ACCELERATED LABORATORY TEST OF BIODETERIORATION

The development of the accelerated laboratory test to study fungal biodeterioration of a cementitious matrix is described in this chapter. Five tests were performed before to define the best methodology. Each test was optimized as a result of the previous one. Therefore, results relating to each test are presented chronologically.

1. Test A

1.1. Set up of the experimental device

1.1.1. Experimental device

Boxes in polypropylene were used for the experimental device (Figure 45). The use of such boxes permits to keep a sterile environment inside it, as they are autoclavable. The experimental device chosen is inspired by the one used in test for wood preservatives involving wood destroying basidiomycetes (NF EN 113). The advantage is that the methodology described was already developed in our laboratory previously. Hence, the procedure was just adapted to our experimental conditions.

1.1.2. Nutrients brought

Stone surfaces will be colonized by micro-organisms if enough humidity and nutrients are available (Kussmaul et al, 1998). For the first test, the idea was that nutritive medium should be brought to trigger and enhance fungal development. The first choice was ported on liquid medium. By this way, the liquid solution could rise continuously by capillarity into the submerged specimens, bringing the humidity for fungal development.

1.1.3. Specimen weathering

Relating to specimens, freshly prepared hydrated cement pastes are characterized by a pH value of 12 or higher. Under such alkaline conditions most micro-organisms are unable to grow (Shirakawa et al, 2003). The progressive carbonation of hydrated cement paste leads to the neutralization of the alkalinity of the water in the matrix pores. This results in a decrease of the surface pH (cf. chap V), thus creating favourable conditions for microbial propagation (Shirakawa et al., 2003). At this stage of the study only carbonation was considered as weathering step. The specimens are sterilized by γ-radiation (30 kGy), which appears to be the most suitable way of sterilization.

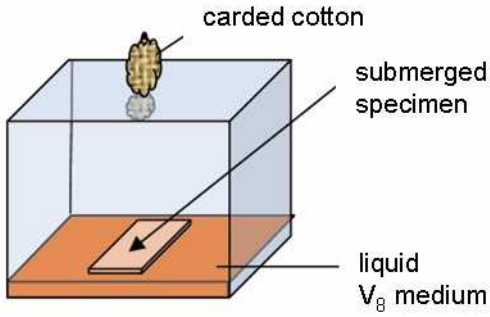


Figure 45: Experimental device developed for the 1st test

1.1.4. Inoculation

Inoculation is performed by means of spores' suspension spread on the whole specimen surface. This way of inoculation is the most widely reported in literature relating to laboratory tests of biodeterioration involving fungi (Oshima et al, 1999; Shirakawa et al, 2003; de Moraes Pinheiro et al., 2003; Urzì and De Leo, 2007). Therefore, experiments can be easily reproducible as the concentration inoculated is exactly known.

1.2. Observations

After seven days of incubation at 24±1°C and 90±5% RH, no fungal growth is noticed on the specimen surface and also in the liquid nutritive medium. Furthermore, the liquid medium became gel like.

From these first observations, it appears that the specimens should not be submerged in liquid medium. In this way, the RH imposed in the culture chamber plus the humidity provided by the presence of liquid medium may result in a too high RH inside the box. Moreover, the specimen may be too damp to trigger an optimal fungal development. These conditions are nearer from culture in liquid medium than one in solid medium. Therefore the experimental conditions withdrew from ones expected.

The first step of optimization will be to change the supply of nutrients: liquid medium will be replaced by solid one.

2. Test B

2.1. Optimization of the experimental device

For this second test, solid nutritive medium covers the box bottom as suggested in NF EN 113. When the spores' suspension is inoculated on the specimen, it will draw along sides and spill on the solid medium. Fungal development should be faster on the nutritive medium than on the specimen surface. Therefore, the presence of nutritive medium should trigger and enhance fungal growth.

Liquid nutritive medium is also spread on the specimen surface. Once it has been absorbed, the specimen is placed vertically in the box, as shown in Figure 46. The suspension of *Alternaria alternata*'s spores are inoculated on the upper face. This way of inoculation was chosen in order to promote the maximum spreading of spores on the specimen, on the two vertical larger sides.

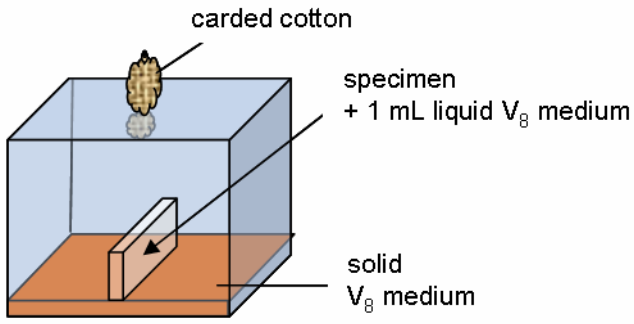


Figure 46: Experimental device for test B

2.2. Observations

After five days of incubation, no fungal growth is noticed on the specimens. Nevertheless, fungal development is observed on the nutritive medium (Figure 47), which indicates that spores inoculated are viable.

After two months of incubation, the entire solid nutritive medium is covered by *Alternaria alternata*. A progressive covering of the specimen was expected at the bottom from the fungal development on the solid nutritive medium, and on the upper face from the germination of spores inoculated. However, no fungal development is noted on the non weathered and the carbonated specimen. The dark colour observed on the specimen surface (Figure 47) corresponds to the spores inoculated and not at all to the fungal development.

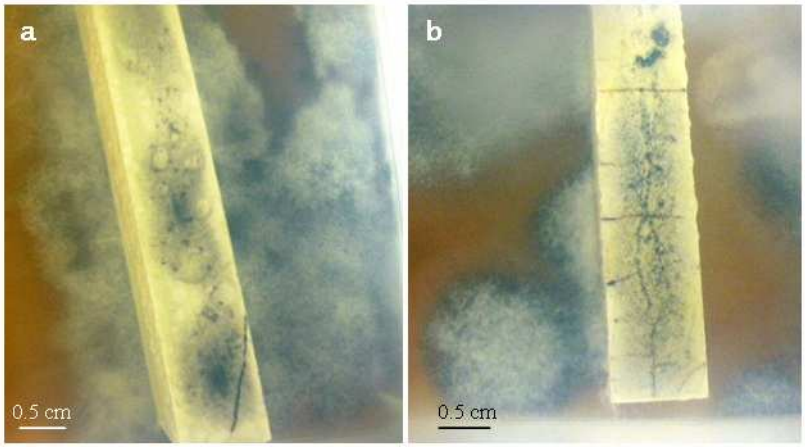


Figure 47: Observations of boxes after 5 days of incubation at 24±1°C and 90±5% RH – (a) Non weathered specimen, (b) carbonated specimen

The vertically disposition of the specimen doesn't seem to be the best choice. Spores are probably not enough spread on the surface. They remained agglomerated which does not promote their germination. Actually, spores inoculation on the larger side should be a better solution: spores could be shared out in a more homogeneous way.

The lack of fungal growth on the specimen could also be explained by another important fact; the surface pH of cement specimens is generally about 12-13. Shirakawa et al (2003) recorded no fungal growth on any of their mortar not exposed to accelerated carbonation. The pH values below or close to 9 allowed the colonisation of mortars by *Cladosporium sphaerospermum*, while pH close to or higher than 10, inhibited such growth (Shirakawa et al., 2003). In the present study, the carbonation operation leads to decrease of surface pH to a

value near 10 (cf chap V). This value remains too high to favour fungal growth. It is necessary to decrease this surface pH to allow the fungus to develop rapidly. With this aim in view, leaching operation was performed on carbonated specimens for the next test. Thus, surface pH of 8.8 was obtained (cf. chap V).

