INFLUENCE OF CEMENT MATERIALS' COMPOSITION ON MICROBIOLOGICAL COLONIZATION OF DAMS

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ABSTRACT

There is scarce information about biological colonization of dams. However, like any other structure, they are subjected to the action of different organisms. In this study, dams, lichens, bryophytes and diatoms have been observed, and it has been noticed that their abundance and distribution is related to the climate conditions and the relative position of the water level. Besides, little is known about the influence of the cement composition on the growth of these organisms, which is important because of the current use of cements with different additions. In order to answer this question, tests have been done using a non lichenized fungus, namely *Aspergillus niger*, because lichen mycobionts grow very slowly in the culture. Cement mortars with different compositions cured during 28 days and subjected to artificial carbonation in the CO_2 chamber were inoculated with spore suspensions of *A. niger* and the degree of colonization was observed after three months under stereo and optical microscope as well as

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Environmental Scanning Electron Microscopy (ESEM). Besides, porosity and pore size of the samples were measured with the mercury intrusion method. It was observed that the higher porosity of the samples with 5% lime filler allowed the fungus to grow more easily. However, this effect was not observed in the samples with higher lime filler percentage, which is attributed to the higher Ca content. Whereas the mortar with puzzolanic additions with the lesser porosity was the least colonized.

Keywords: Dams, biological deterioration, lichens, fungi, *Aspergillus niger*

LIST OF MENTIONED GENERA

Fontinalis, Tillandsia

LIST OF MENTIONED SPECIES

Aspergillus niger, Caloplaca austrocitrina, Limnoperna fortunei

1. INTRODUCTION

There are many factors, like temperature, rain and humidity, that influence the durability of structures and the presence of organisms is one of them. It is well known that after the construction is finished, different species will settle on the new available habitat ranging from microscopic bacteria and cyanobacteria to plants and animals, which may cause biodeterioration: any undesirable change in the properties of a material caused by the vital activities of organisms (Warscheid and Braams, 2000). Organisms will grow on a building and mechanisms and extent of the alteration will depend on the interaction of three factors: (i) the characteristics of the material such as composition, pH, and porosity; (ii) the weather conditions of the location and (iii) the biota, community of organisms, present in the zone. Given the time, different species will colonize the building and develop an ecosystem (Caneva et al., 2003). The above mentioned phenomenon is well known and has been widely researched, since it is a serious threat to many historical buildings and monuments around the world, causing concern among those responsible of heritage conservation (Caneva et al., 2003). Like any other structure, dams are subjected to the action of different organisms, but there is scarce information about microbiological colonization of dams, except Covenham reservoir in South England (Figg et al., 1986) and others from Argentina (Rosato and Traversa, 2000; Rosato, 2008; Traversa et al., 2001). In inspected dams, lichens, bryophytes, and diatoms have been observed (Rosato, 2008).

It is curious to note that lichen species have a clear distribution pattern in the dams of Tandil (Figure 1) and Azul (Buenos Aires province, Argentina): the yellow species grow in the upper part; the grey/green species are in the middle part and the black species in the lower part. This distribution pattern was also described in the Covenham wave Wall (Lincolnshire, U.K.) in a sea environment, and has been attributed to differences in superficial moisture. This is probably the case here, because the concrete retains quite a great percentage of water.

Figure 1. Tandil dam (Buenos Aires province, Argentina).

Figure 2. Map of Argentina showing the location of the mentioned dams.

