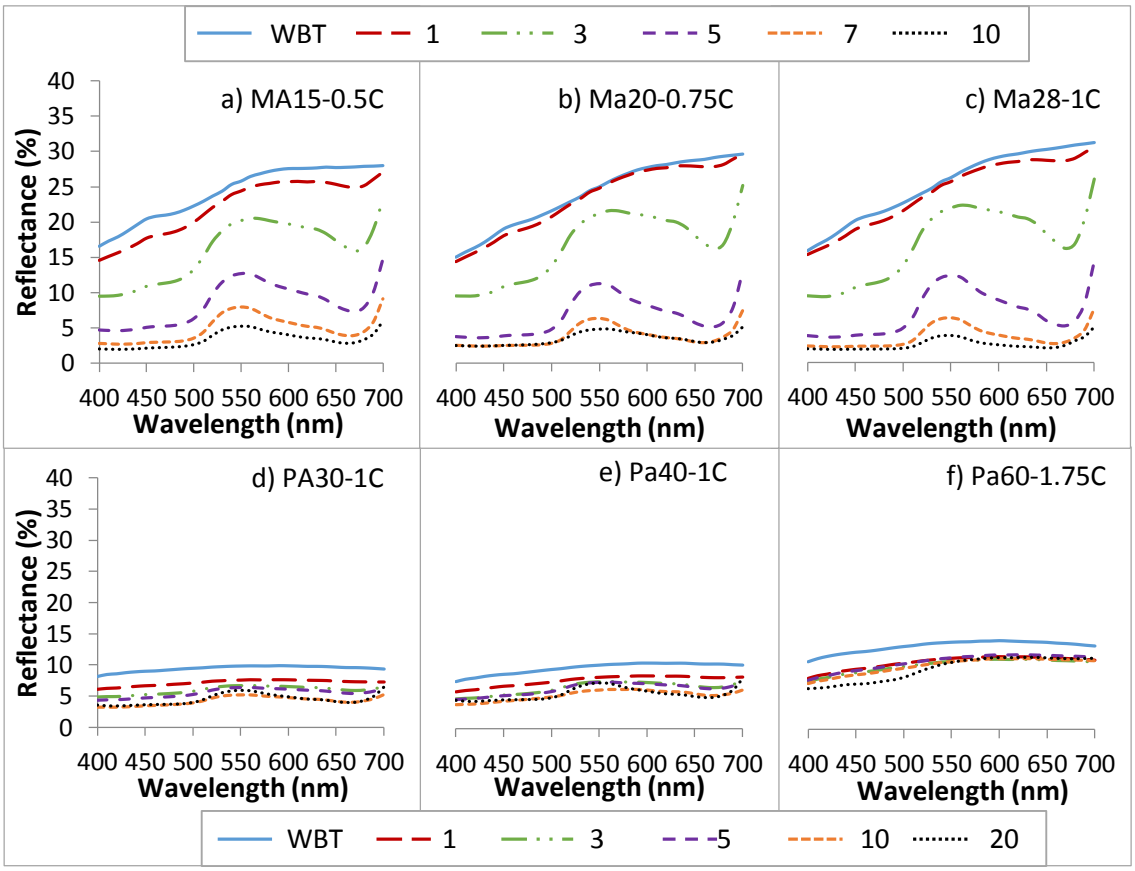

Figure 5.13.- Difference in the drop of reflectance between MPC and OPC specimens in function of the number of weeks subjected to accelerated fouling WBT: wet before test, a) Ma20-B, b) Ma28-C, c) MA15-C, d) Pa40-C, e) Pa60-F and PA30-C.

From graphs of MPC samples (Figure 5.11), it seems that there is no influence of porosity and roughness on biofouling. However, other tests as well as visual examinations showed a different reality. As mentioned before, four different points per specimen were analysed. Furthermore, considering that three replicates were studied, curves correspond to the average of 12 points. Consequently, initial heterogeneities are not detected as Figure 5.14 illustrates. Independent of physical parameters, biofouling of MPC samples started in the upper part of the sample.

A higher heterogeneity was observed for MA15-0.5C specimens due to the highest pore diameter opened to the surface and considering the test was carried out with algae. However, this fact may be different depending on the colonizer organism. In the case of microorganisms as *Chlorella vulgaris*, if the pore diameter were higher than the cell size, organisms would penetrate the specimen and, consequently, spend more time to homogeneously colonised the surface. Then, chemical composition and pH may have more influence than the physical properties when those parameters are suitable for the microorganism.

Depending on the colonizer organisms, suitable physical and chemical properties will be different. For that reason, it is important not lose sight of the fact that microorganisms are the

pioneer colonizers and, consequently, algae like *Chlorella vulgaris* are good pioneer representatives. Furthermore, living organisms modify surface properties during the natural process of colonisation to provide the suitable subtract.

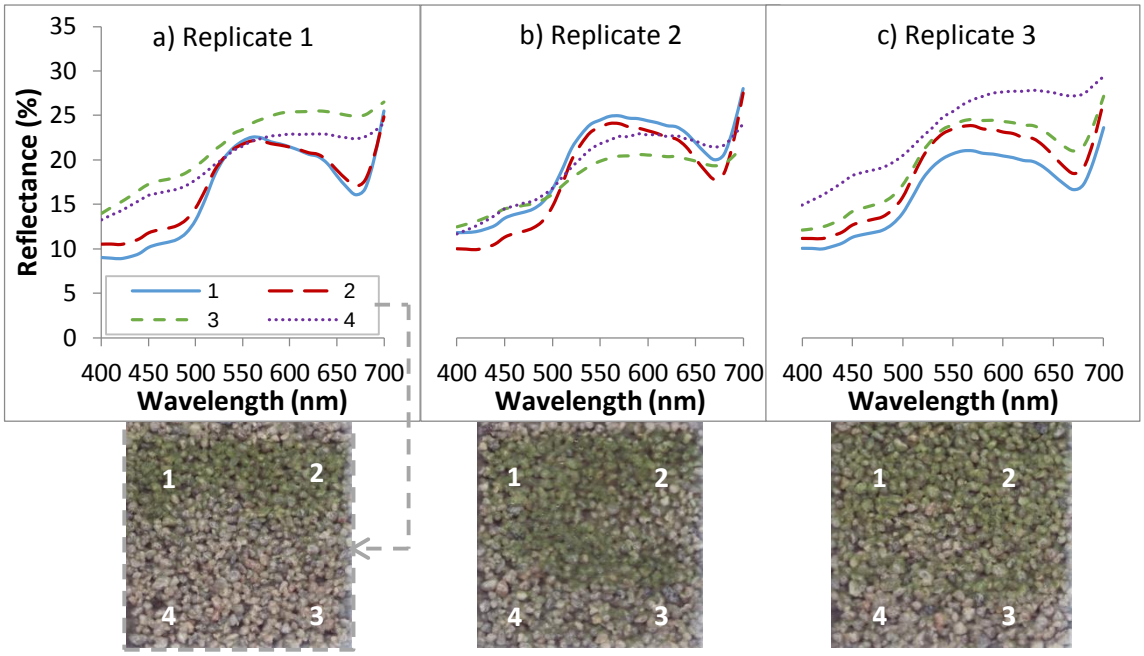

Figure 5.14.- Heterogeneity of reflectance curves for different points (indicated as 1, 2, 3 and 4) and replicates after 2 weeks of accelerated algal fouling.

Results obtained for OPC samples were completely different. The test was prolonged until 20 weeks because no completely fouled surface was detected after 10 weeks. Reflectance curves from specimens prior to the test slightly differ between samples mainly due to the different amount of cement paste and w/c ratio. Biofouling was detected from the second week for Pa40-C and PA30-C, being the samples with the highest porosity and the highest roughness, respectively, although it was undetectable in a visual inspection. Furthermore, fewer reductions in reflectance were detected in comparison to MPC samples.

This fact could be a consequence of the heterogeneity of the fouling of OPC samples as well as for the initial lower reflectance due to natural colour of the samples. Pa60-F samples showed no colonisation on their surface although presence of algae was detected by means of colorimetric measurements due to accumulation of the sprinkling water in some small cavities (as a consequence of air voids). Concerning PA30-C samples, it was detected that algae progressively colonised spaces between aggregates due to the high surface roughness. Furthermore, it is important to notice that biofouling of OPC samples started at the bottom of samples' surfaces. The above may be mainly a consequence of different humidity conditions between the upper and the lower part of OPC specimens.

Other parameters obtained and estimated from colorimetric measurements are shown in Table 5.2. Interesting parameters related to the current objective are parameters L* and a*,

and total difference in colour. Significant changes in lightness were observed for OPC samples, although bigger differences were obtained for MPC samples. Similarly, parameter a* showed a progressive drop, indicating more or less intense biofouling except for Pa60-1.75C. This fact corresponds with previously analysed results, showing no biofouling on these samples. Furthermore, higher total differences in colour where detected for MPC samples, Ma28-1C being the one showing the highest values. This fact might indicate that Ma28-1C specimens are the most bioreceptive ones of all studied samples.

Table 5.2.- Colorimetric measurements.

	Cycle	L^*	a^*	b^*	ΔE	ΔC	ΔH
PA30-1C	0	37,26 ± 2,31	-0,49 ± 0,07	2,34 ± 0,37			
	20	26,79 ± 2,51	-3,18 ± 0,54	7,63 ± 1,23	12,04	5,88	0,84
Pa40-1C	0	37,45 ± 1,10	-0,02 ± 0,12	4,21 ± 0,40			
	20	29,59 ± 1,56	-3,22 ± 0,59	7,49 ± 1,04	9,1	3,94	2,34
Pa60-1.75C	0	43,91 ± 1,57	-0,43 ± 0,10	3,71 ± 0,48			
	20	38,34 ± 1,57	1,34 ± 0,67	10,13 ± 1,78	8,69	6,49	1,51
MA15-0.5C	0	57,29 ± 2,22	1,42 ± 0,53	9,24 ± 1,00			
	10	23,55 ± 5,46	-4,30 ± 0,85	12,59 ± 3,45	34,38	3,95	5,32
Ma20-0.75C	0	55,88 ± 0,95	2,29 ± 0,18	10,93 ± 0,49			
	10	23,02 ± 1,63	-3,12 ± 0,71	9,83 ± 1,50	33,32	-0,85	5,46
Ma28-1C	0	57,08 ± 1,30	2,48 ± 0,24	10,81 ± 0,43			
	10	19,29 ± 1,06	-4,50 ± 0,36	7,55 ± 1,05	38,57	-2,30	7,35

Subsequently, fouling intensity was estimated as mentioned before. Figure 5.15 does not show results corresponding to weeks 6, 7 and 8 due to a problem during the tests, which led to meaningless results. Nevertheless, the graph clearly shows the trend of biofouling. First, MPC samples exhibited higher values and a faster increase of the fouling intensity parameter compared to OPC samples (Figure 5.15). Initially, a fast rise of fouling intensity was observed for all MPC samples. Highest values were obtained for Ma28-1C, arriving to around 10 % and concurring with the reflectance spectra mentioned before. However, a notable drop was observed after week 4.

These results indicates that FI (%) is a useful parameter during the covering process, but can lead to misinterpretation when surfaces are already completely fouled. In the particular case of MPC samples, they were completely fouled after 4 weeks of testing. After these cycles, accumulation of algae on specimens' surface was observed, with the result that surface colour was becoming darker and reflectance was decreasing. In the FI formula, differences between reflectance at 670 nm and 700 nm are considered. Therefore, decreasing the overall reflectance of the sample implies a reduction of this difference (as can be seen in Figure 5.11) and FI ceases to represent the real evolution of biofouling (Figure 5.16).

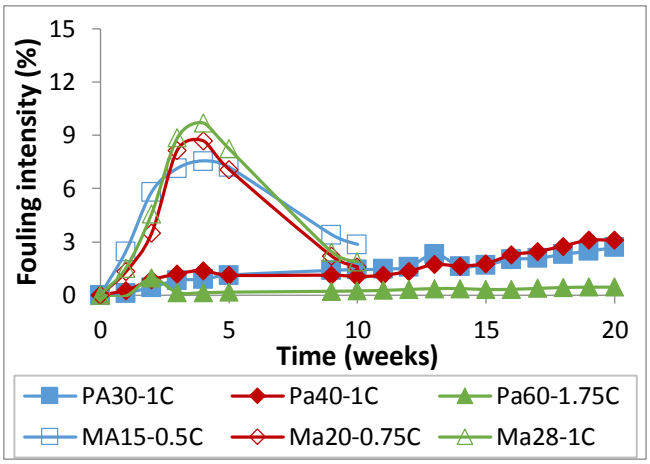

Figure 5.15.- Evolution of fouling intensity (FI). Range of standard errors per dosage during the test: Pa40-1C: 0.19-1.67 %; Pa60-1.75C: 0.01-0.26 %; PA30-1C: 0.01-0.38 %; Ma20-0.75C: 0.06-0.25 %; Ma28-1C: 0.14-0.35 % and MA15-0.5C: 0.15-0.47.

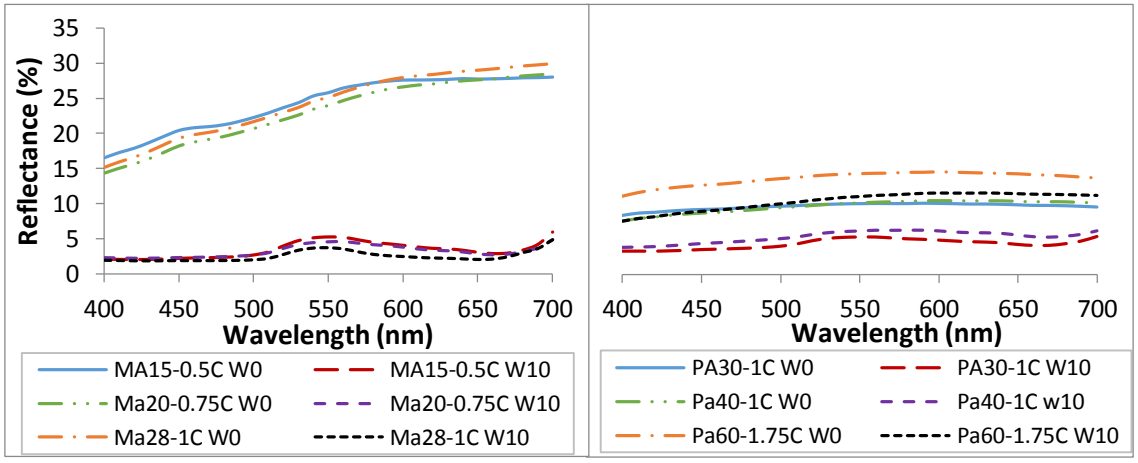

Figure 5.16.- Differences in reflectance curves of MPC (a) and OPC (b) prior to and after 10 weeks of accelerated fouling.

Additionally, OPC samples showed a constant increase trend in fouling intensity although slower than for MPC specimens. Furthermore, this trend may continue further since no complete fouling was observed until 20 weeks. However, the fouling intensity at 2 weeks obtained for OPC samples was only reached after 20 weeks for MPC samples.

Figure 5.16 illustrates the fact that MPC samples suffered an important drop in reflectance curves in comparison with OPC specimens. This progressively darker colour as a consequence of algae accumulation for all MPC specimens indicates that both pH and chemical composition are more suitable for allowing colonisation of *Chlorella vulgaris*. Decrement in lightness (ΔL*) is proposed as a parameter to show a major or minor degree of algal accumulation after complete fouling of specimens' surface.

Subsequently, Figure 5.17 shows the changes in fouled area. Again, the higher bioreceptivity of MPC samples is evident. As mentioned before, a quick fouling of MPC samples

was recorded until complete fouling. Furthermore, the area of fouling for MPC samples increased until around 90 %, while for OPC specimens it remained always lower than 50 %. Nonetheless, it is important to remark that it is not possible to obtain a 100 % of fouling due to pixels corresponding to open porosity.

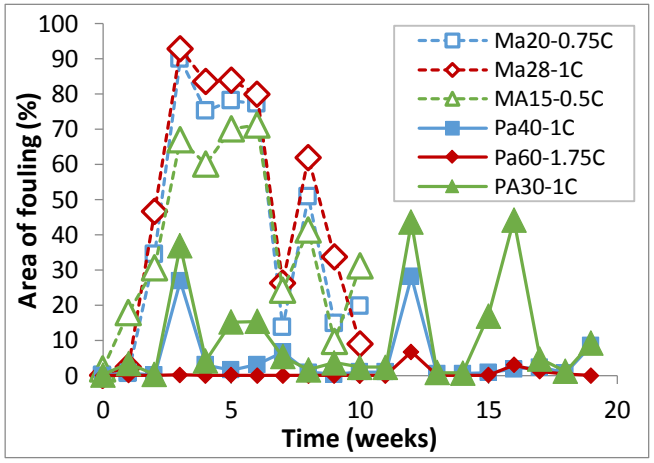

Figure 5.17.- Evolution of the area of biofouling. Range of standard errors per dosage during the test: Pa40-1C: 0.01-11.16 %; Pa60-1.75C: 0.01-1.02 %; PA30-1C: 0.03-5.16 %; Ma20-0.75C: 0.04-7.84 %; Ma28-1C: 0.05-13.67 % and MA15-0.5C: 0.33-10.56 %.

After the sixth week of testing, some incongruent results were recorded due to brightness of the photographs. Moreover, the higher the amount of algae on the surface, the larger the retention of water. Consequently, humidity conditions as well as changes in lightness of the samples caused difficulties to record the evolution of the area of fouling without changing the threshold parameter and/or coordinate.

5.3.2. Biomass quantification

The calibration curve for the non-destructive biomass quantification method is shown in Figure 5.18. Correlation between dry weight and F_0 values follows an exponential tendency and this is coherent with CO_2 availability for the cells in dark conditions. The first stage of the curve corresponds to the fact that cells form a monolayer. In this stage, a linear tendency is observed until there is no more space available and cells are starting to form a multilayer conformation. In this stage, depending on the thickness, just a portion of cells would be in contact with environmental CO_2, and consequently, this method would not be suitable for biomass quantification. Consequently, limit of suitability of the current method should be considered around 320 mg/L (20 mg/cm³) as shown in Figure 5.18.

Figure 5.19 (a) shows the correlation between F_0 and biomass expressed by area (cm²). This correlation should be used with caution because no homogeneous growth is observed in cementitious materials. However, a quantification of dry weight expressed by mg/L has no sense when the surface of building materials is studied. Furthermore, due to heterogeneity of the material and size of *Chlorella vulgaris*, it was decided not to use the basic international units

system (using mg/cm² instead of mg/m²). Figure 5.19 (b) shows biomass quantification taking into account the correlation between dry weight and F_0. The most important and evident results are that Ma28-1C specimens showed the maximum amount of biomass on their surfaces, and no biofouling was recorded on Pa60-F specimens. As shown in Figure 5.19 (b), more algae could be observed on MPC specimens than on OPC specimens.