3. Test C

3.1. Optimization of the experimental device

This test is performed with specimens non weathered, carbonated and leached, and carbonated only. As previously, solid nutritive medium covers the box bottom, V_8 medium or PDA is used for *Alternaria alternata* or *Aspergillus niger* respectively (Figure 48).

Two specimens with the same weathering are placed into each box. Specimens are disposed on thin glass rod in order to avoid direct contact between the solid nutritive medium and specimens.

The way to brought nutrients is also changed. The specimens are soaked with liquid nutritive medium as suggested by Shirakawa et al. (2003). Therefore, nutrients are provided inside the matrix. By this way, water is available to support fungal growth and the fungus may also be forced to penetrate inside the matrix to develop. The question of the necessity to provide regularly nutrients also arises. Some developed tests bring nutrients during incubation (Oshima et al., 1999; Barberousse 2006), while some others don't (Sirakawa et al., 2003; De Moraes Pinheiro et al., 2003; Nielsen et al., 2004). We aim at developing an accelerated test, so optimum fungal development is needed. Therefore, the content of nutrients during the test shouldn't be a limiting parameter. On the other hand, the amount of nutrients available should not hinder interaction between fungi and the matrix.

In the present case, liquid nutritive medium is added on the specimen surface, one millilitre each week.

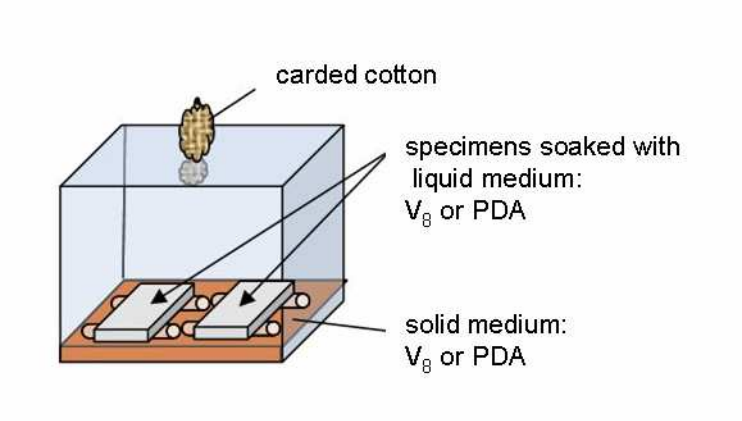


Figure 48: Experimental device for test C

3.2. Observations

Firstly, a rapid fungal development is noticed on the surface of the solid nutritive medium in the box, for *Alternaria alternata* and *Aspergillus niger*. The whole surface is covered after 10 days. This indicates that culture conditions inside the box are compatible with a rapid fungal growth. Nevertheless, no fungal development is observed on the specimen surface, even after two months of incubation. It appears that the experimental device is not optimum, but the way it doesn't is not clear: nutrients are provided to fungi, fungal growth is possible on the solid medium, and spores are visible at the specimen surface. Relating to the relative humidity, it is regulated inside the incubation chamber to 75 or 90±5% for *Aspergillus niger* or *Alternaria alternata* respectively. Inside the box, the RH appears to be suitable for fungal development as fungi grow on the solid nutritive medium.

Specimens with different weathering (non weathered, carbonated then leached, carbonated only) were inoculated. From these observations, no influence of the matrix weathering on the fungal development is noted. At this stage of the study, it could be assumed that fungal development is favoured on the solid medium to the detriment of the specimen surface, no matter the weathering of the matrix. Therefore, a solution to trigger fungal growth on the specimen surface must be determined.

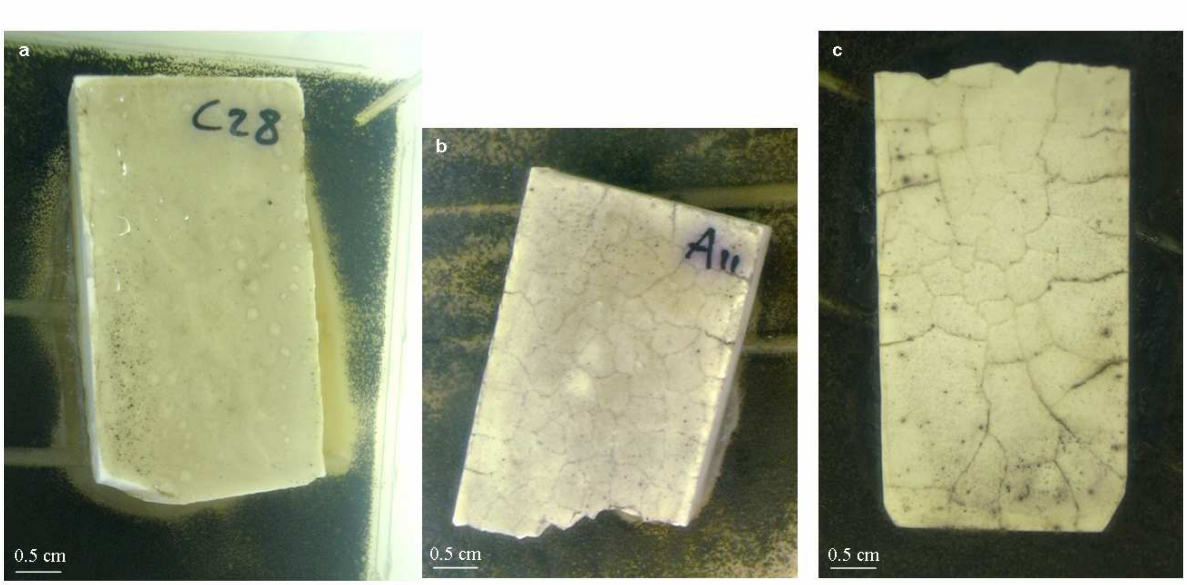


Figure 49: Observations of specimens inoculated with *Aspergillus niger* after 10 days of incubation at 24±1°C and 75±5% RH – (a) Non weathered specimen, (b) carbonated specimen, (c) carbonated then leached specimen

It seems difficult to work more on the matrix weathering to improve its bioreceptivity. Actually, chapter V points out the important matrix' alterations induced by the accelerated weathering (carbonation followed by leaching operation). The surface of carbonated then leached specimens is mainly composed by $CaCO_3$, which yet differs significantly from the initial composition of the matrix (non weathered specimen). Hence, we have first to determine or at least to estimate the real impact of this accelerated weathering on the matrix biodeterioration before to envisage a more extended matrix weathering. That is to say if:

- (i) the deterioration induced by fungal development can be observed and evaluated on the weathered specimens,
- (ii) or the structural and chemical modifications due to the accelerated weathering alters the matrix in such a way that biodeterioration couldn't be more deleterious to the matrix. In this case, while carbonation and leaching occurs in natural environment, the methodology of the accelerated weathering should be reconsidered.

For this reason, the way of the experimental device improvement focuses on the inoculation step rather than on the matrix weathering.

4. Test D

4.1. Optimization of the experimental device

The experimental methodology is similar to the previous one except the way the nutrients are provided. In the present case, specimens are not soaked with liquid medium, but covered by a thin layer of solid nutritive medium (Figure 50). For this, they are rapidly plunged in the solid medium still hot and let to dry under laminar flow hood before inoculation. The test is performed with *Alternaria alternata* and *Aspergillus niger*, on specimens non weathered, carbonated then leached, and carbonated only.