As for the impact of these colonisations, lichens, and other phototrophic organisms may cause mechanic and chemical weathering, but it is only superficial and does not affect the structure. In the case of the Tandil dam in Buenos Aires Province, observations with Scanning Electron Microscope (SEM) showed that the hyphae of the mycobiont, fungal partner of the symbiosis of the lichen *Caloplaca austrocitrina*, penetrate the material. As the hyphae absorb water from the environment, they grow and expand, so the turgor pressure increases, exerting forces on the material and causing damage to microscopic structure of the mortar. This mechanism generates boring channels causing a micropitting (perforations between 10 – 20 µm). Electron dispersive Spectrometry (EDS) revealed that the material affected by lichens had very low calcium content of 5.3%, compared to the 59.4% of the non-colonized concrete, whereas optic microscope observations of thin sections gave as result that *C. austrocitrina* penetrated the cement material up to 1 – 1.5 mm (Traversa et al., 2000; Traversa et al., 2001). However this is considered mostly as an aesthetic problem, not affecting the safety or the operation of the dam; and so, cleaning is not performed, as the costs would be too high, without a clear benefit. The case of flowering plants is different, because their roots exert pressure on the material increasing the size of fissures. However, plants were not observed in the Buenos Aires dams and were not mentioned for Covenham, either. In the case of the Tucumán dam, only small *Tillandsia sp.* was seen, but these are epiphytes, without a well developed root system. A very important biodeterioration problem for dams is the presence of mussels. We must mention the golden mussels (*Limnoperna fortunei*) that invaded the Paraná and Uruguay rivers up to the South of Brazil. These species can reach densities higher than 100,000 ind./m^2 and cause great economic losses due to obstruction of pipelines, interruption of the function of different systems, cleaning, repair, lost profits, etc. Like other mussels, *L. fortunei* has a system to attach it to the substrate by means of an inert sclerotized structure formed by threads called "*byssus*", which penetrates the material of the colonized substrates and can produce deterioration of concrete structures (García et al., 2011).

Sometimes organisms can have a protective action. This was noticed in a fifty – year old dam in Tucumán Province (Argentina), in a zone of subtropical mountain forests: the wall was covered by a thick carpet of mosses (*Fontinalis* sp.) that prevented further erosion by sediments and pebbles carried by the water during the rainy period, when the water flow and stream force increase. In this case, the problems were not caused by the organisms, but the age and quality of the material (Rosato, 2008). The situation mentioned above is attributed to the high porosity of the material, since this means a higher rate of water absorption and a longer water retention period which creates a more favourable condition for the growth of microorganisms, as already observed in sandstone and brick (Rosato, 2010; García and Rosato, 2011). However, no information could be found about the influence of the cement material composition and porosity related to the growth of microorganisms, since the current use of cements is with different additions. At present, the use of cements with additions generates variations in concretes with the same resistance (project resistance) as regards to water absorption, absorption capacity, initial and superficial absorption, percentage of hydration compounds at the same age, etc.

For instance, when portland cement is replaced with lime filler, there is a greater hydration degree in a shorter time without generating an increase in the final volume of hydration product since this addition does not generate hydration products with cementing properties; there is a relative increase of the water/cement (w/c) ratio. As for the addition of slag, their reaction is delayed in time, causing an effective increase of the w/c ratio in the system, lower quantity of initially reactive material, only at early ages. In both cases the porosity and structure of pores is affected, varying according to the percentages of addition (Menéndez et al., 2006). In concrete dams, cement is a material that has to be specified according to the part of the structure of the dam where it will be located. According to the different needs, normal cements with low contents per cubic meter, cements with low hydration heat or cements with different additions are used. In more recent time, the use of additions and replacements, at important percentages that work with sustainability, makes necessary this kind of studies in order to be able to anticipate the influence of biodeterioration on the concrete surface and assess its incidence in the aging or alteration of the material.

The aging process of concrete is a factor of time, causing irreversible phenomena that lead to a decrease of the original confidence margins of hydraulic structures. The rate of aging depends on the quality of the design, technology of constructing the dam, and operating conditions. When aging the reinforced concrete structures are managed, the kind of materials used has to be taken into account to define the critical structures and to assess the degradation factors that may affect their performance. Cement is the central material in this assessment, not only from a structural point of view, but also considering durability standards and even aesthetic factors in the case of a reinforced concrete structure.

In order to make this assessment, accelerated aging techniques are used. A review and an evaluation were conducted of accelerated aging techniques to provide data. These tests can supply data to develop for service life models or that by themselves can be used to predict the service life or performance of reinforced service life models of reinforced concrete structures. The most promising approach for predicting the remaining service life of concrete involves the development of a reference source that contains data and information about the variation of the materials related to the environment conditions and the exposure time would be helpful to predict potential deterioration of critical components of structural concrete in the long term. This would also allow setting limits on hostile environmental exposure for these structures upon the environmental conditions. This why it is important to use accelerated test to assess biological deterioration of materials, as those used by Wiktor et al. (2009) and Prunell et al. (2011).