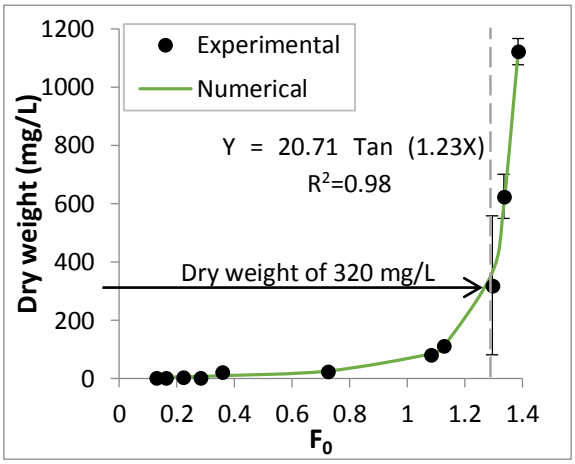

Figure 5.18.- Standard curve for biomass quantification by means of a correlation between dry weight and F_0.

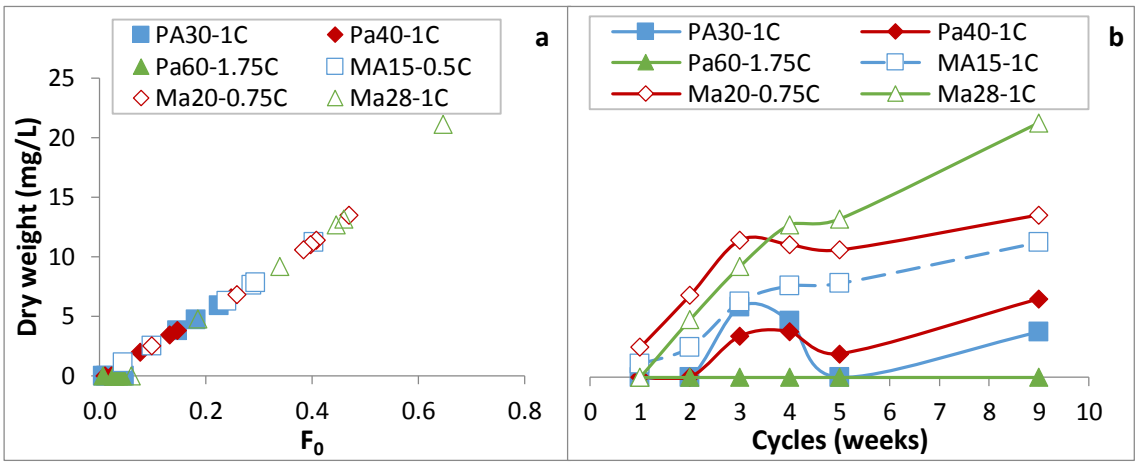

Figure 5.19.- MPC specimens exhibited a higher amount (a) and a faster rate (b) of biomass accumulation during the fouling tests compared to OPC specimens.

Concerning the OPC specimens, Pa40-1C and PA30-1C showed an initial increase of biomass until the third week. Subsequently, a drop of biomass was recorded to finally increase again. However, final biomass for both degrees of bioreceptivity was considerably lower than biomass on MPC specimens. Moreover, Pa60-1.75C had collected no biomass after 9 weeks of testing.

In comparison with the OPC samples, MPC samples showed a faster and more marked increase of biomass. Additionally, the lower biomass observed on MA15-0.5C might be the consequence of higher pore diameter. It was visually detected that sprinkling water (with

nutrients and algae) penetrated through the pores and less retention of algae was recorded. Furthermore, between samples with the same size of aggregates, a higher response to biofouling was observed for Ma28-1C. This fact was also shown in other tests such as fouling intensity, area of fouling (until total coverage of specimens' surface) and ΔE.

Table 5.3 presents an average of F_v/F_m values obtained from weeks 1 to 5. The algae covering both Pa40-1C and PA30-1C samples exhibited good physiological state in terms of photosynthesis from the third and fourth week onwards respectively. (F_v/F_m values higher than 0.5 as mentioned before). The above could indicate that Pa40-1C specimens presented a slightly higher bioreceptivity for *Chlorella vulgaris* than PA30-1C. Additionally, no algal presence was recorded in Pa60-1.75C specimens by means of colorimetric measurements although low levels of dry weight were recorded. F_v/F_m values also indicate the presence of an extremely low amount of algae. Furthermore, these results showed that the algae were under stress (the substrate is not the appropriate) and indicated the low accuracy of the method. MPC specimens presented a higher response, especially for Ma20-0.75C and Ma28-1C specimens, while the values for MA15-0.5C specimens were somewhat lower.

Table 5.3.- F_v/F_m values obtained between cycles 1 and 5 indicating the state of the photosynthetic apparatus.

Cycle (weeks)	PA30-1C	Pa40-1C	Pa60-1.75C	MA15-0.5C	Ma20-0.75C	Ma28-1C
1	0.380 ± 0.055	0.289 ± 0.063	0.198 ± 0.050	0.334 ± 0.017	0.494 ± 0.008	0.438 ±0.025
2	0.427 ± 0.048	0.434 ± 0.041	0.152 ± 0.037	0.487 ± 0.011	0.578 ± 0.003	0.502 ± 0.022
3	0.489 ± 0.052	0.532 ± 0.034	0.171 ± 0.031	0.577 ± 0.007	0.617 ± 0.006	0.581 ± 0.013
4	0.616 ± 0.005	0.622 ± 0.006	0.139 ± 0.029	0.511 ± 0.014	0.553 ± 0.011	0.597 ± 0.005
5	0.526 ± 0.013	0.533 ± 0.022	0.179 ± 0.038	0.485 ± 0.010	0.530 ± 0.010	0.534 ± 0.009

Finally, the influence of a dry period on biomass evolution was evaluated. Figure 5.20 shows F_0 values obtained from 1 to 9 weeks in dry conditions. This graph clearly presents three different stages. First, a drop of F_0 values was observed, which may be a consequence of a decrease in biomass or a protective mechanism to overcome the lack of water, with the algae becoming less photosynthetically active. According to Heber et al. (2007), F_0 for dark-adapted mosses or lichens decrease when the drying process occurs slowly. This reduction of F_0 took place during the first week due to water stress.

Subsequently, the response to fluorescence increased until the second week, which can be considered as a stress indicator similar to what is observed for the case of plants (Lichtenthaler, 1988; Rohácek, 2002). The final decrease of F_0 after week 2 corresponded to the death of the algae (drop of biomass). Furthermore, F_v/F_m values revealed that this method should not be used as a quantification technique when data lower than 0.2 are obtained (Table 5.3 and Table 5.4).

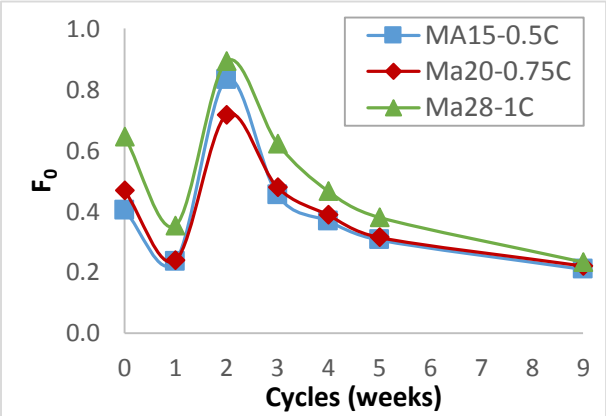

Figure 5.20.- F_0 response to hydric stress.

Considering results obtained for F_0 and F_v/F_m, it was observed that until one week of water stress, a protective mechanism could be occurring, leading to a decrease of both parameters. However, during the second week of water stress, F_0 underwent a sharp increase while F_v/F_m continued decreasing. According to some authors (Heber et al., 2001; Souza et al., 2004; Guo et al., 2006), the above may be related to an advanced stage of stress where PSII is damaged. Finally, in this particular case where no water was applied for 2 weeks, irreversible damage was observed provoking dying of the algae.

Table 5.4.- F_v/F_m values obtained between cycle 1 and 5 under hydric stress.

Cycle (weeks)	MA15-0.5C	Ma20-0.75C	Ma28-1C
1	0.055 ± 0.009	0.133 ± 0.013	0.031 ± 0.005
2	-0.011 ± 0.005	0.012 ± 0.005	-0.019 ± 0.002
3	-0.016 ± 0.003	-0.020 ± 0.004	-0.027 ± 0.001
4	-0.023 ± 0.002	-0.026 ± 0.002	-0.030 ± 0.002
5	-0.020 ± 0.004	-0.018 ± 0.002	-0.025 ± 0.003

5.4. CONCLUSIONS

MPC specimens showed a higher bioreceptivity for *Chlorella vulgaris* than OPC specimens. This is a consequence of the pH and chemical composition which makes this hydraulic binder suitable to allow growth of microorganisms. Furthermore, Ma28-1C appeared to be the most bioreceptive MPC composition, in spite of having the lowest porosity of all samples, and a lower surface roughness than MA15-0.5C.

MPC is more suitable for stimulation of algal colonisation. However, other groups of organisms may have higher affinity for carbonated OPC. Nevertheless, since algae are pioneers of biofouling of cementitious materials, together with bacteria, MPC mortar is likely to be more rapidly colonized. Furthermore, it was observed that Ma28-1C specimens were the most suitable ones in order to stimulate colonisation of *Chlorella vulgaris* in laboratory conditions.

Chemical properties of these specimens seem to have more influence on colonisation than physical properties did. This fact can be seen when comparing OPC and MPC samples.

Furthermore, the clearest difference between different MPC specimens for *Chlorella vulgaris* colonisation is the heterogeneity of initial growth. Due to the higher pore diameter, water containing the algae entered into the specimens, showing a more heterogeneous fouling pattern. Ma20-0.75C and Ma28-1C specimens showed similar results although visual inspections showed a more homogenous and resistant growth and better aesthetic appearance during water stress for Ma28-1C specimens.

Visual inspections revealed a difference in the first stage of colonisation between OPC and MPC specimens. For OPC samples, biofouling started at the bottom side of the specimens, which may be related to the high amount of moisture located in this area. In contrast, MPC specimens showed a better water retention as well as more homogeneous moisture distribution. Consequently, the MPC mortar samples behave like a filter where water penetrated and algae remained on the surface, initially localizing algal fouling in the upper part.

Related to the initiation of biofouling, the presence of algae was detected from the first week on MPC specimens' surface and from the second week on OPC ones. Moreover, MPC samples were completely fouled after 4 weeks, while no complete fouling was observed until 20 weeks for OPC samples.

Concerning the techniques and evaluation criteria used, some considerations should be taken into account. First, results obtained from the fouling intensity analysis revealed that this parameter is not suitable when MPC samples are completely fouled. Once the specimen's surface was covered, the amount of algae was increasing forming different layers and showing as a result a decrease in lightness (L*). The highest decrement in lightness was observed for MPC specimens, resulting in a drop of "fouling intensity" in the range between 62 % and 82 %. Additionally, a more pronounced decrease of the a* parameter was also observed for MPC samples.

A threshold on the a* parameter was used for image analysis and pixels with a* values higher than 120 were considered as fouled. However, problems to obtain realistic values were observed after the sixth week of testing. Once MPC specimens were completely fouled and the thickness of the algae layer was increasing, lightness was decreasing and humidity of the samples increasing. Consequently, humidity caused some brightness in the photographs that made them more difficult to analyse. The current method for algal quantification is suitable until biofouling reached a coverage of about 20 mg/cm^2. Higher amounts of dry weight induce low and insignificant differences on F_0. The mg/cm^2 units are proposed in order to express biomass for surfaces, since expressing biomass relative to volume units is not appropriate for this purpose.

Limitation of the use of this method was also observed when algae were submitted to dry periods. Increases of F_0 values were observed where exposed in dry periods probably showing PSII damage concurring with a drop in F_v/F_m.

6. ANALYSIS OF NATURAL COLONISATION

6.1. INTRODUCTION

Bioreceptivity of a material, climate conditions and the organisms present in an environment are the three big groups of parameters involved in colonisation of materials by living organisms. The first one has been already studied by controlling the remaining parameters in the previous chapter.

The experimental program presented in Chapter 5 was useful as a fast method to compare materials with different bioreceptivity. Parameters such as temperature, relative humidity, day and night regime, the run-off period and the culture solution were controlled. Consequently, having controlled all these parameters, the only variable was specimens' bioreceptivity. However, that situation significantly differs from what happens in real environmental conditions. In that case, not only the specimens differ from one to the other, but also environmental conditions as well as aspects related to living organisms do.

The current chapter provides two new aspects based on the combination of variable climatic conditions, as well as presence and diversity of microorganisms. Concerning to climate conditions, parameters such as temperature, precipitation, sunshine duration (time that direct radiation is higher than 120 W/m²) or direction of the wind will influence colonisation. Moreover, the aforementioned parameters will vary, depending on the location as well as depending on the season. Finally, microorganisms present in the environment differ depending on the geographical situation and different relationships can be found between them. The

effects of competition on the distribution and abundance of two species have been studied in ecology for a long time (Keddy, 1989). Widden (1997) states that a stable coexistence between two species could only occur when competition between them is weak. Consequently, only the main division between competition and coexistence will be considered in this study.

Literature reveals the majority of the studies about bioreceptivity of cementitious materials were carried out exclusively under laboratory conditions although there are recent studies such the one by Tran et al. (2013). However, it is important to simultaneously study natural colonisation. Accordingly, the aim of the current chapter is to evaluate the colonisation of specimens with the same mix design as tested in the previous chapter under environmental conditions. To reach this objective, the following specific objectives are defined:

- Determine mix designs suitability for pioneer colonisers such as environmental bacteria and fungi;
- Assess differences in results due to specimens inclination as well as location;
- Determine the necessity of surficial treatments to accelerate the colonisation process.

6.2. MATERIALS AND METHODS

6.2.1. Locations

Regarding the environmental conditions, both climate conditions as well as quality of the air will influence the colonisation. Therefore, specimens were placed in three different locations: Barcelona city, the Natural Park of Montseny (60 km from Barcelona) and Ghent city. First location corresponds to a contaminated area with Mediterranean climate, the second one to the same climate conditions (although there are some differences due to altitude and proximity of the sea) but in a non-contaminated area and the third in the surroundings of the city of Ghent with oceanic climate (Figure 6.1).

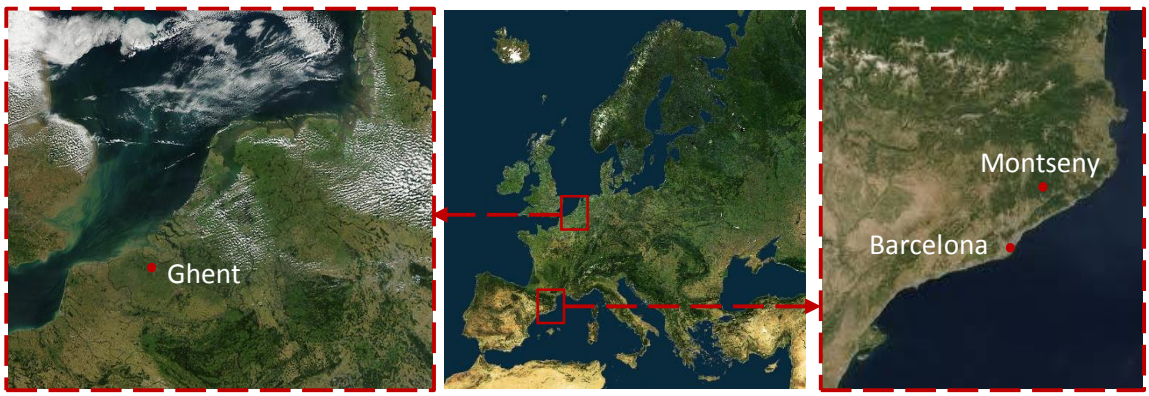

Figure 6.1.- Location of the specimens under environmental exposure.

Mediterranean climate is characterised by general moderate temperatures with hot, dry summers and wet winters with an average 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.

6.2.2. Specimens and setup

According to chapter 5, same specimens' dosages were used for environmental exposure (PA30-1C, Pa40-1C, Pa60-1.75C, MA15-0.5C, Ma20-0.75C and Ma28-1C). Sixty-six replicates per dosage were produced in order to compare results of different locations as well as positions. Both OPC and MPC specimens were cast into 80 x 80 x 20 mm³ polyurethane moulds and demoulded as previously defined. Furthermore, the curing process for both as well as the accelerated carbonation process for OPC specimens was carried out.

Expanded polystyrene plates of 3 cm thickness were cut in order to randomly embed the specimens (Figure 6.2). For that reason, three different layers of expanded polystyrene were used: the first one with the support function and the next two with holes of the specimens' size to fix them inside just leaving one side exposed to the environment. Previous hypothesis was that colonisation in the Mediterranean climate locations would be slower in comparison to the temperate maritime climate. Consequently, two different setup sizes were designed depending on the final location. Furthermore, support plates were produced to place some of the samples horizontally and others vertically.

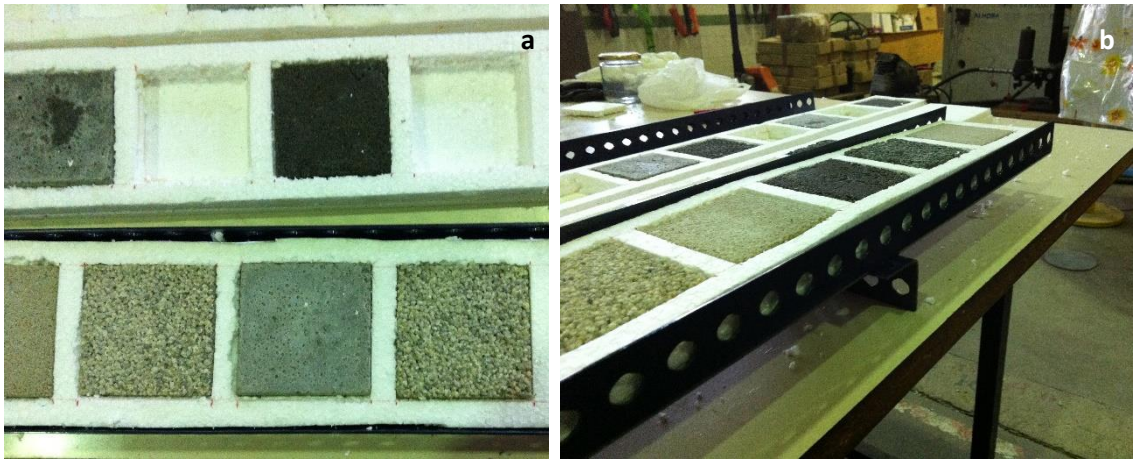

Figure 6.2.- Specimens randomly embedded in the expanded polystyrene plates.