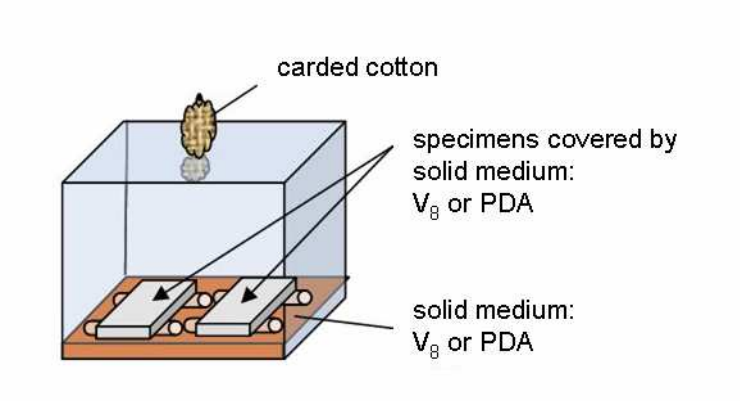


Figure 50: Experimental device for test D

4.2. Observations

As previously, after 10 days of incubation fungal development is noticed on the nutritive medium covering the box bottom for both strains. Now the specimens' surfaces are observed according to the weathering performed. For the non weathered ones, no fungal growth is noted no matter the strain inoculated. For the first time, fungal growth is observed after 10 days of incubation, only for one out of three carbonated specimens inoculated with *Aspergillus niger*. The most interesting point is that on all the carbonated then leached specimens *Alternaria alternata* and *Aspergillus niger*'s growth is observed (Figure 51, Figure 52).

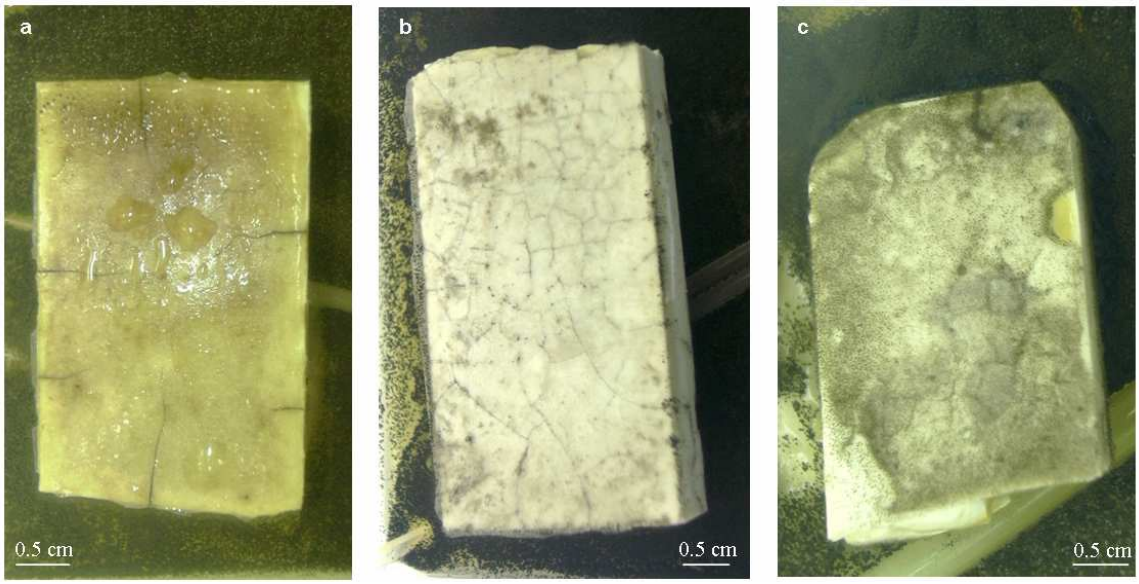


Figure 51: Observations of specimens inoculated with *Aspergillus niger* after 10 days of incubation at 24±1°C and 75±5% RH – (a) Non weathered specimen, (b) carbonated specimen, (c) carbonated then leached specimen

However, after 20 days of incubation, the fungal development observed at 10 days seems to have reached its maximum. After this period, no further growth is noted on the specimen surface.

Figure 52: Observations of carbonated and leached specimens inoculated with *Alternaria alternata* after 10 days of incubation at 24±1°C and 90±5% RH

After three months of incubation, specimens are removed from the box. They are observed with the stereomicroscope and then prepared for the SEM observations. Then a surprising observation was performed for one carbonated specimen for which no *Aspergillus niger* development was noted on the inoculated surface (top face). The bottom surface, separated

from the solid nutritive medium by glass rod was covered by *A.n* (Figure 53b). Nevertheless, this observation is valid just for this single specimen. Nothing comparable was noted for others and for the specimens inoculated with *Alternaria alternata*. Hence, this observation is hardly explainable. The following assumption can be proposed; although specimens are prepared all together, and the accelerated weathering is performed simultaneously on all of them, their chemical composition can change locally one from each others. The carbonation performed on the considered specimen may have led to a surface pH lower than the one generally noted for the other specimens. Therefore, this constitutes favourable condition for the fungal development.