The aim of this study is to compare the growth and development of fungi on cements with different additions and identify the factors that allow a faster colonization by these organisms and find the parameters that can be set in order to inhibit the microorganism's growth.

2. Materials and Method

Tests have been done using a mould, namely *Aspergillus niger*, using a technique similar to that described in Wiktor et al. (2009), allowing a fast development of the fungi and to study the extent of colonization and deterioration. *A. niger* is common in soil and walls of ancient buildings. It is a usual part of the airborne fungal species, due to the production of numerous spores, fast growth and its ability to develop in a wide range of conditions. It is an opportunistic species and not exclusive of heritage buildings, but it can harm the material because it produces citric and oxalic acid that reacts with the free calcium ions present in the substrate to form organic salts, leaving a patina. However, like other fungi and lichens, it cannot grow on fresh cement materials, because of their high pH: the mould can only colonize substrata with pH under 9.5; so it is necessary that the carbonation process has advanced enough to lower the pH to the mould's tolerance range.

As described by Prunell et al. (2011), normal cement mortar test samples dosed with different percentage additions of lime filler and puzzolan (Table 1) were cast in acrylic forms of 11 cm ×12 cm (Figure 3) that yielded 3 specimens of 1 cm ×6.5 cm ×2.5 cm each (Figure 4). Once freed from the mold, test samples were cured during 28 days and subjected to artificial carbonation in the CO_2 chamber during one week. The samples were placed in sterile 500 mL containers prepared with vermiculite covered by a filter paper, wetted with 40

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This method consists in the injection under pressure of a known volume of mercury into the sample. For each pressure interval considered, the observed mercury amount indicates the volume of open pores that have a determined interval of radius size. In this way, an absorption curve can be obtained and the total pore volume can be calculated (ASTM Standard Norm D 4404-84; Moscou and Luby, 1981; Van Brakel et al., 1981). Since mercury enters the bigger pores first and then the smaller ones, the obtained absorption curve allows to know the percentage of the total volume for a given pore radius size and calculate the percentage of the total volume for a given pore radius size interval.

Figure 3. Acrylic mold.

Figure 4. Mortar specimens.

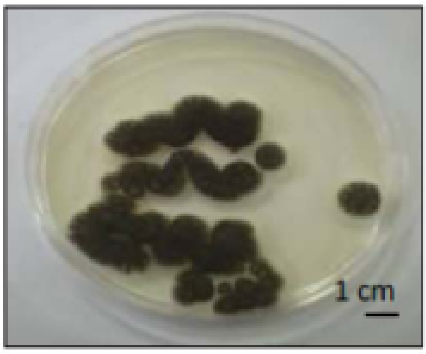

Figure 5. *Aspergillus niger* in culture.

Table 1. Composition of the used experimental mortar mixtures and their dosification

Mixture	Water (g)	Cement (g)	Sand (g)	water/cement material	Filler	Puzzolan
NC	21	35.00	105	0.6	-	-
NCF5	21	33.25	105	0.6	5%	-
NCF10	21	31.50	105	0.6	10%	-
NCF15	21	28.00	105	0.6	20%	-
NCP15	21	29.75	105	0.6	-	15%
NCP15	21	28.00	105	0.6	-	20%
NCP15	21	26.25	105	0.6	-	25%

(NC: Normal Cement; NCLF5: Normal Cement with 5% Lime Filler; NCLF10: Normal Cement with 10% Lime Filler; NCF15: Normal Cement with 15% Lime Filler; NCP15: Normal Cement with 15% Puzzolan; NCP20: Normal Cement with 20% Puzzolan; NCP25: Normal Cement with 25% Puzzolan).