Two 1 x 1 x 0.03 m³ plates were produced to place specimens horizontally in Barcelona and Montseny and one 1 x 1.4 x 0.03 m³ plate for the setup of Ghent. Afterwards, expanded polystyrene to place specimens vertically were cut forming straps of one sample next to the other to place all the specimens in the same position. Twelve 1 x 0.12 x 0.03 m³ straps were produced to place 3 of them in Barcelona, 3 more in Montseny and 6 in Ghent. Subsequently, a metallic support was built in order to fix the expanded polystyrene supports with the embedded specimens. Finally, the complete structure was covered with a net in order to avoid the entrance of animals, which could interfere in the experiment. Furthermore, setups were placed in the way

that vertical specimens will face the north orientation in Montseny and Barcelona (Figure 6.3 and Figure 6.4) and north-west in Ghent (Figure 6.5).

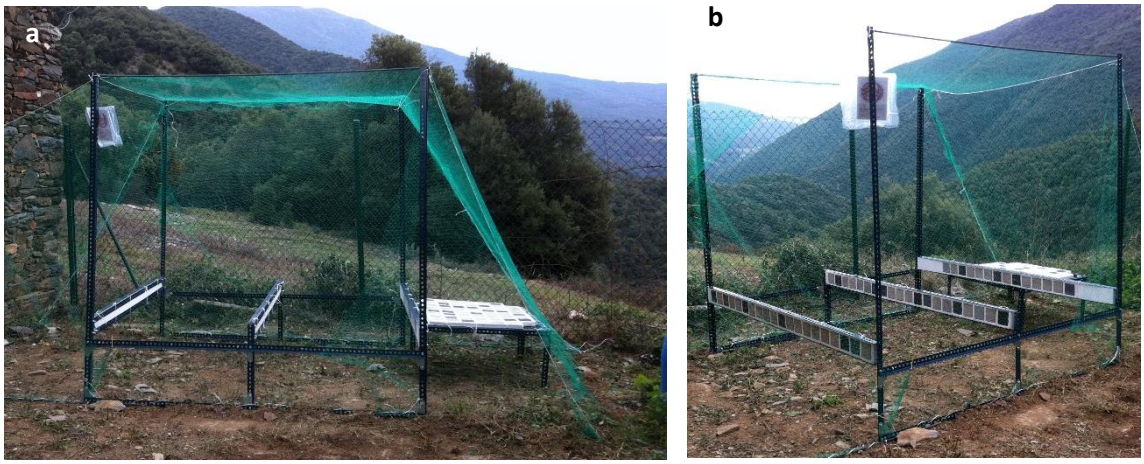

Figure 6.3.- Setup of specimens placed in Montseny.

Figure 6.4.- Setup of specimens placed in Barcelona city.

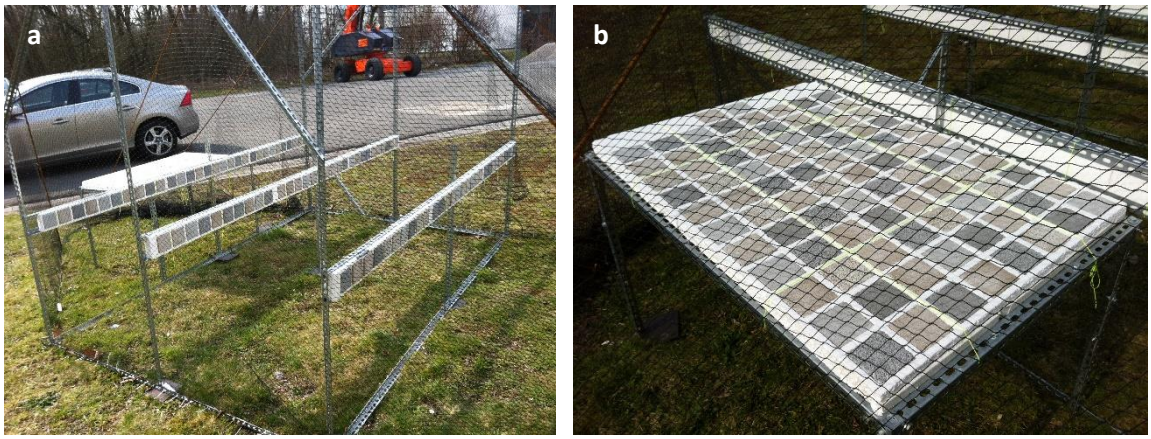

Figure 6.5.- Setup of specimens placed in Ghent city.

Maintenance of the setup areas was carried out in order to avoid interference of surrounding plants. That task was specially done in Montseny for being a mountainous area and also in Ghent, where the setup was placed in a green area at the back side of the Magnel Laboratory for Concrete Research of the Faculty of Engineering and Architecture. Specimens were exposed for approximately 1 year in Barcelona and Montseny, and approximately 6 months in Ghent.

6.2.3. Samples collection and analysis

A wide variety of microorganisms can be found in petrous materials such as natural rocks or cementitious materials. According to Escadeillas et al. (2007), pioneer colonisers are usually bacteria. Those microorganisms have the capability of colonising every surface and allow the colonisation by other organisms. Biofilms formed by bacteria are usually invisible due to the absence of pigments. Then, algae, which are characterised for being autotrophic, form another pioneer group of organisms. In that case, growth is visible and different colours can be observed. Afterwards, fungi can also appear, a group that it is not considered as a pioneer since it is formed by heterotrophic organisms.

The current experimental program aims to give comparative information between different materials' bioreceptivity in the first stage of natural colonisation. In this sense, both visual and invisible colonisation were studied. For the above purpose, detection of photosynthetic organisms by image analysis as well as detection and quantification of the predominant microorganisms by means of SEM, plate counting and biochemical and microscopic analysis were obtained.

First, detection of biological growth due to photosynthetic organisms was determined by means of image analysis. Standardized images of all specimens were taken monthly. A metallic support to standardize the angles as well as the distance between the camera objective and the surface of the specimens was developed. Image analysis was then carried out in order to determine possible changes in the colour of the samples due to colonisation of photosynthetic organisms similarly as it was done in the previous Chapter.

Subsequently, destructive analyses of the specimens were carried out once, at the end of the test (SEM, microorganisms' identification and plate counting). The aforementioned analysis aims to determine the predominant organisms presents on the specimens surface in order to compare different bioreceptivities of the specimens as well as environmental conditions due to location of specimens. Classification and identification of the different groups of organisms, which could be present on specimens' surface, will follow different procedures. First, microorganisms should be grown in specific media depending of the group of organism. For instance, general media for bacteria, fungi or yeasts are selected in order to separate those big groups of organisms. However, there are microorganisms that cannot be grown under laboratory conditions or need special media for their growth. Furthermore, an incubation period

under specific conditions is required after the first stage, in which organisms are sown in the media. Afterwards, pure cultures should be obtained in order to identify the specie or genus.

Different methods can be used for organisms' identification going from classical methods, such as biochemical analysis or microscopic and macroscopic characteristics, to molecular technics, such as the Polymerase Chain Reaction (PCR). In the current experimental program classical methods were used for identification. Taxonomy is the branch of the science concerned with classification of organisms. Accordingly, differential characters should be known in order to determine the species or at least the genus of an organism.

There are plenty of tests for microorganisms' identification although not all of them are useful for the same groups. For instance, bacterial identification usually starts by determining the Gram reaction. By this method, a first screening allows to classify the specie as Gram positive or Gram negative. Then, it is also worthwhile to study the morphology and motility of the cells by means of a microscope although morphology could be affected by the medium on which the organisms are grown and temperature of incubation (Cowan, 1974). Afterwards, biochemical as well as growth ability under different conditions are also useful to identify the species. Regarding identification of fungi and yeasts, microscopic and macroscopic characteristics analysis as well as a biochemical test are usually carried out.

For that purpose two replicates per specimen, position (horizontal or vertical) and location were selected randomly. Then, microorganisms from the surface of the specimens were removed with a cotton swab. Those samples were then seeded in different culture media to detect bacteria, algae and fungi in the Laboratory of Microbiology of the Faculty of Veterinary Medicine (Autonomous University of Barcelona). General culture media used for bacteria detection was Tryptic Soy Agar (TSA). Sabouraud Agar with antibiotics was used for fungi determination, Tryptone Sulfite Neomycin Agar (TSN Agar) and Sulfite Polymixin Sulfadiazine Agar (SPS Agar) for anaerobic bacteria and MacConkey Agar for *Enterobacteriacea* determination. Furthermore, punctual samples of environmental microorganisms present in all three areas were obtained by exposing two Petri dishes, one containing Tryptic Soy Agar and the other one containing Sabouraud Agar with antibiotics. Then, Petri dishes were maintained open next to the setup during 10 minutes to obtain the sample. Composition of all culture media is detailed below.

Once samples were seeded, Petri dishes were incubated under different conditions as detailed in Table 6.1. Afterwards, 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 API 20C AUX gallery.

Table 6.1.- Formula of the culture media in 1 L of distilled water.

Medium	Composition	Quantity (g/L)
Tryptone Soy Agar **Final pH 7.3 ± 0.2 at 37° C** **Incubation: 25° C, 24-48 h**	Casein peptone (pancreatic) Soya peptone (papainic) Sodium chloride Agar	15.0 5.0 5.0 15.0
Sabouraud Agar **Final pH 5.6 ± 0.2 at 25° C** **Incubation: 25° C, 3-4 days**	Peptone Dextrose or glucose Agar	10.0 40.0 15.0
TSN Agar **Final pH 7.0 ± 0.2 at 25° C** **Incubation (in anaerobic conditions): 42° C, 24-48 h**	Casein peptone Yeast extract Sodium sulphite Ferric citrate Neomycin sulphate Polymixin B sulphate Agar	15.0 10.0 1.0 0.5 0.05 0.02 13.5
SPS Agar **Final pH 7.0 ± 0.2 at 25° C** **Incubation (in anaerobic conditions): 37° C, 24-48 h**	Casein peptone Yeast extract Ferric citrate Sodium sulphite Sulfadiazine Polymixin B sulphate Agar	15.5 10.0 0.5 0.5 0.12 0.01 13.0
MacConkey Agar **Final pH 7.1 ± 0.2 at 25° C** **Incubation: 37° C, 24-48 h**	Enzymatic digest of gelatine Enzymatic digest of casein Enzymatic digest of animal tissue Lactose Bile salts mixtures Sodium chloride Neutral red Crystal violet Agar	17.0 1.5 1.5 10.0 1.5 5.0 0.03 0.001 13.5

Moreover, climate data were obtained from the nearest climate station, which will include maximums and minimums or an average of the monthly temperatures (°C) depending on the location, total precipitation per month (l/m^2), sunshine duration (h), and the predominant wind direction. Data corresponding to relative humidity (%) and wind speed (m/s) will be also provided. Data corresponding to those parameters were obtained daily, every 30 minutes in the case of Barcelona and every hour for the weather station in Montseny. However, in order to facilitate the analysis, results were processed in order to obtain monthly values. In this sense, maximum and minimum temperatures per month are provided for the Spanish locations and an average of the mean temperatures for Ghent. The data of precipitation and sunshine duration were cumulated in order to provide the total precipitation and the total duration of sunshine per month. Wind direction data was expressed in degrees and all values were processed in order

to group them into eight directions (north, north-east, east, south-east, south, south-west, west and north-west). Finally, an average of the relative humidity as well as the wind velocity per month was estimated.

Afterwards, information related to air quality will be presented only for the Spanish locations, since it was not possible to obtain the Belgian records. The data considered in the current investigation were concentrations of sulphur dioxide (SO_2, µg/m³), nitrogen oxides (NO_x, µg/m³), carbon monoxide (CO, mg/m³) and ozone (O_3, µg/m³). Those parameters are included in the BOE-A-2011, Real Decreto 102/2011 (Spanish Loyal Legislative Decree) and they can also give extra information to the weather data. In that case, values were obtained daily and the monthly average was calculated.

6.3. RESULTS AND ANALYSIS

In the present section different results will be shown. Three subsections will divide the results by location. Those subsections will include the identification of the most representative species present in all different specimens as well as general information about them will be presented. Moreover, they include an analysis about the representativeness of the results as well as the possible causes and finally, information regarding climate conditions and air quality, comparing the available data for the different locations. Regarding the procedures mentioned in the previous section, photographs of all the specimens were taken. However, no visible growth, the different humidity conditions for specimens at different times or the intensity of the natural light make their comparison difficult (Figure 6.6).

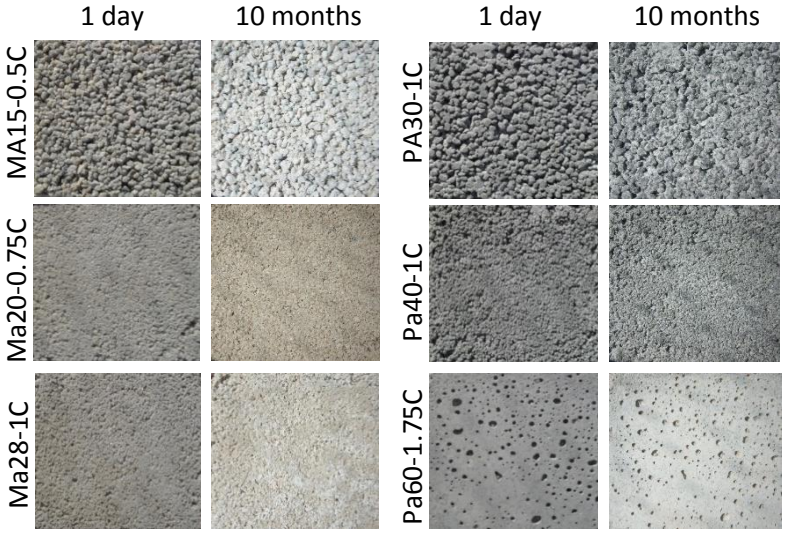

Figure 6.6.- Visual appearance of each dosage in Barcelona city at the beginning and the end of the test.

6.3.1. Barcelona city

Predominant microorganisms

Four different genera of bacteria and ten genera of fungi were identified as the most representative in the punctual environmental sampling. That punctual sampling cannot be considered as a valid result for a period of time since microorganisms distribution varies temporarily as well as geographically. 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. Moreover, fungal populations differ between regions as well as between seasons as happens with bacteria (Shelton et al., 2002).

The identified bacterial genera (species are presented in brackets) were *Aerococcus* (*Aerococcus sp.*), *Bacillus* (*Bacillus subtilis*, others), *Flavobacterium* (*Flavobacterium sp.*) and *Kocuria* (*Kocuria kristinae*, *Kocuria lutea* and *Kocuria rhizophilum*), whose main characteristics are presented in Table 6.2. A total of 78 colony forming units (CFUs) per plate were obtained by direct plate count. With regard to fungi, the genera observed were *Acremonium* (*Acremonium strictum*), *Alter*naria (*Aternaria alternate*), *Aspergillus* (*Aspergillus flavus*, others), Aureobasidium (Aureobasidium pullulans), Cladosporium (*Cladosporium herbarum*), Fusarium (Fusarium moniliforme), Penicillium (Penicillium sp.), Phoma (*Phoma herbarum*), *Rhizopus* (*Rhizopus sp.*) and *Rhodotorula* (*Rhotorula glutinis*). The number of CFUs per plate obtained was 60 for both fungi and yeasts and the taxonomic classification of all genera is presented in Figure 6.7.

Taxonomic classification is a useful tool for the analysis since it will provide information about how similar could be the organisms. For instance, the genus *Aspergillus* and *Penicillium* correspond to the same taxon (Family *Trichocomacea*) and those genera have more similar characteristics than between the genus *Aspergillus* and *Rhizopus*, which correspond to two different divisions. In this sense we could distinguish three main groups, which are *Ascomycota, Zygomycota* and *Basidiomycota*. Inside those groups, six subgroups can be established for *Ascomycota* and one for each one of the other two groups.

Regarding the organisms identified on the specimens surface located in the city of Barcelona, one more genus of bacteria (*Streptococcus*) and three more genera of fungi (*Epicoccum, Gilmaniella* and *Mucor*) were identified. According to the characteristics provided

in Table 6.2, *Streptococcus* species are aerobic and formed by Gram positive spherical cells, which are usually observed in pairs or chains. Cells have a diameter between 0.5 and 1 µm with non-activity catalase nor oxidase. Moreover, they are typically non-motile and non-spore forming. Regarding the fungi, the aforementioned genera are included in colour blue in Figure 6.7. Two of the genera, *Epicoccum* and *Mucor*, form part of families already identified, which is not the case for the genus *Gilmaniella*.

Table 6.2.- Main characteristics of the identified genus of bacteria

Characteristics	*Aerococcus*	*Bacillus*	*Flavobacterium*	*Kocuria*
Shape	Sphere	Rod-shaped	Rod-shaped	Sphere
Size	1-2 µm	0.5-1.5 x 2-6 µm	0.3-0.5 x 2-5 µm	0.7-1.5 µm
Gram	+	+	-	+
Catalase/Oxidase	Weak reaction/-	+/variable	+/+	+/-
Formation	Cells in pairs, tetrads or small clusters	Chains	Chains of 3-4 cells	Cells in pairs, tetrads or small clusters
Motility	-	+	-	-
Spores	-	Endospores	-	-
Aerobic/Anaerobic	Aerobic	Mostly aerobic	Aerobic	Aerobic

Afterwards, Table 6.3 shows the predominant genera found on the specimens placed horizontally. Moreover, results of the plate counts are also provided (CFUs per plate). The number of different genera as well as the genera found per dosage was similar although the quantification showed significant differences between them.

Results presented in Table 6.3 show differences between OPC and MPC specimens as well as between different dosages. Due to the interspecies relationships, it was expected to observe a competition in number between bacteria and fungi. The number of CFUs of bacteria was higher than for fungi in all samples. Nevertheless, the higher CFUs of fungi were observed in the samples with the lowest CFUs of bacteria (Ma20-0.75C) and vice versa for samples from Pa60-1.75C specimens.

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 may be normal to find cells everywhere due to their capabilities. The aforementioned genus has the capability to form endospores, which are small, metabolically dormant cells that 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).