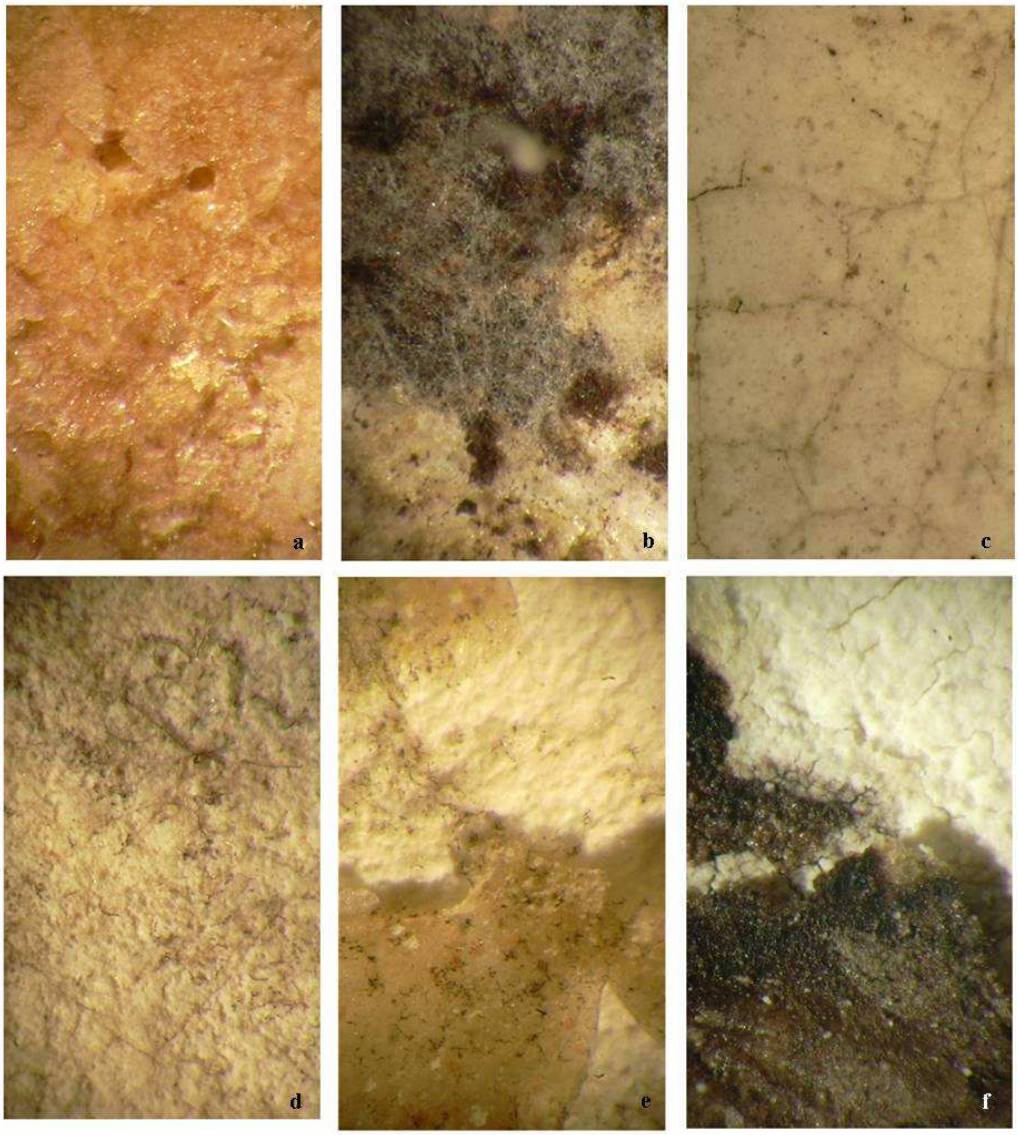


Figure 53: Observations with stereomicroscope (x 50) of the specimen surface after 3 months of incubation – (a), (d) non weathered, (b), (e) carbonated, and (c), (f) carbonated then leached specimen

Stereomicroscope observations show that on all specimens (with or without weathering, and no matter the fungal development observed) the film of agared medium adheres not completely to the specimen surface. The film permits to obtain fungal development on the specimen surface, but also seems to act as an interface or a barrier between fungi and the matrix surface, avoiding a direct interaction between fungi and matrix.

The results of visual and stereomicroscopy assessment of specimen were confirmed with scanning electron microscopy observations. Figure 54a shows spores' chain and hyphae of *Alternaria alternata* which confirms the fungal development previously observed. Actually, inoculation was performed by mean of spores' suspension, so when they are spread on the specimen surface they appear as single cell. Hyphae are the results of spores' germination, and chain of spores resulted from the sporulation. Both observations are due to the fungal development. This growth occurs only superficially on the film, without interfering with the matrix surface. Figure 54b leads to same observations: *Aspergillus niger* grew only on the film surface.

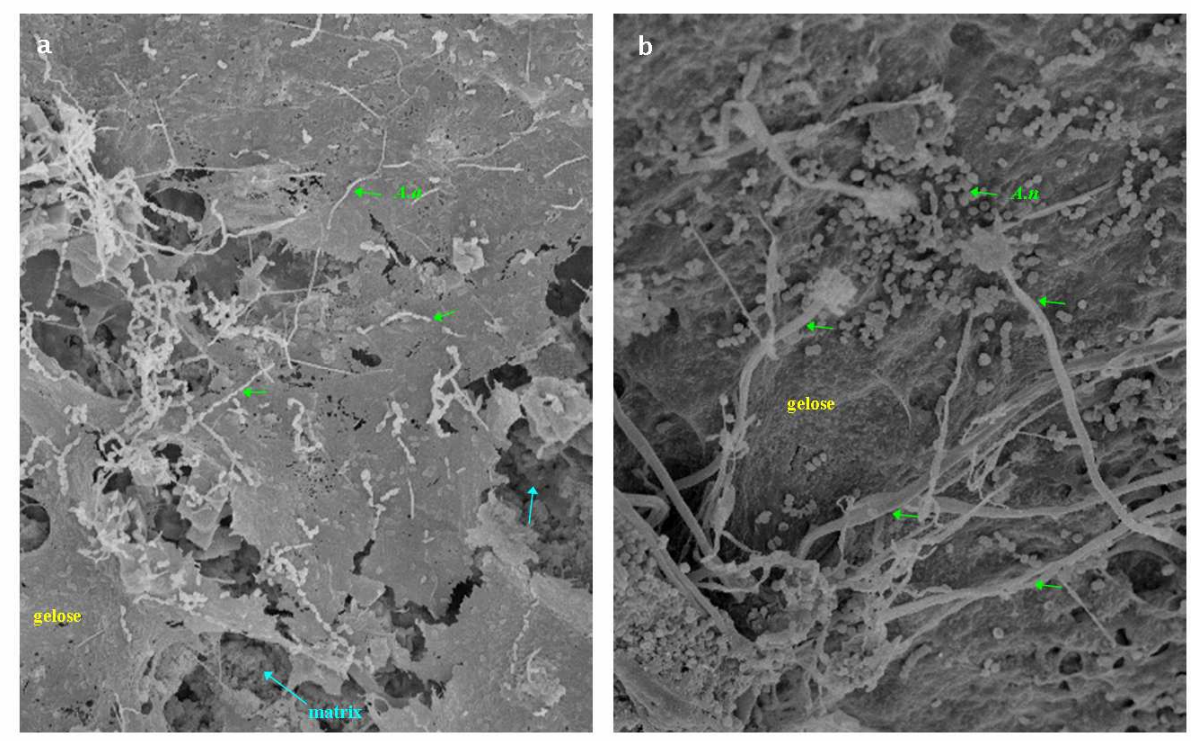


Figure 54: SEM pictures of specimen surface after 6 months of incubation for carbonated then leached specimen inoculated with (a) *Alternaria alternata*, and (b) *Aspergillus niger*

These results show that this methodology allows the fungal development to occur only on the film of solid agared medium and not at all on the surface specimen as it was expected. Nevertheless, it clearly appears that the accelerated weathering of the matrix acts upon the fungal development. Therefore, the fungal growth on the film is mainly observed with the carbonated then leached specimen that is to say to the lowest surface pH. It can be assumed that once the film of solid nutritive medium is in contact with the matrix surface, its pH is balanced with the surface pH of the matrix.

Although the methodology must be improved, it is concluded that the surface pH of the matrix is incontestably a major point to control for the development of the accelerated laboratory test of biodeterioration. This is consistent with results obtained by Shirakawa et al. (2003); they concluded that the degree of carbonation and pH values played a key role in the susceptibility of mortar to fungal colonization. Decrease of surface pH increases considerably matrix bioreciptivity. Carbonation is the most common chemical reaction influencing cement-based materials in natural environmental scenarios (Macias et al., 1997; Gervais et al., 2004) that's why accelerated weathering of matrix is generally performed by carbonation. This accelerated weathering leads to a pH to 8.5 after 120 days of curing (de Moraes Pinheiro et al., 2003) or to a value near 9 after 56 days of curing (Shirakawa et al., 2003). The experimental conditions and sample size should be taken in account for a comparison. Nevertheless, cement based materials are also exposed to the elements (humidity, acidic rain, snow…) which leads to cement compounds leaching (Barbieri Albert, 2002). In our case, carbonation is followed by leaching operation. This allows us to obtain a surface pH about 8.8 after 30 days, and also to have two ways of weathering involved in natural weathering of materials.

So the overall methodology of the test has to be rethought, in particular the inoculation step. This is crucial to obtain a fungal development on the matrix surface in order to study the interactions between fungi and the matrix. The preparation of the matrix does not seem to need further investigations for its improvement at this stage of the study. Besides, the development of the test should be now envisaged from a microbiology point of view. For this, investigations to improve the biodeterioration test are carried out in the department of Microbiology, Genetic and Molecular Sciences of the Sciences University of Messina (Sicily).