The natural puzzolan used is of mineral origin. It is a product of the transformation of volcanic dust and "ashes", incoherent pyroclastic materials formed during explosive eruptions. They are rich in glass and chemically reactive; so they are prone to endogenous reactions, zeolitization and cementation. When submitted to continuous atmospheric action, known as meteorization, it forms a tuff, that is a volcanic rock, more or less consolidated and compact, crystalline, and vitreous. Therefore, it needs to be finely ground down to fine cement, like powder before it can be added to the mixtures. It contains silicates and aluminates that combine with the calcium hydroxide freed during the hydration reactions at environment temperature forming low solubility compounds with agglomerating properties, filling the capillary pores of the concrete. The lime "filler" used is a finely ground calcareous material of inorganic nature and mineral origin, formed mainly by calcium carbonate, with a favourable influence on the properties and behaviour of mortar and concrete, in fresh condition as well as hardened. Its composition is characterized by a limestone of high purity, with $CaCO_3$ content higher than 75%, and a maximum clay content of 1.2%.

The coverage percentages of *A. niger* were measured using a grid that circumscribed six equal parts of the specimen; each part was photographed and the image was used to estimate the coverage percentage of the fungus for each part; the obtained percentages were used to calculate the general average of three specimens for each kind of mixture.

3. Results and Discussion

3.1. Microscope Observations

Images show that the mould *A. niger* was able to grow more on mixture NCLF5, corresponding to an addition of lime filler in a 5% proportion (Figure 6d), whereas the lesser growth of the mould was observed in test samples prepared with mixture NCP25, that made of the addition of puzzolan in 25% proportion (Figure 6n).

It can be also noticed that non-colonized surfaces have a smooth look, whereas the colonized ones seem more irregular and rough (Figures 6a – 6n).

The normal cement NC was covered in 42% (Table 3). The highest coverage percentage was observed on NCLF5 (Normal cement with 5% lime filler), with 56%, whereas the lowest is 11% and corresponds to NCP25, with 25% puzzolan. As already observed by Wiktor et al. (2009), fungi can be inoculated in mortar samples and are able to growth in laboratory conditions, which allows an easier study of the biodeterioration mechanisms caused by them.

However, in this case, we observed differences in mould growth according to the mortar composition. The standard deviations, for each mixture, is about 4.75 and that of the coverage percentages for all mixtures is 17.68, indicating there is indeed a wider data dispersion between the coverage values among different mixtures.

To understand these results, it is important to consider different factors, especially the influence of the calcium proportion and microporosity which will be analyzed in the next sections.

Table 2. Growth variation of the *A. niger* according to the mortar mixture

Mixture	Growth % (average of three specimens)
NC	42
NCLF5	56
NCLF10	49
NCLF15	15
NCP15	49
NCP20	30
NCP25	11

Each observation is the average of three replications.

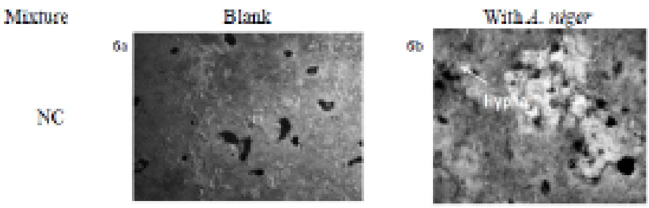

Figure 6. Continued on next page.