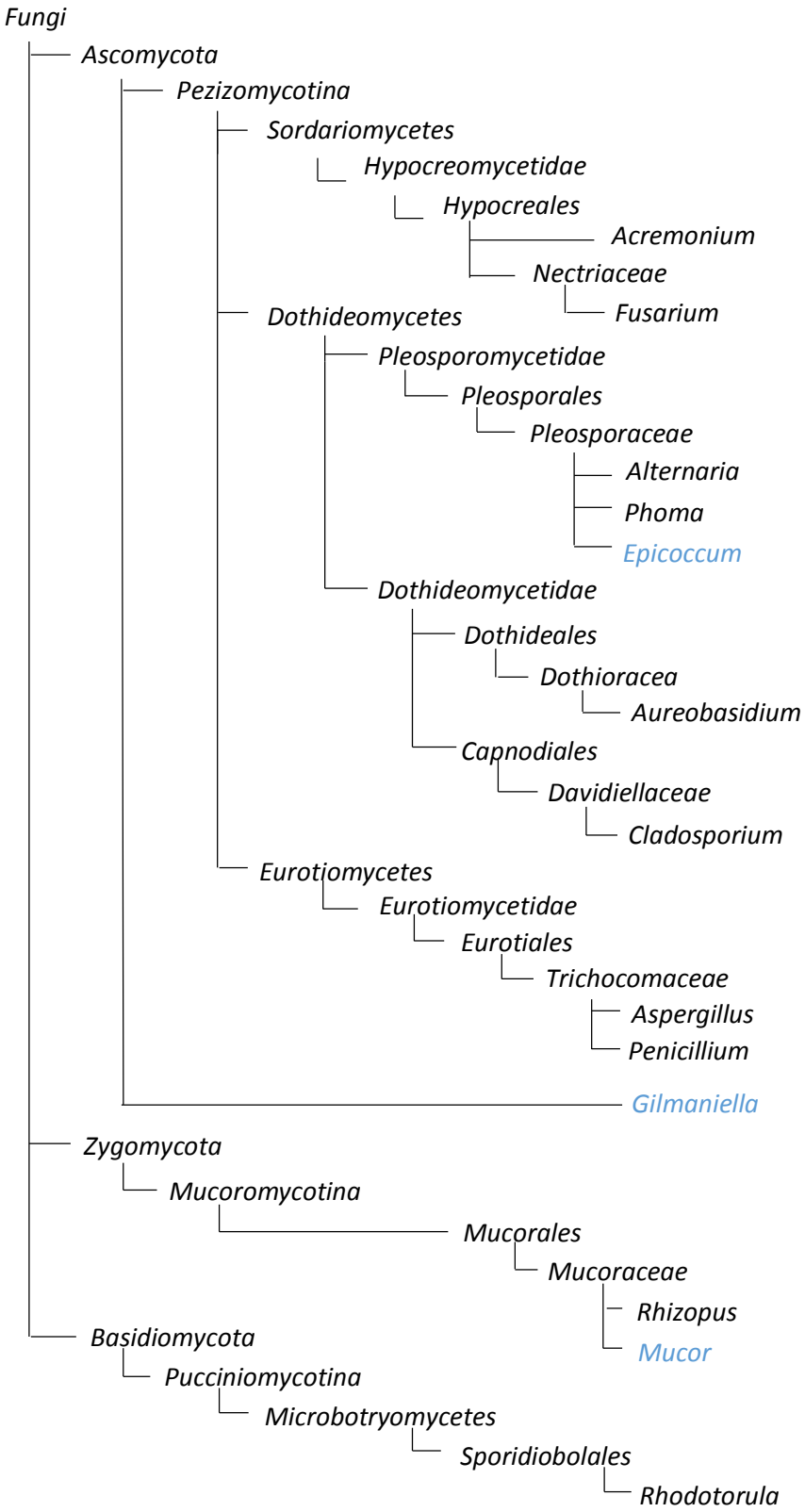

Figure 6.7.- Taxonomy of the identified genera of specimens of Barcelona city. Genera written in blue were found only on specimens' surface.

Table 6.3.- Identification and counting of bacterial and fungal genera on horizontal specimens placed in Barcelona city

Horizontal specimens	Bacteria	CFUs	Fungi	CFUs
PA30-1C	*Aerococcus* *Bacillus* *Flavobacterium* *Kocuria (K. lutea, K. rhizophila, K. kristinae)*	*74*	*Acremonium (A. strictum)* *Cladosporium (C. herbarum)* *Epicoccum (E. purpurascens)*	*8*
Pa40-1C	*Aerococcus* *Bacillus* *Kocuria (K. lutea, K. rhizophila)*	*94*	*Acremonium (A. strictum)* *Alternaria (A. tenuis)* *Cladosporium (C. herbarum)*	*9*
Pa60-1.75C	*Aerococcus* *Bacillus* *Flavobacterium* *Kocuria (K. lutea, K. rhizophila, K. kristinae)*	*170*	*Acremonium (A. strictum)* *Alternaria (A. tenuis)* *Cladosporium (C. herbarum)*	*7*
MA15-0.5C	*Aerococcus* *Bacillus* *Kocuria (K. lutea, K. rhizophila, K. kristinae)* *Streptococcus*	*84*	*Alternaria (A. tenuis)* *Cladosporium (C. herbarum)* *Phoma (P. herbarum)*	*13*
Ma20-0.75C	*Aerococcus* *Bacillus (B. subtilis and others)* *Flavobacterium* *Kocuria (K. lutea)*	*70*	*Acremonium (A. strictum)* *Alternaria (A. tenuis)* *Cladosporium (C. herbarum)* *Gilmaniella (G. humicola)* *Phoma (P. herbarum)*	*22*
Ma28-1C	*Aerococcus* *Bacillus* *Kocuria (K. lutea, K. rhizophila, K. kristinae)*	*108*	*Alternaria (A. tenuis)* *Cladosporium (C. herbarum)* *Phoma (P. herbarum)*	*14*

Regarding microorganisms quantification, results also show that the highest CFUs were obtained for the dosage Pa60-1.75C, which has the lowest R_a-value and the second lowest percentage of voids. However, that fact may be consequence of the aforementioned low roughness, which would favour the removal of the organisms. In contrast, that process will pose a challenge for specimens with high roughness, in which organisms could be placed deeper. The above could imply differences in the predominant genus on a specimen although it is not considered in the current analysis due to the established priorities. Those priorities are to analyse and determine the genera which are able to colonise the most external area of the specimens' surface since the removal was expected to be effective for around 2 mm deep depending on the roughness and the diameter of the open porosity.

Concerning fungal quantification, significant differences are observed between OPC and MPC specimens, where the second ones seems to be more suitable for their growth. 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. In that case in which specimens were placed horizontally and in Barcelona, the highest fungal CFUs per plate were obtained for Ma20-0.75C specimens. Furthermore, they were the ones showing a higher biodiversity. The above may be related not just to the pH but also to properties studied in Chapter 4, where that dosage obtained a relatively low roughness as well as porosity.

Genera *Acremonium, Alternaria* and *Cladosporium* were identified from all the specimens. According to Onions and Brandy (1987), *Acremonium* can grow in a wide range of pH, pH 6 being the optimum and then 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. Then, 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, MPC specimens being more suitable as a support than OPC specimens.

Table 6.4 presents the results obtained for vertical specimens, also showing the most representative genera identified as well as the plate counting. In general, the horizontal position should be the more suitable since gravity would favour deposition of bacteria, spores, organic matter, others. Furthermore, climate conditions such as rain and incident wind will affect also the maintenance of the organisms on the surface. Previous hypotheses consider that wind would affect mostly the 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.

Lower values of colony forming units were obtained, which is a clear consequence of the inclination. Regarding bacterial results, the highest CFUs value was obtained for the dosage Ma28-1C followed again by the Pa60-1.75C dosage. The aforementioned dosages obtained the highest values for quantification of both horizontal and vertical specimens. Regarding fungi, results do not show a clear trend and more replicates may be necessary in order to obtain more representative results.

Finally, SEM images were obtained at the end of the test with the purpose of showing the microscopic appearance of the organisms and the coverage of the specimens. A huge heterogeneity was observed for OPC specimens, where different areas of the specimens showed a completely different appearance. Moreover, bacteria on MPC specimens could not be seen since it was not possible to zoom in with good resolution. The above may be due to some interference between the sample and the sample preparation process.

Table 6.4.- Identification and counting of bacterial and fungal genera on vertical specimens placed in Barcelona city

Vertical specimens	Bacteria	CFUs	Fungi	CFUs
PA30-1C	*Aerococcus* *Kocuria (K. lutea, K. rhizophila, K. kristinae)*	*10*	*Cladosporium (C. herbarum)* *Mucor (M. mucedo)* *Phoma (P. herbarum)*	*10*
Pa40-1C	*Bacillus (B. subtilis and others)*	*2*	*Alternaria (A. tenuis)* *Cladosporium (C. herbarum)*	*2*
Pa60-1.75C	*Aerococcus* *Kocuria (K. lutea, K. rhizophila, K. kristinae)*	*25*	*Acremonium (A. strictum)* *Alternaria (A. tenuis)* *Cladosporium (C. herbarum)* *Phoma (P. herbarum)*	*6*
MA15-0.5C	*Bacillus* *Kocuria (K. rhizophila)*	*9*	-	-
Ma20-0.75C	*Aerococcus* *Bacillus* *Kocuria (K. lutea, K. rhizophila, K. kristinae)*	*11*	*Acremonium (A. strictum)* *Cladosporium (C. herbarum)*	*5*
Ma28-1C	*Aerococcus* *Bacillus* *Kocuria (K. lutea, K. rhizophila, K. kristinae)*	*42*	*Acremonium (A. strictum)* *Alternaria (A. tenuis)* *Cladosporium (C. herbarum)* *Phoma (P. herbarum)* *Rhodotorula (R. glutinis)*	*5*

Figure 6.8 shows a photograph of the colonised surface of a Pa40-1C specimen. Bacteria found are rod-shaped and with a size of around 1.6 x 2 µm, which corresponds with the genus *Bacillus*, the one identified on that specimens. Concerning the two smaller bodies in both sizes of the *Bacillus*, they could be *Aerococcus* due to their size although they were not isolated from those specimens. Moreover, hyphae of fungi were also observed on some specimens as can be seen in Figure 6.9.

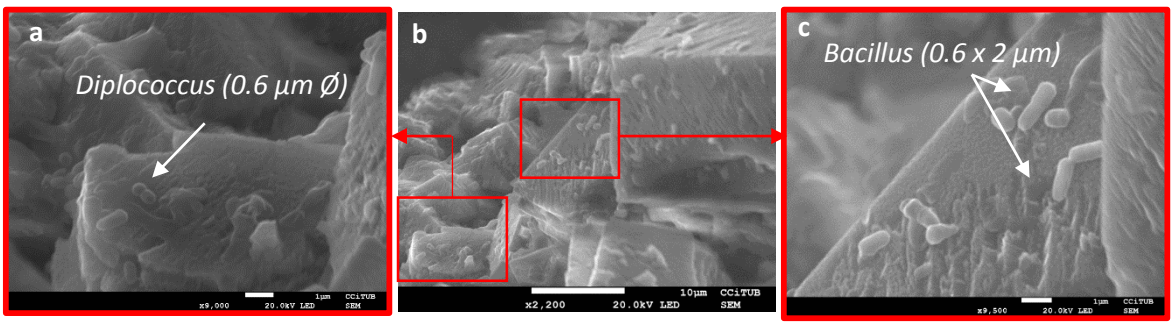

Figure 6.8.- Detail of Pa40-1C specimen with bacteria (b), which may belong to genus Aerococcus (a) and Bacillus (c).

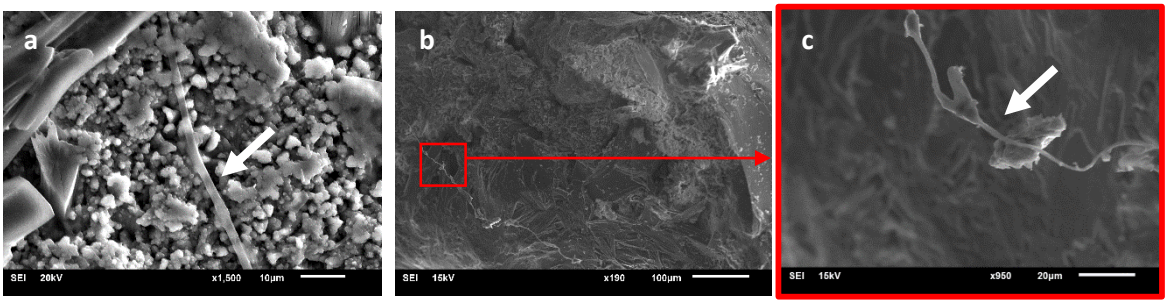

Figure 6.9.- Detail of a fungal hyphae (white arrow) on (a) Pa40-1C and on (b, c) Ma20-0.75C.

Weather and air quality data

Figure 6.10 shows climate data corresponding to the city of Barcelona and a weather station placed in less than 1 km from the specimens' location. Maximum temperatures were recorded between 20.9° C (February) and 33.2° C (June) while the minimum ranged from -0.5° C (February) and 16.3 C (July and August). March was the rainiest month of the studied period, recording 133 l/m² (it can also be expressed as 133 mm), although for most of the months a precipitation lower than 50 l/m² was recorded. Regarding relative humidity, values ranged from 57.5 % to 70 % and the sunshine duration was mostly between 190 and 330 hours per month. Finally, the wind direction was to the west, oscillating between north-west and south-west and the monthly average of wind speed was 2.3 m/s.

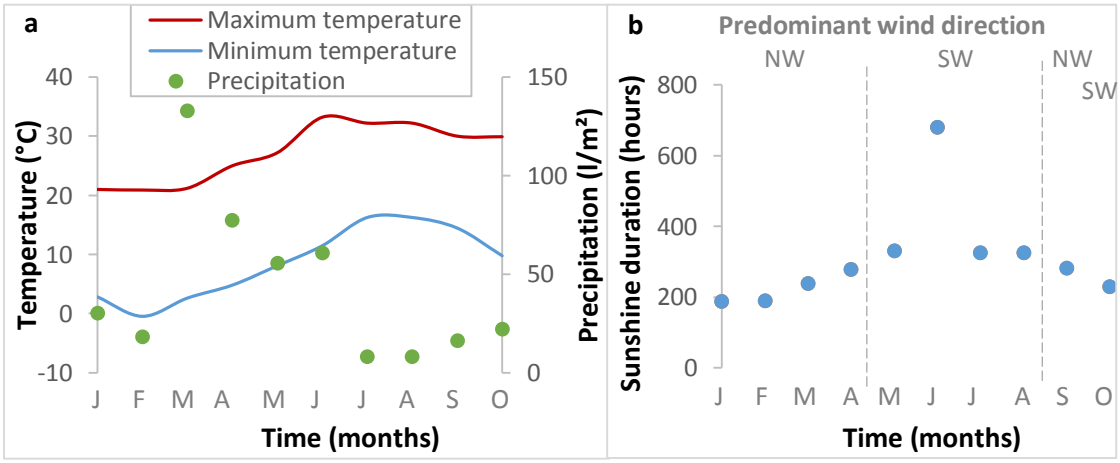

Figure 6.10.- Monthly climate data for Barcelona city from January to October 2013 corresponding to (a) temperature, precipitation, (b) sunshine duration and predominant wind.

Atmospheric pollution levels also affect the colonisation and development of organisms (Nuhoglu et al., 2006). Sulphur dioxide 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 also promotes the oxidation of

the sulphur dioxide (Johansson et al., 1988). The above is applicable to OPC specimens although not for MPC since the chemical composition is completely different. Concerning to NO_x, Mancinelli and McKay (1983) states that NO has a bacteriostatic effect although NO_2 decreases the effect of air pollution on MPC.

Figure 6.11 shows information regarding air quality of Barcelona city. Figure 6.11 (a) shows four different curves corresponding to SO_2, NO, NO_2 and the sum of NO and NO_2 (NO_x) levels during the experimental program period. Additionally, Figure 6.11 (b) shows CO and O_3 monthly levels. NO_x levels ranged from 27 µg/m³ to 65.4 µg/m³ with a monthly average of 46.2 µg/m³. Furthermore, recorded NO_2 levels were significantly higher than NO levels and low SO_2 levels were obtained. According to the previous explanation, that fact may stimulate a higher colonisation and development of specimens in the city of Barcelona. Accordingly, SEM images recorded plenty of particles attached on the surface (Figure 6.12). Based on previous works (Nuhoglu et al., 2005), all those particles may be consequence of the environmental pollutants.

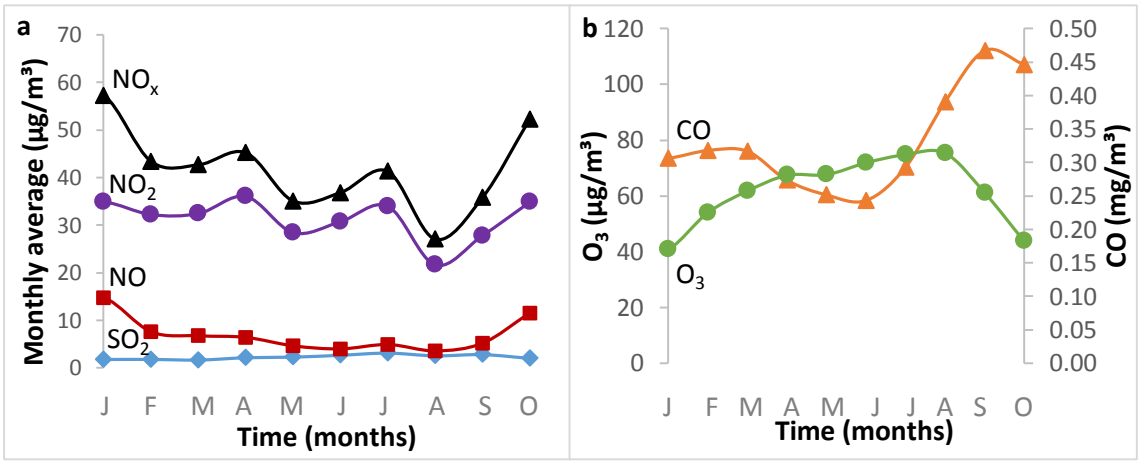

Figure 6.11.- Air quality at Barcelona city in terms of amounts of (a) sulphur dioxide (SO_2), nitrogen oxides (NO, NO_2, NO_x), (b) monoxigen carbon (CO) and ozone (O_3).

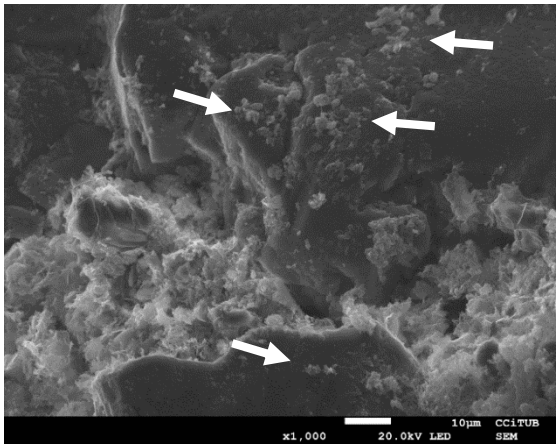

Figure 6.12.- Surface particles (white arrows) on Ma20-0.75C specimen due to air pollution of Barcelona city.

CO levels are expressed in mg/m³ and the values obtained ranged from 0.2 to 0.5 mg/m³ obtaining the highest value in September. Regarding ozone levels, values ranged from 33.4 to 75.3 µg/m³, recording the highest level in August.