5. Test E

5.1. Improvement of the experimental device

5.1.1. Conditioning of boxes

The present test is developed in collaboration with Prof. Clara Urzì and Dr. Filomena De Leo. In the laboratory, incubator with temperature regulation only is available. Relative humidity can't be exactly adjusted. So the first modification performed on the experimental device is the use of vermiculite directly in the box (Figure 55). Keeping the vermiculite wet will bring constantly humidity necessary for fungal development inside the box. A sheet of paper separates the specimens from the vermiculite to avoid the direct contact. For sterilization, specimens are exposed overnight to UV lamp. Two specimens with the same weathering are set in each box.

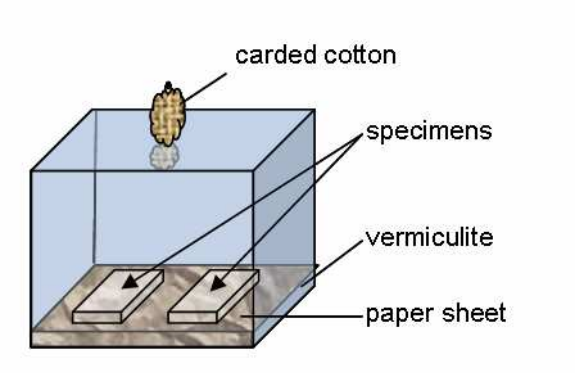


Figure 55: Experimental set-up for test E

5.1.2. Inoculation

Four fungal strains are studied in this test: *Alternaria alternata* (melanin producer hyphomycete), *Aspergillus niger* (acidogenic hyphomycete), *Exophiala* sp. (yeast-like fungus), *Coniosporium uncinatum* (meristematic fungus).

Inoculation is performed by mean of suspension of fungal units and not only spores suspension. Firstly because it would have no sense to talk about spores relating to meristematic and yeast like fungi as they don't sporulate. Then fungi can develop from spores but also from hyphal fragment. Therefore, relating to *Alternaria alternata* and *Aspergillus niger*, which sporulate, this way of inoculation should increase the potential of development.

5.1.3. Results

The results obtained are first described. They are presented following analytical techniques used: direct observation, stereomicroscopy, staining with periodic acid Schiff's reagent, scanning electron microscopy. They are then connected and compared to be discussed.

5.1.3.1.Direct observations

Observations are classified according to the weathering of the matrix.

- **Non weathered specimens**

Figure 56 presents direct observations of the non weathered specimens performed after four weeks of incubation. No microbial growth is noticed on all exposed specimens. *Coniosporium uncinatum* cells are dark pigmented and taller than those of other fungi inoculated. Hence, this results in the presence of black spots on the specimen. Moreover, when the suspension of cells is inoculated on the non weathered specimens, it does not penetrate immediately into the matrix. Thus, cells can agglomerate on the surface, resulting in observation of black spots. These spots are, in this case, absolutely not due to the fungal development.

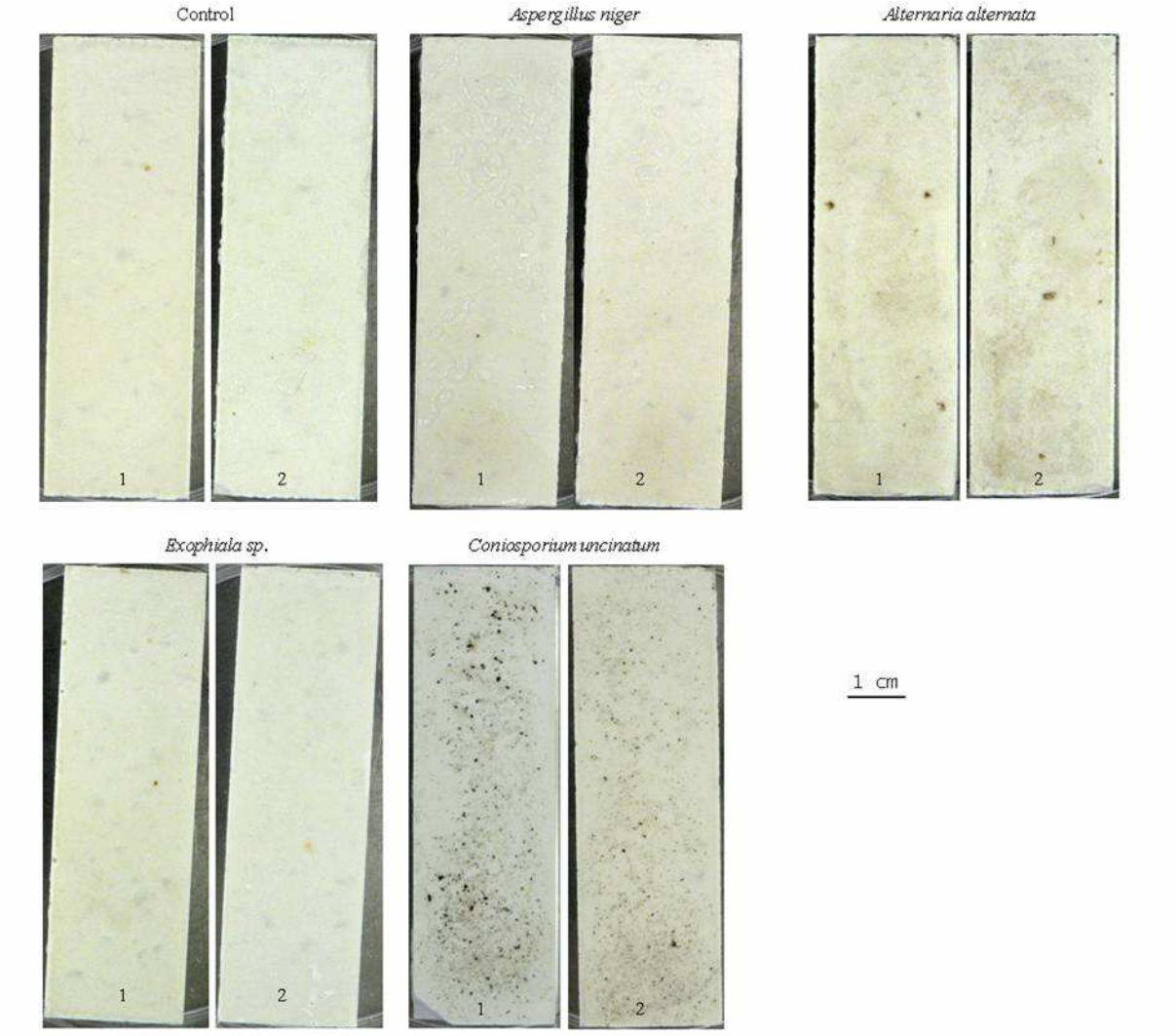


Figure 56: Direct observations of the non weathered specimens after 4 weeks of incubation

- **Carbonated specimens**

Figure 57 shows direct observations performed on the carbonated specimens just after inoculation (T0) and after four weeks of incubation (T4). No microbial growth is noted on the control and specimens inoculated with *Exophiala* sp., *Aspergillus niger*. A fungal development is observed on one specimen inoculated with *Coniosporium uncinatum*. It appears after three weeks of incubation and looks like small dark area of about 1cm diameter. Nevertheless, it doesn't seem to be characteristic of *Coniosporium uncinatum* development, but looks more like *Alternaria alternata*. While boxes are handled with lot of care, they remain very close during experiment, so a fungal contamination can easily occurs. This should be confirmed with microscopic observations. Interesting results are obtained with the specimens inoculated with *Alternaria alternata*. Therefore, a fungal development is noticed after one week only of incubation for one specimen. For the second specimen inoculated, it appears after three weeks of incubation. The fungus grows until the fourth week. It appears as dark area, scarcely spread on the specimen surface. Hydrated cement paste are heterogeneous material, and there are likely to exist micro-regions that could be prone to preferential fungal colonisation due to e.g. differences in the carbonation rate. Differences in specimen porosity would lead to different rates of CO_2, which in turn would produce varying degree of carbonation. This would result in differences in the pH values in the micro-niches, thus influencing fungal growth (Shirakawa et al., 2003). The fungal spreading may also reflect the distribution of fungal cells on the specimen surface after inoculation.