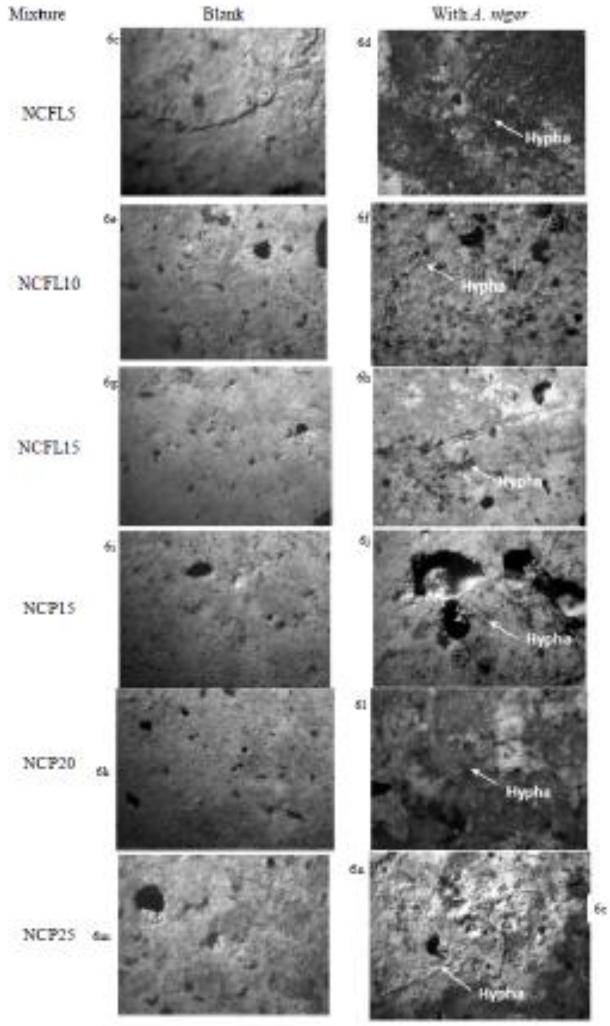

Figure 6. Comparison of colonized mortar specimens inoculated with *A. niger* and those non-colonized.

3.2. Environmental Scanning Electronic Microscope Observations

NC (normal cement) has a smooth surface, with small pores, about 4 – 5 µm diameter and scarce hyphae of *A. niger*, about 10 and 12 µm diameter (Figure 7). NCLF5 corresponds to cement with lime filler 5% (Figure 7b). Its surface is irregular and porous. There are numerous small pores 2 – 3 µm diameter, about 59% of the total counted on the picture, others more scarce 14 – 18 µm, 24%, and bigger ones between 30 – 40 µm diameter, 15.5%, with only a big pore 80 µm, 1.5%. In this case it can be noted that the mould colonization is abundant. NCP25 is cement with puzzolan 25% (Figure 7d). Its surface is very smooth, with few small pores having 2 – 5 µm diameter, and the colonization is scarce, with 8 – 10 µm diameter hyphae. Except for NCLF5, there is very little variation in pore size. There is also slight variation in the diameter of the hyphae. It is also noted in further observations that the mould tended to grow in clusters around the mixture's clasts (Figures 7d – 7j), but the growth is lesser in mixture NCLF15, with high content of filler, NCP20 and NCP25, with 20% and 25% puzzolan, respectively. Here it was observed that hyphae had 10 – 12 µm diameter but only 7 – 8 µm on the mixtures NCLF15, NCP20 and NCP25. This pattern indicates that the fungus tends to grow in the areas with a higher content of free calcium ions.

3.3. Electron Dispersive Spectroscopy Microanalysis

Samples were also analyzed with EDS to assess their elemental composition. Observing the composition of the mortars, we find the calcium percentage decreases with the increasing amount of puzzolan (Table 4).

Table 4. Chemical composition of the mortar samples

Element	NC	NCLIF5	NCLF10	NCLF15	NCP15	NCP 20	NCP25
C	11.82	9.15	8.26	11.63	7.25	8.75	11.88
O	48.03	41.38	44.47	41.58	38.93	46.79	52.58
Na	-	0.39	-	-	-	-	-
Mg	0.31	0.40	0.73	0.19	-	-	-
Al	-	1.32	0.36	0.13	0.83	0.15	-
Si	1.50	3.63	2.69	1.31	3.29	0.86	0.70
Ca	38.05	42.78	43.48	45.16	48.55	42.98	34.84
Fe	-	0.95	-	-	1.15	0.47	-

This could explain why the mould grows so scarcely on these mixtures. In contrast, there is no increase of growth in the mixtures with increasing percentages of lime filler; but rather the contrary. This indicates that the important characteristic for the mould is the free calcium available as hydroxide and soluble salts rather than the total calcium percentage.

3.4. Mercury Intrusion Porosimetry

This technique allows to measure pore volume and size. In this case only three specimens were tested: normal cement NC, NCLF 5 (with 5% filler), and NCP25 (with puzzolan 25%). Since mercury intrusion porosimetry allows knowing the distribution of the size pores of the material, it is interesting to analyze this aspect and see how it may affect the growth of the microorganisms.

For this reason, the materials with the lowest (NCP25) and the highest fungal coverage (NCLF5) were chosen and compared to the normal cement (NC), to look for differences in the microporosimetry, especially at the pore sizes related to the diameter of the hyphae (Table 5).