6.3.2. Natural Park of Montseny

Predominant microorganisms

Same species of bacteria were found as in the city of Barcelona with the exception of *Flavobacterium*, a genus that was not found in Montseny. Regarding fungi, less environmental biodiversity was observed for the punctual environmental sampling comparing the genera identified from the environment of Barcelona city and the natural park in Montseny. Moreover, the genus *Epicoccum*, which was only found on PA30-1C specimens, was identified from the environmental sample of Montseny.

Table 6.5 shows the results obtained for the specimens placed horizontally in the natural park of Montseny. For that location, less colonisation was expected 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).

The only genus for both bacteria and fungi not found on Barcelona city specimens was the yeast *Saccharomyces* (*Fungi, Ascomycota, Saccharomycotina, Saccharomycetes, Saccharomycetidae, Saccharomycetales, Saccharomycetaceae*), which was only isolated from specimens PA30-1C. First, less colony forming units were quantified from the specimens in Montseny with the exception of the dosage Pa60-1.75C, which recorded also the highest CFUs of fungi.

Regarding bacterial results, genus *Bacillus* and *Kocuria* appeared as the most ubiquitous since they are present on almost all specimens. In the current case, genus *Aerococcus* is less representative since it was just identified from two different dosages.

Concerning to fungi, lower CFUs were counted and similar results in terms of variability and number of genera were obtained. Comparing those results with the ones obtained for specimens placed in Barcelona city, three different genera were not found on horizontal specimens nor on vertical ones. Those genera were *Saccharomyces*, which was only found on PA30-1C specimens, and *Penicillium* and *Fusarium*, which were only found on MPC specimens. Moreover, no organism of the genus *Gilmaniella* was identified. According to results obtained for Barcelona specimens, the genus *Epicoccum* was only found on OPC specimens. The above may be consequence of their higher affinity for alkaline substrates (Schol-Schwarz, 1959).

Results from specimens placed vertically in Montseny are presented in Table 6.6. According to previous results, CFUs found for both bacteria and fungi were less on vertical

specimens than in horizontal ones. However, significantly higher values were obtained for MA15-0.5C specimens.

Table 6.5.- Identification and counting of bacterial and fungal genera on horizontal specimens placed in Montseny (Unc.: uncountables)

Horizontal specimens	Bacteria	CFUs	Fungi	CFUs
PA30-1C	*Aerococcus* *Bacillus* *Flavobacterium* *Kocuria (K. lutea, K. rhizophila, K. kristinae)*	47	*Acremonium (A. strictum)* *Alternaria (A.tenuis)* *Cladosporium (C. herbarum)* *Epicoccum (E. purpurascens)* *Rhodotorula (R. glutinis)* *Saccharomyces*	6
Pa40-1C	*Bacillus* *Kocuria (K. rhizophila)*	3	*Cladosporium (C. herbarum)* *Epicoccum (E. purpurascens)*	3
Pa60-1.75C	*Bacillus* *Flavobacterium* *Kocuria (K. lutea, K. rhizophila, K. kristinae)*	Unc.	*Acremonium (A. strictum)* *Alternaria (A. tenuis)* *Cladosporium (C. herbarum)*	35
MA15-0.5C	*Bacillus* *Kocuria (K. lutea, K. rhizophila, K. kristinae)*	45	*Alternaria (A. tenuis)* *Aspergillus (A. flavus)* *Aureobasidium (A. pullulans)* *Cladosporium (C. herbarum)* *Penicillium (P. rugulosum)* *Rhodotorula (R. glutinis)*	6
Ma20-0.75C	*Aerococcus* *Bacillus* *Kocuria (K. lutea)*	8	*Alternaria (A. tenuis)* *Cladosporium (C. herbarum)* *Fusarium (F. moniliforme)* *Penicillium (P. rugulosum)*	6
Ma28-1C	*Bacillus (B. subtilis and others)* *Kocuria (K. lutea, K. kristinae)*	33	*Alternaria (A. tenuis)* *Fusarium (F. moniliforme)*	2

Only the most ubiquitous genera, *Aerococcus, Bacillus* and *Kocuria*, were identified as the most representative groups. In accordance to bacterial results, low fungal growth was observed. A new genus was identified from both OPC and MPC specimens, *Aureobasidium*. The aforementioned genus was found in the punctual environmental sample of Barcelona city although was not found on any specimen. In contrast, it was not found in the punctual environmental sample in Montseny, but it was on specimens' surface. That fact shows that results obtained from environmental samples could not be generalised to the complete experimental program duration.

In general, just a CFU per genus and plate was found with exception of MA15-0.5C specimens. Table 6.4 showed no fungal growth on MA15-0.5C specimens which significantly differ from those results. A more intensive experimental work in terms of colonisation evolution for both specimens' surface and environment as well as more replicates would be necessary in order to justify those results.

Table 6.6.- Identification and counting of bacterial and fungal genera on vertical specimens placed in Montseny

Vertical specimens	Bacteria	CFUs	Fungi	CFUs
PA30-1C	*Aerococcus* *Bacillus (B. subtilis)*	2	*Acremonium (A. strictum)* *Cladosporium (C. herbarum)* *Rhodotorula (R. glutinis)*	3
Pa40-1C	*Bacillus* *Kocuria (K. rhizophila)*	3	*Aureobasidium (A. pullulans)* *Fusarium (F. miniliforme)*	2
Pa60-1.75C	*Aerococcus* *Bacillus (B. subtilis)* *Kocuria (K. lutea, K. rhizophila, K. kristinae)*	5	*Alternaria (A. tenuis)* *Aureobasidium (A. pullulans)* *Cladosporium (C. herbarum)* *Phoma (P. herbarum)*	4
MA15-0.5C	*Aerococcus* *Bacillus* *Kocuria (K. rhizophila)*	30	*Aureobasidium (A. pullulans)* *Cladosporium (C. herbarum)* *Phoma (P. herbarum)*	30
Ma20-0.75C	*Bacillus (B. subtilis)*	1	*Alternaria (A. tenuis)* *Fusarium (F. moniliforme)*	2
Ma28-1C	*Aerococcus*	3	*Aureobasidium (A. pullulans)* *Cladosporium (C. herbarum)*	2

Regarding SEM analysis, pollen grains of different species were found on specimens placed in the Natural Park of Montseny. Pollen from three different species were identified as can be seen in Figure 6.13. Grains of pollen shown in Figure 6.13 (a) were not easily identified due to the high level of collapse. However, according to the flora present in the natural park of Montseny and the morphology and size observed in the photograph, it may belong to a plant of the genus *Papaver*. The grain on the left may correspond to a polar view while the one on the right may match with an equatorial view, in which the exine is divided in three lobes. Pollen grains are relatively small, with sizes ranging from 15 µm to 35 µm, tricolpate, isopolar and radiosymmetrical (Trigo et al., 2008).

Grain 1 in the Figure 6.13 (b) might belong to the genus *Cedrus*, whose pollen grains are big (ranging from 37 to 52 µm), heteropolar, bisymmetrical with a rough surface (Trigo et al., 2008). Finally, grains labelled as 2 in Figure 6.13 (b) may belong to the genus *Lactuceae* due to

their size ranging from 20 µm to 45 µm. Moreover, they are tricolpate, isopolar, radiosymmetrical and reticulate.

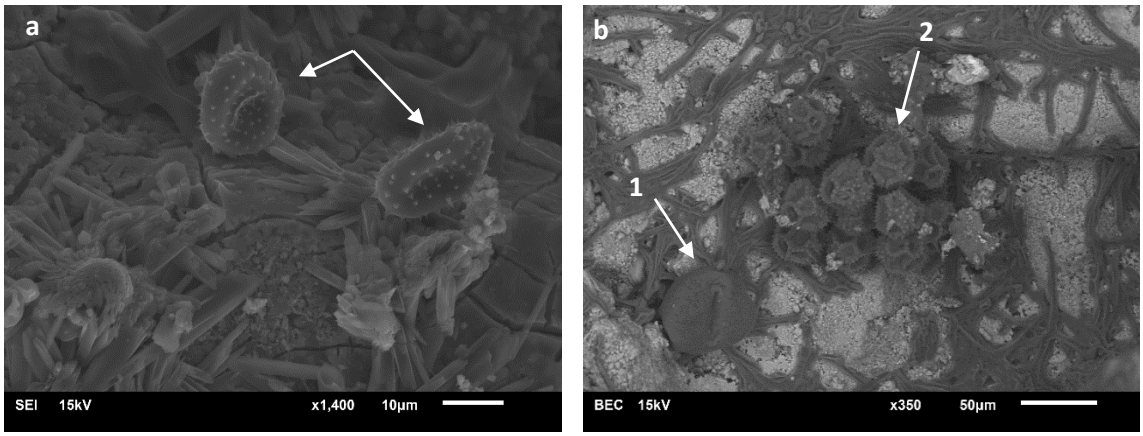

Figure 6.13.- Collapsed pollen grains found on Pa40-1C specimens.

Weather and air quality data

Figure 6.14 shows weather data from the Natural Park of Montseny, where specimens were placed next to the weather station. Recorded maximum temperatures ranged from 16.1° C (February) to 31.3° C (August), while minimums went from -2.7° C (February) to 14.5° C (July). The precipitation recorded at the Natural Park of Montseny ranged from 24 l/m² to 132.4 l/m², obtaining the highest value in March. Relative humidity ranged from 63.6 % to 78.8 % with a monthly average of around 69 %. Regarding sunshine duration, it was similar as in Barcelona with the exception of June, recording higher values in that last location. Finally, predominant wind direction was similar for both locations although the average of the wind speed per month was considerably lower (1.1 m/s).

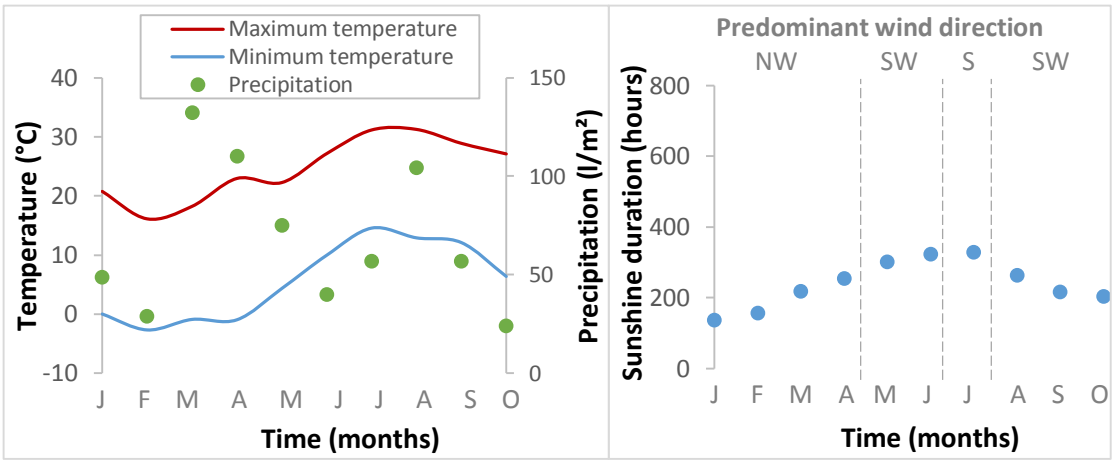

Figure 6.14.- Monthly climate data for Montseny from January to October 2013 corresponding to (a) temperature, precipitation, (b) sunshine duration and predominant wind.

Figure 6.15 shows data corresponding to the air quality of the natural Park of Montseny. Two graphs are presented in which SO_2 and NO_x curves are shown in Figure 6.15 (a) and CO and

O_3 curves in Figure 6.15 (b). Distinction between curves corresponding to NO and NO_2 are not shown due to the low NO contribution.

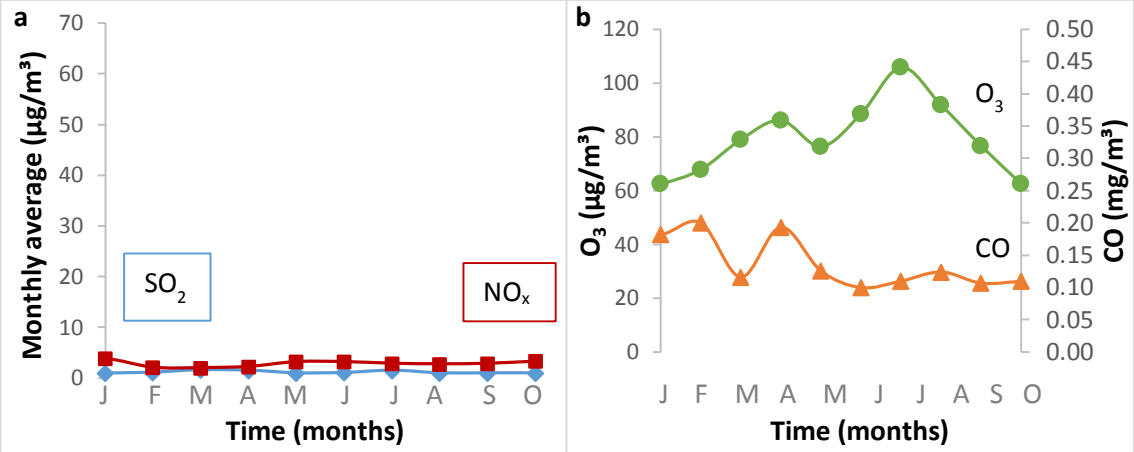

Figure 6.15.- Air quality in Montseny in terms of amounts of SO_2, NO_x, CO and O_3.

6.3.3. Ghent city

No bacterial nor fungal genera were identified from environmental samples for the city of Ghent, which may be consequence of the procedure. The environmental samples were obtained differently in this case due to plates' contamination. The followed method was used for sampling by means of a pump connected to a sterilised glass recipient full of sterilised saline solution, sucking air and organisms to impact with the recipient walls and finally fall into the solution (Rosas et al., 2004).

Table 6.7 shows the results obtained on horizontal specimens placed in Ghent. Bacterial quantification was not carried out and negative or low results are shown. Furthermore, there are no results corresponding to the MA15-0.5C specimens due to the loss of the samples. Concerning to bacteria, a low variability of genera was found. Between them, the most ubiquitous genera coinciding with results from Barcelona and Montseny specimens were also found. Afterwards, also *Flavobacterium* and *Streptococcus* were found on OPC specimens but not on MPC specimens which differs from previous results for the other two locations.

Fungal results show different quantification for yeasts (genus *Rhodotorula* and *Saccharomyces*) and the rest of genera. That is mainly due to the significant difference between presence of yeast and the others. Yeasts were found on OPC specimens and the number of CFUs was significantly higher than in any of the specimens studied until here. Yeasts are responsible of fermentation processes and those are favoured in acidic media. Furthermore, species of the genus *Saccharomyces* grow well at pH around 8 (Praphailong and Fleet, 1997) and consequently OPC specimens may be more suitable for that genus. However, it is not the case of *Rhodotorula* since the species *R. glutinis* has an optimal pH for growth around 5.2 (Martínez et al., 2006). The above may be due to the previous presence of bacteria which would provide the acidic pH suitable for their presence. Regarding the other fungal genera, *Alternaria* and *Penicillium* were

only identified from MPC specimens, which may be consequence of the lower pH in comparison to OPC specimens.

Table 6.7.- Identification and counting of bacterial and fungal genera on horizontal specimens placed in Ghent city (NQ.: non-quantified)

Horizontal specimens	Bacteria	CFUs	Fungi	CFUs
PA30-1C	*Bacillus* *Streptococcus*	NQ	*Cladosporium* *Fusarium*	2
			Rhodotorula *Saccharomyces*	> 10^5
Pa40-1C	*Bacillus* *Streptococcus*	11	*Fusarium*	20
			Saccharomyces	> 10^5
Pa60-1.75C	*Bacillus* *Flavobacterium* *Streptococcus*	NQ	*Fusarium* *Penicillium*	100
			Saccharomyces	> 10^5
Ma20-0.75C	*Aerococcus* *Bacillus* *Kocuria (K. lutea)*	NQ	*Alternaria (A. tenuis)* *Cladosporium (C. herbarum)* *Fusarium (F. moniliforme)* *Penicillium (P. rugulosum)*	6
Ma28-1C	*Bacillus (B. subtilis and others)* *Kocuria (K. lutea, K. kristinae)*	NQ	*Alternaria (A. tenuis)* *Fusarium (F. moniliforme)*	2

Table 6.8 shows the last results corresponding to quantification and identification, which correspond to vertical specimens placed in the city of Ghent. Results showed a lower diversity of bacteria and absence of them in two specimens, Pa60-1.75C and Ma20-0.75C. In contrast, higher CFUs were found for fungi and that fact may be the reason of the lower presence of bacteria in terms of diversity. High presence of fungi may be forming a layer, which could be hindering the removal of the underlaying formed by bacteria. On the other hand, competition relationships due to the presence of non-culturable bacteria on specimens placed in Ghent may reduce the biodiversity of bacteria obtained by means of those procedures.

Fungal growth results were not the ones expected for different reasons. First, Saccharomyces was found on both OPC and MPC specimens which may be due to the presence of different species with different requirements. Then, only yeasts were identified from OPC specimens with lower roughness (Pa40-1C and Pa60-1.75C) and in the extreme case of Pa60-1.75C where no bacteria were obtained. Again, the possibility of presence of non-culturable microorganisms could be the cause of those results, although the great number of CFUs of yeasts may lead to the fact of a problem with the microorganisms' removal procedure. Finally, the

genus *Rhizopus* was identified only on one of the specimens (Ma20-0.75C) and it was not identified on specimens in Barcelona nor in Montseny.

Table 6.8.- Bacterial and fungal genera on vertical specimens placed in Ghent city and plate counting (NQ: non-quantified)

Vertical specimens	Bacteria	CFUs	Fungi	CFUs
PA30-1C	*Flavobacterium*	NQ	*Cladosporium* *Fusarium*	2
			Rhodotorula *Saccharomyces*	> 10^5
Pa40-1C	*Bacillus* *Flavobacterium* *Kocuria*	NQ	*Saccharomyces*	> 10^5
Pa60-1.75C	-	-	*Saccharomyces*	> 10^5
MA15-0.5C	*Flavobacterium* *Kocuria*	NQ	*Fusarium*	3
			Saccharomyces	> 10^5
Ma20-0.75C	-	-	*Cladosporium* *Fusarium* *Rhizopus* *Saccharomyces*	230
Ma28-1C	*Bacillus*	11	*Fusarium* *Penicillium*	24

As it was already mentioned before, sampling was done in Ghent and all samples were sent to Barcelona for their analysis. Consequently, an in situ analysis would be interesting in order to determine the validity of those results.

Weather and air quality data

Less information regarding that location was obtained although differences between the duration of the Spanish tests and the one developed at Ghent make not possible to compare Ghent results with the others.