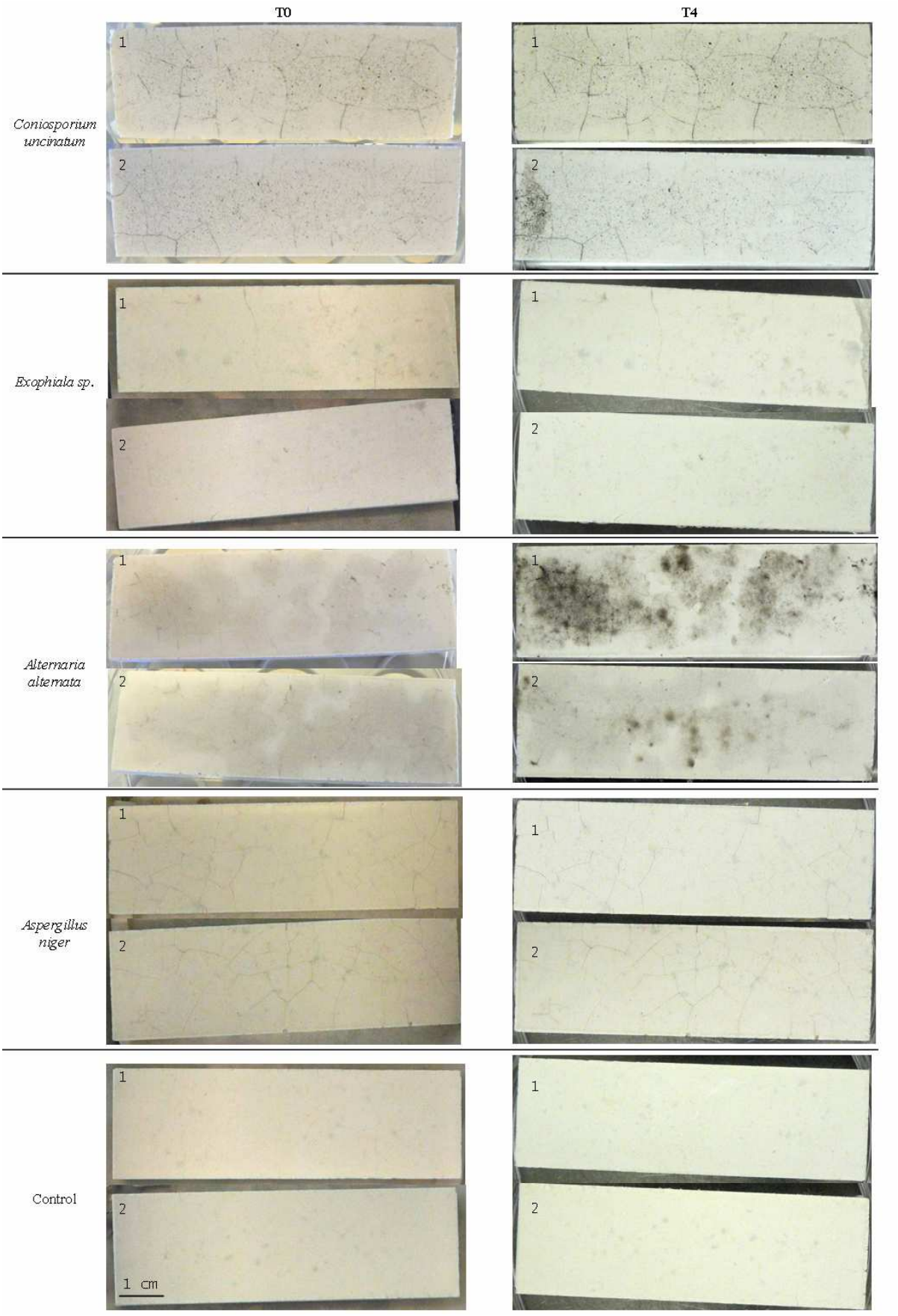


Figure 57: Direct observations of the carbonated specimens

- **Carbonated then leached specimens**

Figure 58 presents direct observations performed on the carbonated then leached specimens. Microbial development is noticed on all the specimens after one week of incubation only. For the specimens inoculated with *Aspergillus niger*, *Exophiala* sp. and surprisingly also controls, it appears as pink coloured spots which seem to grow along the specimen cracks. It is certainly due a bacterial contamination. Moreover, the specimens were inoculated in two series with one day of interval: firstly inoculation with *Aspergillus niger*, *Exophiala* sp., and controls were performed, then the day after with *Alternaria alternata* and *Coniosporium uncinatum*. The bacterial contamination occurred probably during the inoculation of the first series. Nevertheless, this contamination remains localized on restricted area and doesn't spread all over the surface.

Relating to controls, the bacterial contamination is not the only one. For one specimen, a small fungal development is noted after two weeks of incubation. It is characterized by the development of white mycelium on about 0.5 cm diameter.

For the specimens inoculated with *Aspergillus niger*, except the bacterial contamination, no microbial growth is observed.

Exophiala sp. growth is noticed after the first week on both specimens inoculated. The bacterial contamination doesn't seem to prevent the fungal development. The growth is more pronounced on the periphery area of the specimen surface.

Alternaria alternata development is observed since the first week of incubation. It is characterized first with mycelial development and then sporulation is observed. The growth appears more homogeneously than for the carbonated only specimens. Others fungal developments are noted, different from the *Alternaria alternata*'s one. It should be fungal contamination. Each week, specimens are not kept in sterile atmosphere during the stereomicroscope observations. While the time of contact with the not sterile air is a maximum shorten, a contamination is still possible.

The development of *Coniosporium uncinatum* is observed since the first week of incubation. It is characterized by the increase of the number and the size of the black spots. Mycelial growth is noted as the incubation time increase. As with *Alternaria alternata*, the specimens are certainly contaminated by some other fungi. Microscopic observations should inform us more precisely on the kind of fungi. The development seems to occur all over the surface, rather homogeneously.