The total pore volume of the samples were 92.2 mm^3 for sample NC; 116.2 mm^3 for NCLF5; and 103.2 mm^3 for NCP25

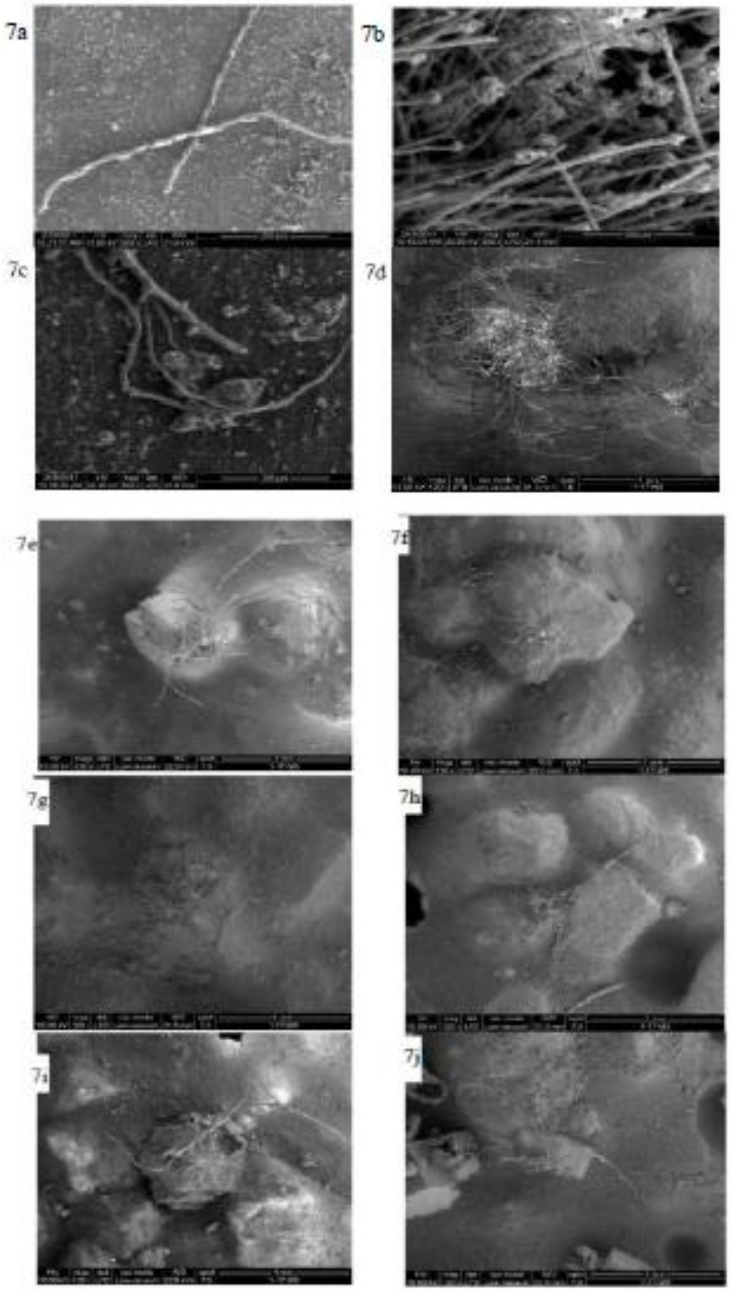

Figure 7. Growth of *A. niger* according to mortar additions observed under ESEM (7a: Growth on normal cement NC; 7b: Growth of moulds on cement with 5% filler NCLF5;7c: Growth on cement with 25% puzzolan NCP25; 7d: Growth on normal cement mortar NC; 7e: Growth on cement mortar with 5% filler NCLF5; 7f: Growth on cement with 10% filler NCLF10; 7g: Growth on cement with 15% filler NCLF15, 7h: Growth on cement mortar with 15 % puzzolan NCP 15; 7i: Growth on cement mortar with 20 % puzzolan NCP20; and 7j: Growth cement mortar with 25 % puzzolan NCP25).