Figure 6.16 shows information related to mean monthly temperatures, total monthly precipitation, sunshine duration and predominant wind direction. Figure 6.16 (a) shows mean temperatures ranged from 1 to 20° C and the mean monthly precipitation without considering May was 44.2 l/m². However, May obtained precipitation values, which increased the average until 55.9 l/m² by recording a monthly precipitation of 126 l/m². The above also contributes to the local relative humidity, which monthly average was around 72 % ranging from 64.5 % to 84.5 %.

Additionally, Figure 6.16 (b) shows sunshine duration, which ranged from 58.6 hours to 267.7 hours with variable wind directions. In that case, wind may have a greater influence due to the variations recorded in the test period. Regarding wind speed, the monthly average obtained was 3.3 m/s.

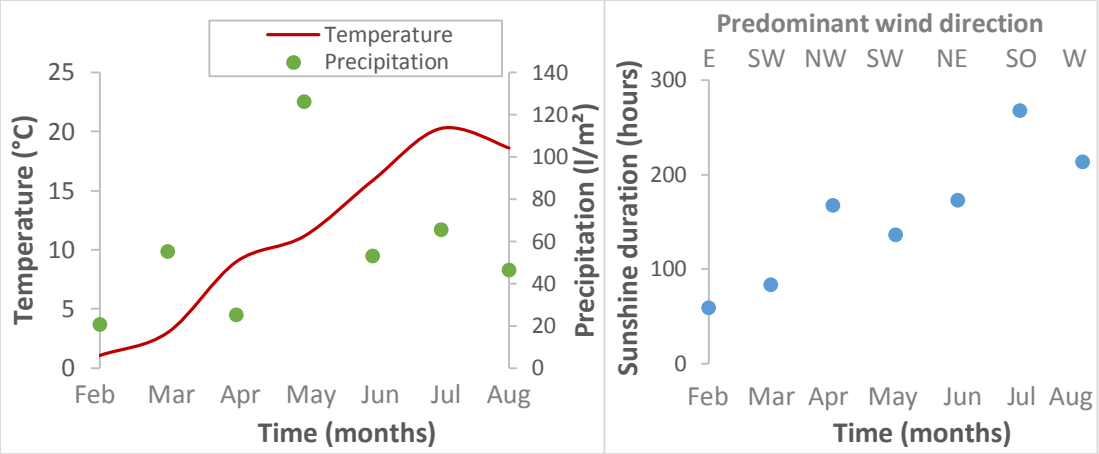

Figure 6.16.- Data of (a) monthly mean temperatures and precipitation as well as (b) sunshine duration and predominant wind direction in Ghent.

6.3.4. Comparison between locations

Regarding predominant microorganisms, 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 genus found for both location were similar. Moreover, the lower presence of microorganisms on specimens placed vertically was constant in all locations. Usually, higher amounts of aerial living organisms are present in the air of a city than of a forest. Their dispersion could go over long distances although it depends on the environmental conditions. Rain and low-speed wind favour the deposition of aerial particles (microorganisms, dust, others). In contrast, high-speed wind may transport the particles further.

Accordingly, the impact of the raindrops and the direct wind at high speeds may produce the detachment of the organisms from a surface. 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, 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.

Comparing the Spanish location, similar results between Barcelona city and the Natural Park of Montseny were obtained for the maximum and minimum temperatures. Both maximum and minimum temperatures were lower in comparison to results obtained for Barcelona city, which makes sense due to the altitude. The weather station from Barcelona is placed at an altitude of around 80 m while the one from Montseny is placed at around 1000 m. Precipitation

was generally higher at the Natural Park of Montseny, recording a monthly average of around 68 l/m² while in Barcelona it was around 43 l/m².

In summary, the mean temperature, sunshine duration and wind speed were higher in Barcelona city for the presented period. In contrast, relative humidity as well as precipitation was lower. Consequently, environmental conditions from 2013 made it difficult to state which of both locations was more suitable in terms of colonisation. On one hand, rain favours the deposition of the aerial microorganisms but it also leads to their detachment from the surfaces due to the raindrops impact. Therefore, more data regarding rain, such as speed or the impact force, would be necessary in order to determine the weight of both effects. The wind direction parameter has significance for the specimens placed vertically. However, it was similar for both locations and favourable since low direct incidence was recorded.

Regarding air quality, NO_x and SO_2 levels in Montseny were significantly lower than the levels obtained in Barcelona city, especially results obtained for NO_x concentrations. In addition, recorded CO levels were quite similar although O_3 levels were higher in Montseny in terms of monthly average. The above makes sense since the environment of Montseny is less contaminated than the one of Barcelona city.

6.4. CONCLUSIONS

The current chapter is a first approach to the analysis of the natural colonisation of the produced materials by pioneer organisms. A combination of the identification of the predominant genus, their quantification in terms of UFCs, the analysis of the weather conditions and the air quality of three different locations has been carried out although not all parameters were obtained for the Ghent city location.

Genus diversity of bacterial growth was similar for all three locations. Quantitative results of Barcelona city and Montseny reveal higher bacterial colonisation in urban areas than in natural ones, which corresponds to previous hypothesis. Quantitative comparison with specimens placed in Ghent was not carried out due to the different time of the tests between locations.

Regarding fungi, similar genus diversity was obtained between Barcelona city and Montseny in which low presence of yeasts was recorded. However, results obtained for the Ghent location show a predominant presence of yeasts, mainly from the genus *Saccharomyces* and in high amounts. Comparison between both Spanish locations reveal the highest colonisation in urban areas due to a major presence of aerial microorganisms. Furthermore, it is difficult to establish differences between different dosages and it would be necessary to carry out a more extensive experimental program in this sense.

Weather data as well as parameters related to air quality also contributes to the fact that urban areas may be more suitable for the colonisation of the selected materials by pioneer

microorganisms. The above might contribute to evolution of the colonisation allowing the emergence of other groups of living organisms.

7. CONCLUSIONS AND FUTURE PERSPECTIVES

7.1. INTRODUCTION

The work here presented provides information regarding the possibility of using a cementitious material as biological substratum. In that sense, the investigation resulted in an international patent concerning an application for construction of multi-layered panels (PCT/ES2013/070438). The invention relates to a cement-based multilayer assembly that can be used as a biological support for building facades or other structures. The structure of the panel comprises a first layer, which consists of conventional concrete and it is responsible for the structural function of the panel. Moreover, production of this layer would depend on project requirements. Subsequently, there is a second layer with the main function of protecting the first one. This layer would have a waterproofing capability and could also improve adhesion between first and third layer. Then, the third layer is the one with an enlarged bioreceptivity and the one related to the current study. Furthermore, thanks to this improvement of bioreceptivity, rain water retention as well as stimulation of colonisation by living organisms will be achieved. Finally, the last layer would be a discontinuous one in order to allow different designs of the surface. Areas without this layer would allow organisms to colonise the surface and the retained water will maintain local humidity. Exit of water is then redirected to these holes promoting better local conditions for colonising organisms.

The structure of the current chapter corresponds to the conclusions obtained from the current work as well as the suggestions and future research lines. Therefore, the main conclusions obtained in this doctoral thesis are presented in the current chapter and they are

divided in general and specific conclusions. General conclusions (section 7.2) correspond to the main objectives presented in Chapter 1. Then, section 7.3 refers to the specific conclusions, linked with the specific objectives also presented in Chapter 1. Finally, in some suggestions for future research are presented in section 7.4.

7.2. GENERAL CONCLUSIONS

The interest of integrating nature into the cities in construction envelopes is not recent, and technology has experienced great advances in the last years. However, several aspects still require further research. For that reason a rather generalist thesis was outlined to obtain a first approach of a complete different perspective, which answers to the general objective: provide a possibility of using a structure surface as biological substratum.

Results showed that the proposed approach of developing structures allowing biological growth in their surface is possible. The above suggests this could be considered as a novel solution for the industry to improve aspects such as costs, integration and maintenance. Regarding the main objective, the work was focused on two main issues: modification of the chemical as well as physical properties of the cementitious materials to be used as biological growth substratum, and the evaluation of the suitability of the materials for colonisation by living organisms under both laboratory and environmental conditions. This section presents the general conclusions obtained for each one of them in response to the general objectives defined in Chapter 1 as follows:

- Regarding the first main issue, it was demonstrated that both chemical and physical properties of a cementitious material can be modified in a controlled manner. Considerations given in the literature regarding the more influencing parameters for biological colonisation (porosity, roughness and pH) were taken into account and were considered as priorities. Consequently, a wide variety of materials bioreceptivities were characterised and six mix designs were selected.

- Concerning the second main issue, positive results were obtained for the colonisation evaluation. Significant differences were recorded for different mix designs under laboratory conditions and interesting results were also obtained under environmental conditions.

To sum up, the current thesis demonstrates the possibility of using stone materials as a biological substratum by means of modification of their bioreceptivity, which was the main objective of this work. However, further research is required due to the innovative nature of the proposed solution as is presented below.

7.3. SPECIFIC CONCLUSIONS

In view of the specific objectives, for each one of the subjects studied in this thesis, several results and improvements were detailed in previous chapters. The most relevant conclusions are described below divided into the two main issues: the modification of the cementitious material and the evaluation of the colonisation.

7.3.1. Cementitious material

- A slight decrease of the pH of Ordinary Portland Cement mortars was obtained when adding boric acid to the mixtures, which was far from the neutrality. Concentrations lower than 2 % over cement weight slowed down the setting and also affected the flexural and compressive strengths, which were significantly reduced. Moreover, higher concentrations stopped the hydration process. Consequently, the use of boric acid additions to decrease the pH of OPC mortars was discarded. No effect of the addition of oxalic acid in the mixtures was recorded for acid concentrations from 0 to 13 % over cement weight. In consequence, its use was also discarded.

- Different Magnesium Phosphate Cement mixtures, in terms of cement composition, provide an acceptable range of pH within 5.8 and 7. Characterisation of all different formulations provided acceptable physic-chemical properties as well as flexural and compressive strengths results for their use as mortar. Regarding the benefits of using MPC, lower water consumption was recorded due to the remarkable lower water demand when comparing that hydraulic binder and Ordinary Portland Cement. Additionally, no presence of dangerous compounds for human health (during production) or structural durability was observed. However, results suggest that the current mixes should be preferably used for non-structural elements.

- Lower $NH_4H_2PO_4$:MgO ratios (P:M ratios) are more interesting due to better physico-mechanical properties as well as from an economic point of view. After the characterization, the P:M ratio of 1:2 with 6 % borax addition was discarded since results between that formulation and P:M ratios of 1:1.75 with 6 % borax addition were quite similar and a better workability was observed for P:M ratios of 1:1.75. Consequently, the P:M ratio 1:1.75 with a 6 % borax addition was selected.

- An objective-oriented design was used to obtain different roughness and porosity. Variations of the type of cement, the granular skeleton, the water to cement ratio and the amount of cement paste were combined in order to produce different materials' bioreceptivities. The methodology used for the selection of the cement paste amounts was based on the estimation of the minimum amount of cement paste required for joining all the aggregates and obtaining the highest macroporosity. That methodology worked perfectly for OPC mortars although it was not appropriate for MPC mortars probably due to the fluidizer effect of the compound used as a retarder, borax.

- Control of the aforementioned parameters was easier for OPC mortars, which may be connected to the drawbacks of the dosage method for MPC mortars. Significant differences were obtained regarding pore diameter distribution between OPC and MPC mortars. OPC samples showed a more continuous pore size distribution while MPC presented extremely low percentages of pores with diameter lower than 50 nm. The above indicates different water absorption as well as water retention patterns for the different dosages, which may also indicate different colonisation patterns between them.

7.3.2. Biological growth

- MPC specimens showed a higher bioreceptivity for pioneer colonisers such as *Chlorella vulgaris* than OPC specimens under laboratory conditions. Carbonated OPC specimens did not obtained complete biofouling up to 20 weeks of accelerated testing, while some MPC specimens were totally covered after 4 weeks. However, no visible colonisation was observed on specimens exposed to the ambient environment for any of the three selected locations (Barcelona city, Natural Park of Montseny and Ghent city) although specimens placed in Ghent were exposed for a shorter period. Genera diversity of bacterial growth between the three locations did not show significant differences. However, the most significant difference on fungal growth diversity was obtained for the location of Ghent, were a predominant presence of yeasts was recorded.

- The methodologies of evaluation of biofouling used in this work were adapted from previous studies regarding the biodeterioration of stone materials. In consequence, those methodologies are suitable for the initial stages of biofouling but no information for later stages was obtained from literature. The fouling intensity parameter was useful for the analyses until complete coverage of the specimens' surface. It appeared to become useless after that event, due to the drop in lightness (L*). The influence of the aforementioned problem was also evident for the estimation of the covered area. Moreover, the use of the PAM fluorometry for biomass quantification by measuring the chlorophyll fluorescence was useful and can be used until a biomass of 20 mg/cm². However, the use of the above-mentioned technique under environmental conditions should be considered cautiously. The above is due to the fact that the factor F_0 (minimum fluorescence intensity under dark conditions) does not only increases when biomass increase but also when the organisms are subjected to hydric stress.

- Considering results obtained under laboratory conditions, Ma28-1C specimens appeared to be the most bioreceptive MPC composition for the algae since they were completely biofouled in the shortest time (4 weeks) and with the highest homogeneity. However, MA15-0.5C may obtain better results for longer times under environmental conditions since those high homogeneous configurations could lead to durability problems regarding the biological growth. The above is due to the fact that biomass would increase until a certain point in which the detachment of the organisms may occur as in nature.

Regarding the results from environmental specimens, no significant differences were obtained when comparing specimens with different mix designs and a more comprehensive experimental program should be carried out.

- The influence of the inclination on the colonisation was verified, in which horizontal surfaces are easily colonised than vertical ones. Furthermore, greater colonisation of specimens placed in Barcelona city than for the Natural Park Montseny was obtained. Weather and other parameters related to air quality contributed to the fact that urban areas may be more suitable for the colonisation of the selected materials by pioneer microorganisms. Consequently, the above may contribute to the emergence of other groups of living organisms.

- Results obtained for the specimens tested under environmental conditions show the necessity of the application of extra treatments in order to accelerate the colonisation process. Specimens from the Spanish locations did not show any visible growth up to 1 year of exposition, which suggests the necessity of an extra actuation for a further acceleration. Results for the same period of time were not yet obtained for the Belgian location and consequently, it is not possible to establish whether additional measures are necessary.

7.4. FUTURE PERSPECTIVES

In spite of the advances described in the previous section, there still exists a lot of space for further studies considering the low availability of previous works on this topic and the innovative nature of the current one. Those studies may be on the subjects treated in this thesis and on many other possible subjects related to the possibility of using stone-like construction materials as a substratum for living organisms. Based on that, this section presents some suggestions for future researches and experimental campaigns.

- Studies regarding the use of different Magnesium Phosphate Cements or their production from industrial by-products should be carried out in order to reduce costs.

- Improvement of water absorption and retention properties of the mortars should be achieved. In that sense, two different possibilities may be considered: addition of specific products for the purpose such as watergels or expanded clay, or development of a coating, which reduces the transpiration of the material avoiding the loss of the stored water.

- The mechanisms for accelerating the natural colonisation of those materials should be studied. Application of surficial temporary coatings such as culture media or pre-inoculation of autochthonous organisms as source of organic matter to improve the establishment of pioneer colonisers are two possible procedures for the aforementioned purpose. Additionally, the durability of the biological growth should be also studied.

- It is important to extend the experimental program regarding natural colonisation under environmental conditions in two ways: duration of the specimens' exposition and frequency of the tests. A more frequent analysis as well as the use of more advanced techniques such as genomic biotechnologies may provide extra information for a better understanding of the colonisation process.

- Studies in terms of industrial application should be carried out. The application of those materials for the purpose of allowing biological growth should not be considered for structural elements, but they can be used as an extra layer of a structural element. The above suggests the possibility of using those materials for multi-layered elements, which may imply further research.

REFERENCES

Anderson, L.M., Mulligan, B.E., Goodman, L.S., *Effects of vegetation on human response to sound,* Journal or Arboriculture, 1984, 10(2).

Ariño, X., Ortega-Calvo, J.J., Gomez-Bolea, A., Saiz-Jimenez, C., *Lichen colonisation of the Roman pavement of Baelo Claudia (Cádiz, Spain): biodeterioration vs bioprotection,* Science of the Total Environment, 1995, 167: 353-363.

ASTM C596-09, *Standard Test Method for Drying Shrinkage of Mortar Containing Hydraulic Cement*, ASTM; 2009.

ASTM C642-13, *Standard test method for density, absorption and voids in hardened concrete,* ASTM; 2013.

Attwell, K., *Urban land resource and urban planting – case studies from Denmark,* Landscape and Urban Planning, 2000, 52: 145-163.

Azmi, O.R. and Seppelt, R.D., *Fungi of the Windmill Islands, continental Antarctica. Effect of temperature, pH and culture media on the growth of selected microfungi,* Polar Biology, 1997, 18 (2): 128-134.

Babichenko S., Leeben A., Poryvkina L., Shalapyonok A., Seppällä J., *Variability of Chlorella sp. fluorescence in response to different nitrogen conditions*, International Journal of Remote Sensing, 2001, 22: 403–414.

Baldocchi, D.D., Hicks, B.B., Camara, P., *A canopy stomatal resistance model for gaseous deposition to vegetated surfaces,* Atmospheric Environment (1967), 1987, 21 (1): 91-101.

Barberousse, H., Lombardo, R.J., Tell, G., Couté, A., *Factors involved in the colonisation of building façades by algae and cyanobacteria in France,* Biofouling, 2006, 22: 69-77.

Barberousse H., Ruot B., Yéprémian C., Boulon G., *An assessment of façade coatings against colonisation by aerial algae and cyanobacteria*, Building and Environment, 2007, 42: 2555–2561.

Baumann, N., *Ground-nesting birds on green roofs in Switzerland: Preliminary observations,* Urban Habitats, 2006, 4: 37-50.

Berrini, M, and Bono, L., *Urban Ecosystem Europe Report 2007,* Ambiente Italia Research Institute, 2007.

Berthelin, J., *Microbial weathering processes*, In: Krumbein, W.E. (Eds.), Microbial Geochemistry. Blackwell Scientific, Oxford, UK, 1983. pp. 223–262.

Bidwell, R.G.S. and Fraser, D.E., *Carbon monoxide uptake and metabolism by leaves,* Canadian Journal of Botany, 1972, 50 (7): 1435-1439.

Bolund, P. and Hunhammar, S., *Ecosystem services in urban areas,* Ecological Economics, 1999, 29: 293-301.

Böger P., *Das Strukturproteid aus Chloroplasten einzelliger Grünalgen und seiner Beziehung zum Chlorophyll*, Flora 1964, 154: 174–211.

Boncukcuoğlu R., Yılmaz M.T., Kocakerimb M.M., Tosunoğlua V., *Utilization of borogypsum as set retarder in Portland cement production*, Cement and Concrete Research, 2002, 32 (3): 471-475.