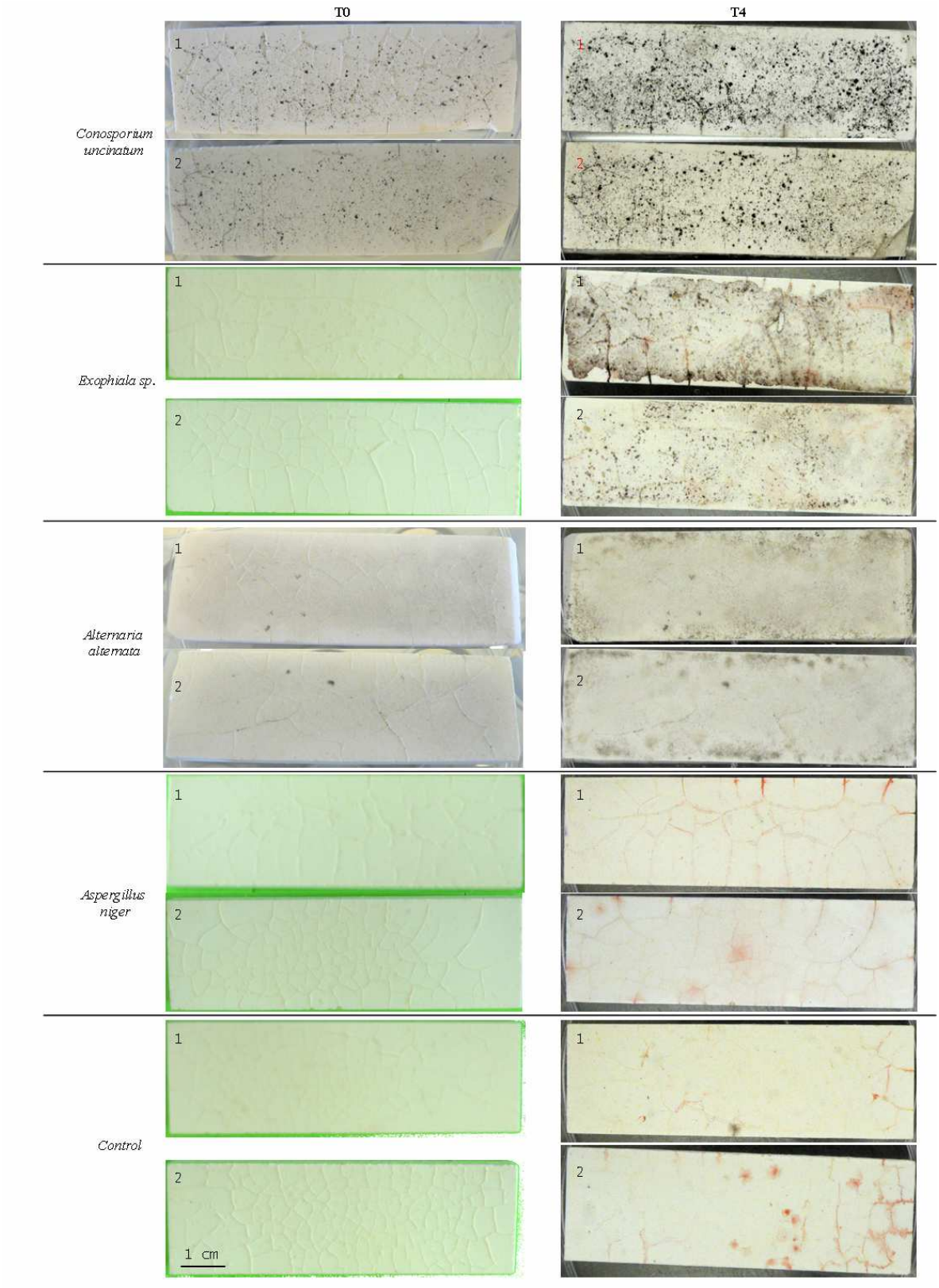


Figure 58: Direct observations of the carbonated then leached specimens – (T0) after inoculation, (T4) after four weeks of incubation

5.1.3.2.Stereomicroscope observations

The observations performed with stereomicroscopy confirm and complete the direct observations.

- **Non weathered specimen**

Figure 59 presents the stereomicroscopic observations performed on the non weathered specimens, just after inoculation (T0) and after four weeks of incubation (T4). The absence of microbial growth is confirmed for all the non weathered specimens. Spores and fungal cells are visible on the specimen surfaces. Moreover, no fungal development is observed on the paper sheet on which the specimens are disposed in the box. Therefore, the assumption that the suspension could flow away along the sides during inoculation can reasonably be ruled out.

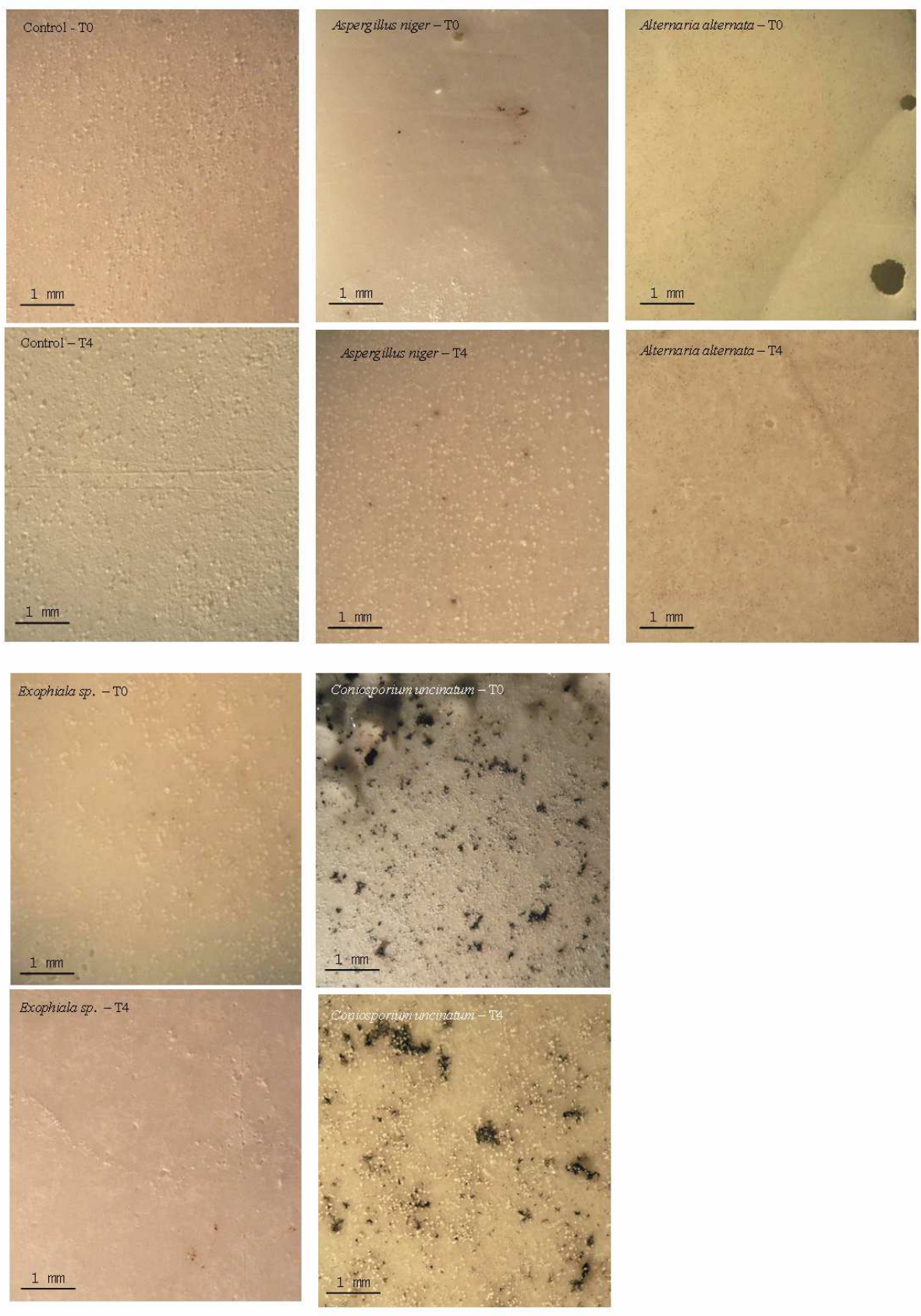


Figure 59: Observation with stereomicroscope of the non weathered specimens – T0: just after inoculation; T4: after 4 weeks of incubation

- **Carbonated specimens**

Figure 60 shows observations of the carbonated specimens performed with the stereomicroscope. Fungal contamination is observed at the third week of incubation on one carbonated specimen inoculated with *Exophiala* sp. . It appears as white mycelium localized on surface of about 0.5 cm diameter.