At the analyzed percentages, the normal cement sample has the lowest porosity; the additional proportions in the studied mortars only cause a dilution effect. When the percentage of pore volume at the defined pore radius size interval is examined, another factor appears: normal cement (NC) has the highest proportion of big pores, whereas NCP25, normal cement with 25% puzzolan, has the highest percentage of small and very small pores. Their percentages for NCCF5, normal cement with 5% lime filler, are intermediate between the other two kinds of mixtures, except for the percentages of the total pore volume calculated for the pores between 1,000-10,000 Å.

However, the relevant pore size to consider regarding fungal colonization is the interval of 10,000 – 100,000 Å (1 – 10 μm), because the hyphae have a diameter of about 10 – 12 μm. NC has 2.2% against 0.9% of both NCLF5 and NCP25. In one hand, pore size distribution does not explain the differences of fungal growth between these two kinds of mixtures. In the other hand, NCLF5 has a good proportion of pores bigger than 10 μm, not measured by this method but observed in the ESEM images.

Concrete dams can be colonized by different organisms that can deteriorate them (Figg et al., 1986; Rosato and Traversa, 2000; Rosato, 2008). The kind of the organisms present and the extent of the damage will depend not only on the weather conditions, but also on the characteristics of the material. Wiktor et al. (2009) cultured moulds on mortar samples and described the mechanisms of biodeterioration. These mechanisms are common for fungi and lichens (Caneva et al., 2003; Traversa et al., 2000; Traversa et al., 2001). This culture method was adapted and in the preliminary study we observed growth differences according to different mortars used as substrate (Prunell et al., 2011). In the case of mortars with filler, the presence of bigger pores and the higher calcium concentration appears as favourable for the more intensive growth of the mould. As for the mortars with puzzolan addition, the mixture does not end the reaction at the age of study to close the porosity of the mortar at the used proportion, but the presence of calcium hydroxide enhances the growth of the mould, but in a clearly less intensive way than the mixture with filler.

Table 5. Variation of the percentage of the total volume for each pore radius size range (Microporosity analysis)

% of the total pore volume for pore radius size range (Å)	NC	NCLF5	NCP25
<10	23.0	24.3	30.2
10 – 100	43.5	43.7	42.7
100 – 1,000	25.9	21.7	18.3
1,000 – 10,000	5.4	9.4	6.9
10,000 – 100,000	2.2	0.9	0.9

CONCLUSION

It was postulated that the higher porosity of the samples with 5% lime filler allowed the fungi to grow more easily. However, this effect was not observed in the samples with higher lime filler percentage whereas the mortars with puzzolanic additions, having lesser porosity, were least colonized.

It must be mentioned that the hyphae had a smaller diameter when growing on mixtures with 15% lime filler or higher percentages of puzzolan (20% – 25%). When the microporosity is examined, the normal cement has a higher percentage of the pore volume in the 10,000 – 100,000 Å, pore radius range from 1 µm to 10 µm, the size where fungal hyphae (3 – 12 µm diameter) would fit. NCLF5 and NCP25 have the same percentage of the pore volume at that pore radius range.

Therefore, microporosity does not explain the difference in fungal growth between NCLF5 and NCP25. Anyway, NCLF5 has the highest percentage of the pore volume in the 1,000 – 10,000 Å pore radius ranging from 0.1 µm to 1 µm. It is possible that the smaller hyphae (3 µm diameter) have small tips that can get into these pores that are later disrupted as the hyphae grow.

Mixtures NCLF10, NCLF15, NCP15, and NCP20 have high calcium values whereas NCP25 has the lowest percentage. This may explain the difference of fungal growth between NCLF5 and NCP25, but do not account for the lesser growth of the mould on the other mixtures. However, since calcium salts have low solubility, a higher calcium proportion means that only small a part will be dissolved and the rest will be in excess. The concentration of calcium ions is an important factor for fungal growth, but only as long as there is soluble calcium and not an excess of calcium.

Therefore, as a recommendation, the porosity and the free calcium ions of the mortar should be kept as low as possible, especially when building a structure that will be permanently wetted, as it happens with dams. This can be achieved by a careful dosing of the mixture and by keeping the water – cement relationship to a minimum.

However, it is important to remember this will not prevent the growth of microorganisms, but delay their appearance.

Another fact to consider is that following the recommendations indicated above would mean lower maintenance costs required by a more compact structure, with the benefit of an increased durability.

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