Bovallius, A., Bucht, B., Roffey, R., Änas, P., *Three-year investigation of the natural airborne bacterial flora at four localities in Sweden,* Applied and Evironmental Microbiology, 1978, 35 (5): 847-852.

Cámara, B., de los Ríos, A., García-del-Cura, M.A., Galván, V., Ascaso, C., *Dolostone bioreceptivity to fungal colonisation,* Materiales de Construcción, 2008, 58: 113-124.

Chiesura, A., *The role of urban parks for the sustainable city,* Landscape and Urban Planning, 2004, 68: 129-138.

Cocke D.L., Mollah M., Vempati R.K., Hess T.R., *Multitechnique approach to understanding the microstructure of cement-based systems*, Materials Research Society Symposium Proceedings 1995, 370: 279-284.

Cofman, R.R. and Davis, G., *Insect and avian fauna presence on the Ford assembly plant ecoroof,* 3rd North American Green Roof Conference: Greening rooftops for sustainable communities, Washington, DC. 4-6 May 2005. Pp. 457-468.

Coutinho, M.L., Miller, A.Z., Gutierrez-Patricio, S., Hernandez-Marine, M., Gomez-Bolea, A., Rogerio-Candelera, M.A., Philips, A.J.L., Jurado, V., Saiz-Jimenez, C., Macedo, M.F., *Microbial*

communities on deteriorated artistic tiles from Pena National Palace (Sintra, Portugal), International Biodeterioration and Biodegradation, 2013, 84: 322-332.

Cowan, S.T., *Manual for the identification of medical bacteria,* Cambridge University Press, 1974. ISBN: 0-521-20399-6.

Crispim, C.A., Gaylarde, P.M., Gaylarde, C.C., Neilan, B.A., *Deteriogenic cyanobacteria on historic buildings in Brazil detected by culture and molecular techniques,* International Biodeterioration and Biodegradation, 2006, 57: 239-243.

Currie, B.A. and Bass, B., *Estimates of air pollution mitigation with green plants and green roofs using the UFORE model,* Urban Ecosystems, 2008, 11 (4): 409-422.

Cutler, N. and Viles, H., *Eukaryotic microorganisms and stone biodeterioration,* Geomicrobiology journal, 2010, 27: 630-646.

Czech, B., Krausman, P.R., Devers, P.K., *Economic associations among causes of species endangerment in the Inuted States*, BioScience, 2000, 50: 593-601.

De Belie, N., Monteny, J., Beeldens, A., Vincke, E., Van Gemert, D., Verstraete, W., *Experimental research and prediction of the effect of chemical and biogenic sulfuric acid on different types of commercially produced concrete sewer pipes,* Cement and Concrete Research, 2004, 34 (12): 2223-2236.

de los Ríos, A., Cámara, B., del Cura M.A. García, Rico, V.J., Galván, V, Ascaso, C., *Deteriorating effects of lichen and microbial colonisation of carbonate building rocks in the Romanesque churches of Segovia (Spain),* Science of the Total Environment, 2009, 407: 1123-1134.

De Muynck, W., Maury Ramirez, A., De Belie, N., Verstraete, W., *Evaluation of strategies to prevent algal fouling on White architectural and celular concrete,* International Biodeterioration and Biodegradation, 2009, 63: 679-689.

Demirbas, A. and Karslioglu S. *The effect of boric acid sludges containing borogypsum on properties of cement*. Cement and Concrete Research, 1995, 25 (7): 1381–1384.

Deruelle, S., *Rôle du support dans la croissance des microorganisms,* Materials and Structures, 1991, 24: 163-168.

Dilani, A., *Design and Health: the Therapeutic Benefits of Design*. Svensk Byggtjanst, Stockholm, 2001. ISBN: 978-91-7357-507-2.

Dinsdale, S., Pearen, B., Wilson, C., *Feasibility Study for Green Roof Application on Queen's University Campus*. Queen's Physical Plant Services, 2006.

Dubosc, A., Escadeillas, G., Blanc, P.J., *Characterization of biological stains on external concrete walls and influence of concrete as underlying material,* Cement and Concrete Research, 2001, 31 (11): 1613-1617.

Dukes, W.H., *Conservation of stone: causes of decay,* Architects' Journal, 1972, 156: 429-432.

Dunnet, N. and Kingsbury, N., *Planting green roofs and living walls*. Timber Press, Inc. Portland, Oregon, 2004. ISBN: 0-88192-640-X.

Earth Pledge Foundation, *Green Roofs. Ecological Design and Construction.* Schifer Books. Atglen, 2005. ISBN: 0-7643-2189-7.

Economist Intelligence Unit, *European Green City Index, Siemens AG, Munich, Germany, 2009. Ref.: A19100-F-P152-X-7600.*

Eggert A., Häubner N., Klausch S., Karsten U., Schumann R., *Quantification of algal biofilms colonising building materials: chlorophyll a measured by PAM-fluorometry as a biomass parameter*, Biofouling, 2006, 22 (1/2): 79–90.

Elbeyli I.Y., Derun E.M., Gülen J., Pişkin S., *Thermal analysis of borogypsum and its effects on the physical properties of Portland cement*, Cement and Concrete Research, 2003, 33 (11): 1729-1735.

Escadeillas, G., Bertron, A., Blanc, P., Dubosc, A., *Accelerated testing of biological stain growth on external concrete walls. Part 1: Development of the growth tests,* Materials and Structures, 2007, 40 (10): 1061-1071.

Favero-Longo, S.E., Isocrono, D., Piervittori, R., *Lichens and ultramafic rocks: a review,* Lichenologist, 2004, 36: 391-404.

Favero-Longo, S.E., Borghi, A., Tretiach, M., Piervittori, R., *In vitro receptivity of carbonate rocks to endolithic lichen-forming aposymbionts,* Research in Microbiology, 2009, 113: 1216-1227.

Fernandes, P., *Applied microbiology and biotechnology in the conservation of stone cultural heritage materials,* Applied Microbiology and Biotechnology, 2006, 73: 291-296.

Ferri L., Lottici P.P., Lorenzi A., Montenero A., Salvioli-Mariani E., *Study of silica nanoparticles — polysiloxane hydrophobic treatments for stone-based monument protection,* Journal of Cultural Heritage, 2011, 12 (4): 356–363.

Formosa, J., Chimenos, J.M., Lacasta, A.M., Haurie, L., Rosell, J.R., *Novel fire-protecting mortars formuled with magnesium by-products,* Cement and Concrete Research, 2011, 41 (2): 191-196.

Fuller, R.A. and Gaston, K.J., *The scaling of green space coverage in European cities*, Biology letters, 2009, 5: 352-355.

Gaylarde, C., Silva, M.R., Warscheid, Th., *Microbial impact on building materials: an overview,* Materials and Structures, 2003, 36 (5): 342-352.

Getter, K.L. and Rowe, B.D., *The role of extensive green roofs in sustainable development,* Hortscience, 2006, 41 (5): 1276-1285.

Giannantonio, D.J., Kurth, J.C., Kurtis, K.E., Sobecky, P.A., *Molecular characterizations of microbial communities fouling painted and unpainted concrete structures,* International Biodeterioration and Biodegradation, 2008, 63: 30-40.

Giannantonio, D.J., Kurth, J.C., Kurtis, K.E., Sobecky, P.A., *Effects of concrete properties and nutrients on fungal colonisation and fouling,* International Biodeterioration and Biodegradation, 2009, 63: 252-259.

Gorbushina, A.A., *Life on the rocks,* Environmental Microbiology, 2007, 9: 1613-1631.

Gorbushina, A.A., Broughton, W.J., *Microbiology of the atmosphere-rock interface: how biological interactions and physical stresses modulate a sophisticated microbial ecosystem,* Annual Review of Microbiology, 2009, 63: 431-450.

Grant, G., Engleback, L., Nicholson, B., Gedge, D., Frith, M., Harvey, P., *Green roofs: their existing status and potential for conserving biodiversity in urban areas.* English Nature Research Reports,2003, 498: 9-59.

Grant, G., *Green roofs and façades.* HIS BRE Press, 2006. ISBN: 978-1-86081-940-7.

Grimm, N.B., Faeth, S.H., Golubiewski, N.E., Redman, C.L., Wu, J., Bai, X., Briggs, J.M., *Global Change and Ecology of Cities*, Science, 2008, 319: 756-760.

Guillitte, O., *Cinétique de la colonisation végétle de matériaux composites de construction* (PhD thesis) Faculte des Sciences Agronomiques de Gembloux, Belgium, 1993.

Guillitte, O., *Bioreceptivity: a new concept for building ecology studies,* Science of the Total Environment, 1995, 167 (1-3): 215-220.

Guillitte, O. and Dreesen, R., *Laboratory chamber studies and petrographical analysis as bioreceptivity assessment tools of building materials,* 1995, 167 (1-3): 365-374.

Guo Y.P., Zhou H.F., Zhang L.C., *Photosynthetic characteristics and protective mechanisms against photooxidation during high temperature stress in two citrus species*, Scientia Horticulturae, 2006, 108: 260-267.

Hall D.A., Stevens R., El Jazairi B., *Effect of water content on the structure and mechanical properties of magnesia-phosphate cement mortar*, Journal of the American Ceramic Society 1998, 81: 1550-1556.

Hall D.A., Stevens R., El-Jazairi B., *The effect of retarders on the microstructure and mechanical properties of magnesia-phosphate cement mortar*, Cement and Concrete Research 2001, 31: 455-465.

Hartig, T., Mang, M., Evans, G.W., *Restorative effects of natural environment experience,* Environment and Behaviour, 1991, 23: 3-26

Hawkes, D., *Towards the sustainable city*, Renewable Energy, 1995, 6 (3): 345-352.

Heber U., Bukhov N.G., Shuvalov V.A., Kobayashi Y., Lange O.L., *Protection of the photosynthetic apparatus against damage by excessive illumination in homoiohydric leaves and poikilohydric mosses and lichens*, Journal of Experimental Bototany, 2001, 52: 1999–2006.

Heber U., Azarkovich M., Shuvalov V., *Activation of mechanisms of photoprotection by desiccation and by light: poikilohydric photoautotrophs*, Journal of Experimental Botany, 2007, 58 (11): 2745-2759.

Heidt, V. and Neef, M., *Benefits of urban green space for improving urban climate,* Ecology, planning, and management of urban forests, 2008, 84-96. ISBN: 978-0-387-71424-0.

Henry J.L., *Optimización del control de la resistencia del hormigón de la obra de la depuradora del Besós mediante ultrasonidos*. Dissertation Work, Universitat Politècnica de Catalunya. Barcelona, Spain, 2003.

Hernández M.S., Goñi S., Puertas F., Guerrero A., Palacios M., Dolado J.S., *Synergy of T1-C3S and β-C2S Hydration Reactions*, Journal of the American Ceramic Society 2011, 94 (4): 1265-1271.

Hess, D., *Plant Physiology,* Springer Study Edition. ISBN: 978-3-642-80813-5.

Hjelmroos, M., *Relationship between airborne fungal spore presence and weather variables: Cladosporium and Alternaria,* GRANA, 1993, 32 (1): 40-47.

Hoppert, M., Flies, C., Pohl, W., Günzl, B., Schneider, J., *Colonisation strategies of lithobiontic microorganisms on carbonate rocks,* Environmental Geology, 2004, 46 (3-4): 421-428.

Hosker, R.P., Lindberg, S.E., *Review: Atmospheric deposition and plant assimilation of gasses and particles,* Atmospheric Environment, 1982, 16 (5): 889-910.

Hoyano, A., *Climatological uses of plants for solar control and the effects on the thermal environment of a building,* Energy and Buildings, 1988, 11 (1-3): 181-199.

Hueck, H.J., *The biodeterioration of the materials as part of hylobiology,* Material und Organism, 1965, 1: 5-34.

Iyengar S.R. and Al-Tabbaa A., *Development Study of a low-pH Magnesium Phosphate Cement for Environmental Applications*, Environmental Technology 2007, 28: 1387-1401.

Jo, H.K., *Impacts of urban greenspace on offsetting carbon emissions for middle Korea.* Journal of Environmental Management, 2002, 64: 115-126.

Johansson, C., Rodhe, H., Sanhueza, E., *Emission of NO in a tropical savannah and a cloud forest during the dry season,* Journal of Geophysical Research Atmospheres, 1988, 93 (D6): 7180-7192.

Johnston, J. and Newton, J., *Building Green, a guide to using plants on roofs, walls and pavements.* Major of London, 2004. ISBN: 1-85261-637-7.

Jones, B.L. and Cookson, J.T., *Natural atmospheric microbial conditions in a typical suburban area,* Applied and Environmental Microbiology, 1983, 45 (3): 919-934.

Kabisch, N. and Haase, D., *Green spaces of European cities revisited for 1990-2006*, Landscape and Urban Planning, 2013, 110: 113-122.

Kaltenbach, F., *Living walls, vertical gardens – from the flowerpot to the planted system façade*, Detail nr 12, 2008, 1454-1463.

Keddy, P.A., *The competition,* Springer, London, UK, 1989. ISBN: 978-0-41231-350-9.

Kenworthy, J.R., *The eco-city: ten key transport and planning dimensions for sustainable city development,* Environment and Urbanization, 2006, 18 (1): 67-85.

Klein N., *El rol físico del agua en mezclas de cemento Portland* (PhD thesis) Universitat Politècnica de CatalunyaBarcelona, Spain: Universitat Politècnica de Catalunya, 2012.

Köhler, M., *Long-term vegetation research on two extensive green roofs in Berlin,* Urban Habitats, 2006, 4: 3-26.

Kolb, R. W. and Schwartz, D. (2009) *Introduction, in Corporate Boards: Managers of Risk, Sources of Risk* (eds R. W. Kolb and D. Schwartz), Blackwell Publishing Ltd., Oxford, UK. doi: 10.1002/9781444315882.ch.

LAINE, M.M., JORGENSEN K.S., KIVIRANTA H.S., VARTIAINEN T.S., JOKELA J.K., ADIBI A.K., SALKINOJA-SOLONEN M.K., *Bioaerosols and particles release during composting of contaminated sawmill soil*, Bioremediation Journal, 1999, 3 (1): 47-58.

Lambertini, A., Ciampi, M., Leenhardt, J., *Vertical Gardens,* Verba Volant. U.S.A., 2007. ISBN: 978-1-905216-07-9.

Langerberg W.J., Sutton, J.C., Gillespie, T.J., *Relation of Weather Variables and Periodicities of Airborne Spores of Alternaria dauci,* Phytopathology, 1977, 67: 879-883.

Laurie, I.C., *Nature in cities, the natural environment in the design and development of urban green space, John Wiley & Sons Ltd. 1977. ISBN 0-471-99605-X.*

Lee, A.C.K. and Maheswaran, R., *The health benefits of urban green spaces: a review of the evidence*, Journal of Public Health, 2011, 33 (2): 212-222.

Li, Z., Ding, Z., Zhang, Y., *Development of sustainable cementitious materials,* In: Proceedings of the international workshop on sustainable development and concrete technology. USA: Center for Transportation Research and Education, Iowa State University, 2004. pqp. 55–76.

Lieber W. and Richartz W., *Einfluß von TriaÈthanolamin, Zucker und Börsäure auf das Erstarren von Zementen,* Zement-Kalk-Gips, 1972, 61 (9): 403-409.

Lighthart, B. and Shaffer, B.T., *Bacterial flux from chaparral into the atmosphere in mid-summer at a high desert location,* Atmospheric Environment, 1994, 28 (7): 1267-1274.

Lighthart, B. and Shaffer, B.T., *Airborne bacteria in the atmospheric surface layer: temporal distribution above a grass seed field,* Applied and Environmental Microbiology, 1995, 61 (4): 1492-1496.

Lighthart, B., *Mini-review of the concentration variations found in the alfresco atmospheric bacterial populations,* Aerobiologia, 2000, 16: 7-16.

Lichtenthaler H.K., *Chlorophylls and carotenoids: pigments of photosynthetic membranes*, Methods in Enzymology 1987, 148: 349–382.

Lichtenthaler H.K., *In Vivo Chlorophyll Fluorescence as a Tool for Stress Detection in Plants, Applications of Chlorophyll Fluorescene in Photosynthesis Research*, Stress Physiology, Hydrobiology and Remote Sensing, 1988. Pp. 129–142.

Little, P., *Deposition of 2.75, 5.0 and 8.5 µm particles to plant and soil surface,* Environmental Pollution, 1977, 12: 293-305.

Lodish, H., Berk, A., Zipursky, S.L., Matsudaira, P.,Baltimore, D., Darnell, J., *Molecular Cell Biology*, 4th edition, W. H. Freeman, New York, 2000. ISBN: 0-7167-3136-3.

Loh, S., *Living walls – a way to green the built environment,* BEDP Environment Design Guide. Tec 26. August 2008. Pp. 1-7.

Lourenço, P.B., Luso, E., Almeida, M.G., *Defects and moisture problems in buildings from historical city centres: a case study in Portugal,* Building and Environment, 2006, 41 (2): 223-234.

Mancinelli, R.L. and McKay, C.P., *Effects of nitric oxide and nitrogen dioxide on bacterial growth,* Applied Environmental Microbiology, 1983, 46 (1): 198-202.

McCartney, H.A. and Lacey, M.E., *The relationship between the release of ascospores of Sclerotinia sclerotiorum, infection and disease in sunflower plot in the United Kingdom,* GRANA, 1991, 30: 486-492.

Mansch, R. and Bock, E., *Biodeterioration of natural stone with special reference to nitrifying bacteria,* Biodegradation, 1998, 9 (1): 47-64.

Manso, S., Mestres, G., Ginebra, M.P., De Belie, N., Segura, I., Aguado, A., *Development of a low pH cementitious material to enlarge bioreceptivity,* Construction and Building Materials, 2014a, 54: 485-495.

Manso, S., De Muynck, W., Segura, I., Aguado, A., Steppe, K., Boon, N., De Belie, N., *Bioreceptivity evaluation of cementitious materials designed to stimulate biological growth,* Science of the Total Environment, 2014b, 481: 232-241.

Martínez, C., Gertosio, C., Labbe, A., Pérez, R., Ganga, M.A., *Production of Rhodotorula glutinis: a yeast that secretes α-L-arabinofuranosidase,* Electronic Journal of Biotechnology, 2006, 9 (4): 407-413.

Maxwell D.P., Falk S., Trick C.G., Huner N.P.A., *Growth at Low Temperature Mimics High-Light Acclimation in Chlorella vulgaris*, Plant Physiology, 1994, 105: 535-543.

Maxwell K. and Johnson G.N., *Chlorophyll fluorescence: a practical guide,* Journal of Experimental Botany, 2000, 51 (345): 659–668.

McDonnell, M.J., Pickett, S.T.A., Groffman, P., Bohlen, P., Pouyat, R.V., Zipperer, W.C., Parmelee, R.W., Carreiro, M.M.,Medley, K. *Ecosystem processes along an urban-to-rural gradient*, Urban Ecosystems, 1997, 1 (1):21-36.

McKinney, M.L., *Urbanization, biodiversity, and conservation*, BioScience, 2002, 52: 883-890.

Mentens, J., Raes, D., Hermy, M., *Green roofs as a tool for solving the rainwater runoff problem in the urbanized 21st century?* Landscape and Urban Planning, 2006, 77: 217-226.

Mestres, G and Ginebra, M.P., *Novel magnesium phosphate cements with high early strength and antibacterial properties,* Acta Biomateralia, 2011, 7 (4): 1853-1861.

Miller, A.Z., Dionísion, L., Macedo, M.F., *Primary bioreceptivity: a comparative study of different Portuguese lithotypes,* International Biodeterioration and Biodegradation, 2006, 57: 136-142.

Miller, A.Z., Laiz, L., Gonzalez, J.M., Dionísion, L., Macedo, M.F., Saiz-Jimenez, C., *Reproducing stone monument photosynthetic-based colonisation under laboratory conditions,* Science of the Total Environment, 2008, 405: 278-285.

Miller, A.Z., Dionísion, A., Laiz, L., Macedo, M.F., *The influence of inherent properties of building limestones on their bioreceptivity to phototrophic microorganisms,* Microbial Ecology, 2010, 60 (1): 55-68.

Miller, A.Z., Rogerio-Candelera, M.A., Laiz, L., Wierzchos, J., Ascaso, C., Sequeira Braga, M.A., Hernández-Mariné, M., Maurício, A., Dionísion, A., Macedo, M.F., Saiz-Jimenez, C., *Laboratory-induced endolithic growth in calcarenites: Biodeteriorating potential assessment,* Annals of Microbiology, 2009, 59: 1-9.

Mindess, S., Young, J.F., Darwin, D., *Concrete (2nd edition),* Prentice Hall PTR, 2003. ISBN: 978-0130646323.

Misra A.N., Misra M., Singh R., *Chlorophyll Fluorescence in Plant Biology*, Biophysics, Oxford, 2009. Pp. 171–192.

Nielsen, T.S. and Hansen, K.B., *Do green areas affect health? Results from a Danish survey on the use of green areas and health indicators*, Health & Place, 2007, 13 (4): 839-850.

Nuhoglu, Y., *The harmful effects of air pollutans around the Yenikoy thermal power plant on architecture of Calabarian pine (Pinus brutila Ten.) needles,* Journal of Environmental Biology, 2005, 26: 315-322.

Nuhoglu, Y., Oguz, E., Uslu, H., Ozbek, A., Ipekoglu, B., Ocak, I., Hasenekoglu, I., *The accelerating effects of the microorganisms on biodeterioration of stone monuments under air pollution and continental-cold climatic conditions in Erzurum, Turkey,* Science of the Total Environmentl, 2006, 364 (1-3): 272-283.

Oberndorfer, E., Lundholm, J., Bass, B., Coffman, R.R., Doshi, H., Dunnet, N., Gaffin, S., Köhler, M., Liu, K.K.Y., Rowe, B., *Green roofs as urban ecosystems: Ecological structures, functions and services,* BioScience, 2007, 57 (10): 823-833.

Observatorio de la Sostenibilidad de España, *Sostenibilidad local: Una aproximación urbana y rural*, Informe temático, 2009. NIPO: 770-08-129-3.

Onions, A.H.S. and Brady, B.L., *Taxonomy of Penicillium and Acremonium,* Chapter 1 from Biotechnology Handbooks 1: *Penicillium* and *Acremonium*, Peberdy, J.F. (ed.). ISBN: 0-306-42345-6.

Ortega-Calvo, J.J., Ariño, X., Hernandez-Marine, M., Saiz-Jimenez, C., *Factors affecting the weathering and colonisation of monuments by phototrophic microorganisms,* Science of the Total Environment, 1995, 167: 329-341.

Ottelé, M., van Bohemen, H.D., Fraaij, A.L.A., *Quantifying the deposition of particulate matter on climber vegetation on living walls,* Ecological Engineering, 2010, 36 (2), 154-162.

Ottelé, M., *The Green Building Envelope. Vertical Greening* (PhD thesis) Technical University of Delft, Delft, Holland, 2011.

Papida, S., Murphy, W., May, E., *Enhancement of physical weathering of building stones by microbial populations,* International Biodeterioration and Biodegradation, 2000, 46: 305-317.

Paul, M.J. and Meyer, J.L., *Streams in the urban landscape*, Annual Review of Ecology and Systematics, 2001, 32: 333-365.

Pérez, G., *Façanes vegetades. Estudi del seu potencial com a sistema passiu d'estalvi d'energia, en clima mediterrani continental* (PhD thesis) Polytechnical University of Catalonia, Barcelona, Spain, 2010.

Pérez, G., Rincón, L., Vila, A., González, J.M., Cabeza, L.F., *Green vertical systems for buildings as passive systems for energy savings,* Applied Energy, 2011, 88 (12): 4854-4859.

Perini, K., Ottelé, M., Fraaij, A.L.A., Haas, E.M., Raiteri, R., *Vertical greening systems and the effect on air flow and temperatura on the building envelope,* Building and Environment, 2011, 46 (11): 2287-2294.

Piervittori, R., Salvadori, O., Laccisaglia, A., *Literature on lichens and biodeterioration of stoneworks I,* Lichenologist, 1994, 26: 171-192.

Piervittori, R., Salvadori, O., Laccisaglia, A., *Literature on lichens and biodeterioration of stoneworks II,* Lichenologist, 1996, 28: 471-483.

Piervittori, R., Salvadori, O., Laccisaglia, A., *Literature on lichens and biodeterioration of stoneworks III,* Lichenologist, 1998, 30: 263-277.

Pitts, B., Hamilton, M.A., McFeters, G.A., Stewart, P.S., Willse, A., Zelver, N., *Color measurement as a means of quantifying surface biofouling,* Journal of Microbiological Methods, 1998, 34 (2): 143-149.

Popovics S., Rajendran N., Penko M., *Rapid Hardening Cements for Repair of Concrete*, Materials Journal 1987, 84 (1): 64-73.

Portillo, M.C., Gazulla, M.F., Sanchez, E., Gonzalez, J.M., *A procedure to evaluate the resistance to biological colonisation as a characteristic for product quality of ceramic roofing tiles,* Journal of the European Ceramic Society, 2000, 31 (3): 351-359.

Praphailong, W. and Fleet, G.H., *The effect of pH, sodium chloride, sucrose, sorbate and benzoate on the growth of food spoilage yeasts,* Food Microbiology, 1997, 14 (5): 459-468.

Pretty, J., Griffin, M., Sellens, M., Pretty, C., *Green exercise: complementary roles of nature, exercise, diet in physical and emotional well-being and implications for public health policy*. CES Occasional Paper 2003-1. University of Essex (www2.essex.ac.uk/ces).

Priego, C., *Naturaleza y Sociedad. El valor de los espacios verdes urbanos*, Ministerio de Medio Ambiente y Medio Rural y Marino, 2011. ISBN: 978-84-491-1078-8.

Prieto, B. and Silva, B., *Estimation of the potential bioreceptivity of granitic rocks from their intrinsic properties,* International Biodeterioration and Biodegradation, 2005, 56: 206-215.

Pugh, T.A.M., MacKenzie, A. R., Whyatt, D., Hewitt, C.N., *Effectiveness of Green Infrastructure for Improvement of Air Quality in Urban Street Canyons,* Environmental Science and Technology, 2012, 46 (14): 7692-7699.

Qiao, F., Lin, W., Chau, C.K., Li, Z., *Property assessment of magnesium phosphate cement,* Key Engineering Materials, 2009a, 400-402: 115-120.

Qiao, F., Chau, C.K., Li, Z., *Setting and compressive strength characteristics of magnesium phosphate cement paste,* Advances in Cement Research, 2009b, 21 (4): 175-180.

Ramachandran V.S., *Hydration of cement – role of triethanolamine*, Cement and Concrete Research 1976, 6 (5): 623-631.

Rebolledo N., *Aplicación de técnicas no destructivas al estudio del hormigón fresco,* (Master thesis), Universidad Politécnica de Madrid. Madrid, Spain, 2010.

Reponen, T., Wileke, K., Ulevicius, V., Grinshpun, S.A., Donnelly, J., *Techniques for dispersion of microorganisms into air,* Aerosol Science and Technology, 1997, 27 (3): 405-421.

Rohácek K., *Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships*, Photosynthetica, 2002, 40 (1): 13–29.

Rotmans, J. and Van Asselt, M., Vellinga, P., *An integrated planning tool for sustainable cities,* Environmental Impact Assessment Review, 2000, 20: 265-276.

Saiz-Jimenez, C., Ariño, X., Ortega-Calvo, J.J., *Mechanism of Stone deterioration by photosynthesis-based epilithic biofilms.* In: De Cleene M, editor. Interactive physical weathering and bioreceptivity study on building stones, monitored by Computerized X-Ray Tomography (CT) as a potential non-destructive research tool. Protections and Conservation of the European Cultural Heritage. Research Report nº 2. Ghent: Science Information Office, 1995. Pp. 25-62.

Saiz-Jimenez, C., *Biodeterioration vs biodegradation: the role of microorganisms in the removal of pollutans deposited on historic buildings,* International Biodeterioration and Biodegradation, 1997, 70 (2-4): 225-232.

Salisbury, F. and Ross, C., *Plant Physiology*, Wadsworth Publishing Company, 1992. ISBN: 0-534-15162-0.

Sarkar A.K., *Phosphate cement-based fast-setting binders*, American Ceramic Society Bulletin 1990, 69: 234-238.

Schol-Schwarz, M.B., *The genus Epicoccum Link,* Transactions of the British Mycological Society, 1959, 42 (2): 149-173.

Shaffer, B.T., Lighthart, B., *Survey of culturable airborne bacteria at four diverse locations in Oregon: urban, rural, forest and coastal,* Microbial Ecology, 1997, 34: 167-177.

Shelton, B.G., Kirkland, K.H., Flanders, W.D., Morris, G.K., *Profiles of airborne fungi in buildings and outdoor environments in the United States,* Applied and Environmental Microbiology, 2002, 68 (4): 1743-1753.

Shirakawa, M.A., Gaylarde, C.C., Gaylarde, P.M., John, V., Gambale, W., *Fungal colonisation and succession on newly painted buildings and the effect of biocide,* Microbiology Ecology, 2002, 39 (2): 165-173.

Singh N.K., Mishra P.C., Singh V.K., Narang K.K., *Effects of hydroxyethyl cellulose and oxalic acid on the properties of cement*, Cement and Concrete Research, 2003, 33 (9): 1319-1329.

Smith, W.H. and Staskawicz, B.J., *Removal of atmospheric particles by leaves and twigs of urban trees: Some preliminary observations and assessment of research needs,* Environmental Management, 1977, 1 (4): 317-330.

Song, Y., *Ecological city and urban sustainable development,* Procedia Engineering, 2011, 21: 142-146.

Soudée, E. and Péra, J., *Mechanism of setting reaction in magnesia-phosphate cements,* Cement and Concrete Research, 2000, 30: 315-321.

Souza R.P., Machado E.C., Silva J.A.B., Lagôa A.M.M.A., Silveira J.A.G., *Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (Vigna unguiculata) during water stress and recovery*, Environmental and Experimental Botany, 2004, 51: 45-56.

Starks, T.L. and Shubert, L.E., *Colonisation and succession of algae and soil-algal interactions associated with disturbed areas,* Journal of Phycology, 1982, 18 (1): 99-107.

Stephens, C., *Bacterial sporulation: A question of commitment?,* Current Biology, 1998, 8 (2): R45-R48.

Stigsdotter, U.K., Ekholm, O., Schipperijn, J., Toftager, M., Kamper- Jorgensen, F., Randrup, T.B., *Health promoting outdoor environments – Associations between green space, and health, health-related quality of life and stress based on a Danish national representative survey,* Scandinavian Journal of Public Health, 2010, 38 (4): 411-417.

Taylor H.F.W., *Cement Chemistry*. Academic Press Inc. London; 1990.

Tiano, P., *Biological deterioration of exposed works of art made of stone,* In: L.H.G. Morton (Ed.), The Biodeterioration of Constructional Materials, Biodeterioration Society, Occasional Publication 3, Lancashire Polytechnic, UK, 1986. Pp. 37-44.

Tiano, P., Accolla, P., Tomaselli, L., *Phototrophic biodeteriogens on lithoid surfaces: an ecological study,* Microbial Ecology, 1995, 29: 299-309.

Tiano, P., *Biodeterioration of monumental rocks: decay mechanisms and control methods,* Science and Technology of Cultural Heritage, 7: 19-38.

Tomaselli, L., Lamenti, G., Bosco, M., Tiano, P., *Biodiversity of photosynthetic microorganisms dwelling on stone monuments,* International Biodeterioration and Biodegradation, 2000, 46: 251-258.

Tong, Y. and Lighthart, B., *Solar radiation is shown to select for pigmented bacteria in the ambient outdoor atmosphere,* Photochemistry and Photobiology, 1997, 65 (1): 103-106.

Tran, T.H., Govin, A., Guyonnet, R., Grosseau, P., Lors, C., Garcia-Diaz, E., Damidot, D., Devès, O., Rout, B., *Influence of the intrinsic characteristics of mortars on biofouling by Klebsormidium flaccidum,* International Biodeterioration and Biodegradation, 2012, 70: 31-39.

Tran, T.H., Govin, A., Guyonnet, R., Grosseau, P., Lors, C., Garcia-Diaz, E., Damidot, D., Devès, O., Rout, B., *Avrami's law based kinetic modelling of colonisation of mortar surface by alga Klebsormidium flaccidum,* International Biodeterioration and Biodegradation, 2013, 79: 73-80.

Trigo, M.M., Jato, V., Fernández, D., Galán, C., *Atlas Aeropalinológico de España,* Universidad de León, España, 2008. ISBN: 978-84-9773-403-5.

Ulrich, R.S., *The Biophilia Hypothesis*, Island Press, 1993. ISBN: 1-55963-147-3.

UNE-EN 196-1:2005, *Methods of testing cement – Part 1: Determination of strength*, AENOR; 2005.

UNE-EN 196-2:2006, *Methods of testing cement – Part 2: Chemical analysis of cement*, AENOR; 2006.

UNE-EN 196-3:2005, *Methods of testing cement – Part 3: Determination of setting times and soundness*, AENOR; 2005.

UNE-EN 196-9:2011, *Methods of testing cement – Part 9: Heat of hydration – semi-adiabatic method,* AENOR; 2011.

UNE-EN 1015-3:2000, *Methods of test for mortar for masonry. Part 3: Determination of consistence of fresh mortar (by flow table),* AENOR; 2000.

UNE 7088:1955, *Compactness determination in the arids for mortars and concrete,* AENOR; 1955.

UNE 80105:1986, *Test methods of cements. Physical analysis. Density determination by the liquid displacement pycnometer*, AENOR; 1986.

UNE 80230:2010, *Methods of testing cement. Chemical analysis. Alternative methods*, AENOR; 2010.

UNE 83227:2005, *Admixtures for concrete, mortars and grouts. Determination of pH*, AENOR; 2005.

United Nations, Department of Economic and Social Affairs, Population Division, *World Urbanization Prospects. The 2001 Revision.* Data Tables and Highlights. 2002. Working paper No. ESA/P/WP.173.

United Nations, Department of Economic and Social Affairs, Population Division, *World Population Prospects. The 2008 Revision.* Highlights. 2009. Working paper No. ESA/P/WP.210.

Valls S., *Estabilización física y química de los lodos de depuradora de aguas residuales y de material de demolición para su utilización en ingeniería civil* (PhD thesis), Universitat Politècnica de Catalunya, Barcelona, Spain, 1999.

Valls S. and Vàzquez E., *Stabilisation and solidification of sewage sludges with Portland cement*, Cement and Concrete Research 2000, 30: 1671-1678.

van Eijk R.J. and Brouwers H.J.H., *Modelling the effects of waste components on cement hydration*. Waste Management (Oxford), 2001, 21 (3): 279–284.

Van Renterghem, T. and Botteldooren, D., *In-situ measurements of sound propagating over extensive green roofs,* Building and Environment, 2011, 46 (3): 729-738.

Wagh A.S., *Chemically bonded phosphate ceramics*, Twenty-First Century Materials with Diverse Applications. Elsevier BV. Belgium: Amsterdam; 2004.

Widden, P., *Competition and the fungal community,* Chapter 9 from *The mycota*, Springer. ISBN: 3-540-58005-0.

Wilson, E.O., *Biophilia*, Harvard University Press, 1984. ISBN: 0-674-07442-4.

Wong, N.H., Tan, A.Y.K., Chen, Y., Sekar, K., Tan, P.Y., Chan, D., Chiang, K., Wong, N.C., *Thermal evaluation of vertical greenery systems for building walls,* Building and Environment, 2010, 45 (3): 663-672.

Yang Q. and Wu X., *Factors influencing properties of phosphate cement-based binder for rapid repair of concrete*, Cement and Concrete Research 1999, 29 (3): 389-396.

Yang Q., Zhu B., Zhang S., Wu X., *Properties and applications of magnesia-phosphate cement mortar for rapid repair concrete*, Cement and Concrete Research 2000, 30: 1807-1813.

Yousuf M., Mollah A., Vempati R.K., Lin T.C., Cocke D.L., *The interfacial chemistry of solidification/stabilization of metals in cement and pozzolanic material systems*, Waste Management 1995, 15 (2): 137-148.

PUBLICATIONS

Articles

Manso, S., Mestres, G., Ginebra, M.P., De Belie, N., Segura, I., Aguado, A., *Development of a low pH cementitious material to enlarge bioreceptivity,* Construction and Building Materials, 2014a, 54: 485-495.

Manso, S., De Muynck, W., Segura, I., Aguado, A., Steppe, K., Boon, N., De Belie, N., *Bioreceptivity evaluation of cementitious materials designed to stimulate biological growth,* Science of the Total Environment, 2014b, 481: 232-241.

Conference proceedings

Manso, S., Segura, I., Aguado, A., *Influencia de la preparación de la muestra en la determinación de pH en pastas, morteros y hormigones*, Anais do 54º Congreso Brasileiro do Concreto CBC2012, Maceió (Brasil), October 2012.

De Belie, N., Wang, J., De Muynck, W., Manso, S., Segura, I., *Microbial interactions with mineral building materials,* 8th International Symposium on Cement and Concrete ISCC-2013, Nanjing (China), September 2013.

Patents

Manso S, Segura I, Aguado A, Conjunto multicapa en base cemento, aplicable como soporte biológico para fachadas de edificios u otras construcciones, Patent PCT/ES2013/070438, 28 July 2013.