The fungal development noticed on the specimen inoculated with *Coniosporium uncinatum*, is the result of a contamination. The spores are produced in chain, and are very similar to those of *Alternaria alternata* (Figure 60l). Moreover, both fungi (*Coniosporium uncinatum* and *Alternaria alternata*) were inoculated in the same time. Theses considerations linked at the closeness of boxes during the observations, lead to think that the contamination is certainly due to *Alternaria alternata*.

Relating to the specimens inoculated with *Alternaria alternata*, the growth is characterized by mycelial development and production of spores (Figure 60j). Nevertheless, the observations performed after four weeks of incubation show that the fungal development is not exclusively due to *Alternaria* growth. Therefore, spores which differ in shape and grouping from those of *Alternaria alternata* are noticed on the specimen surface (Figure 60k). Their development remained localized in restricted area and do not prevent *Alternaria alternata* growth. However, these strains were not identified.

As previously, fungal cells and spores are observed on the specimen surface for which no fungal growth was noticed. Furthermore, fungal development was also noted in some cases. So the absence of fungal growth can’t be explained by a possible too low suspension concentration.

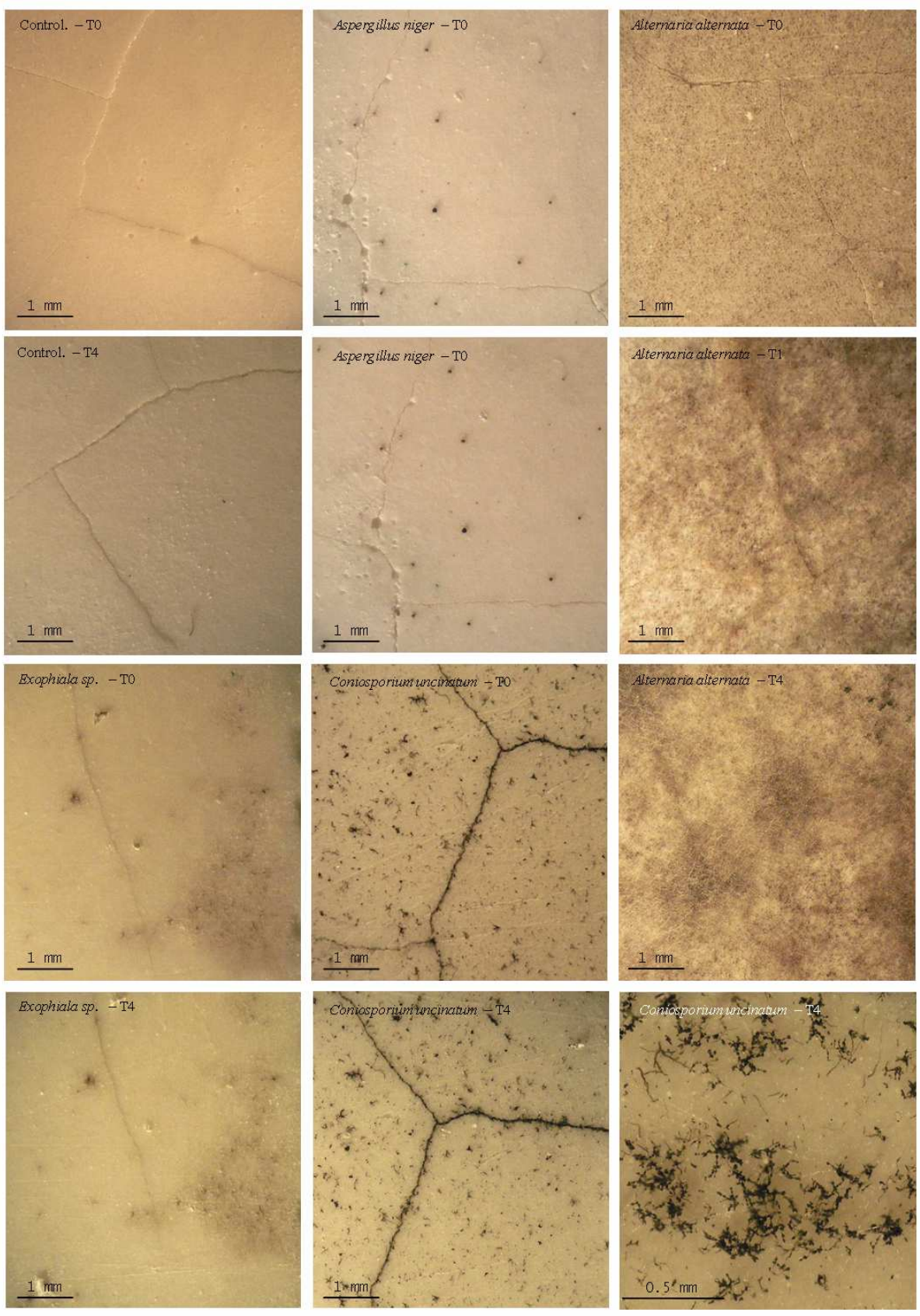


Figure 60: Observations performed with stereomicroscope of the carbonated specimens – (T0) just after inoculation, (T1) after one week of incubation, (T4) after four weeks of incubation

- **Carbonated then leached specimens**

Figure 61 presents the stereomicroscopic observations performed on the carbonated then leached specimens after inoculation and after four weeks of incubation. The pink coloured contamination observed for the controls and the specimens inoculated with *Aspergillus niger* has creamy like aspect which comforts the assumption of bacterial contamination. Moreover, this contamination seems to grow along the cracks (Figure 61c, Figure 61g, Figure 61h). After two weeks of incubation, fungal contamination is also noticed for the control, appearing as white mycelium localized on small area (Figure 61d). These observations point out the inefficiency of the sterilization with exposition to UV lamp.

Relating to the specimens inoculated with *Alternaria alternata*, the growth is observed since the first week of incubation. It develops more mycelium and produces fewer spores than on the carbonated only specimen. Moreover, after four weeks of incubation; fungal contamination is noticed (Figure 61l).

Exophiala sp. growth is not spread all over the surface but rather occurs from numerous spots. It seems characteristic of the yeast like development. A fungal contamination, the same than the one observed for the carbonated specimens is also noticed.

A homogeneous growth of *Coniosporium uncinatum* is observed on the specimen surface after one week of incubation. White mycelium and spores are also noticed. They are certainly due to a fungal contamination. Moreover, among them, some spores very similar in size and shape to those of *Alternaria alternata* are noted (Figure 61t). As previously mentioned the contaminations noted don’t seem to prevent the fungal growth of the inoculated strain.

These observations show that the fungal development appears to be favoured as the pH surface decreases. pH values seem to play a key role in the susceptibility of mortars to fungal colonisation (Shirakawa et al., 2003).

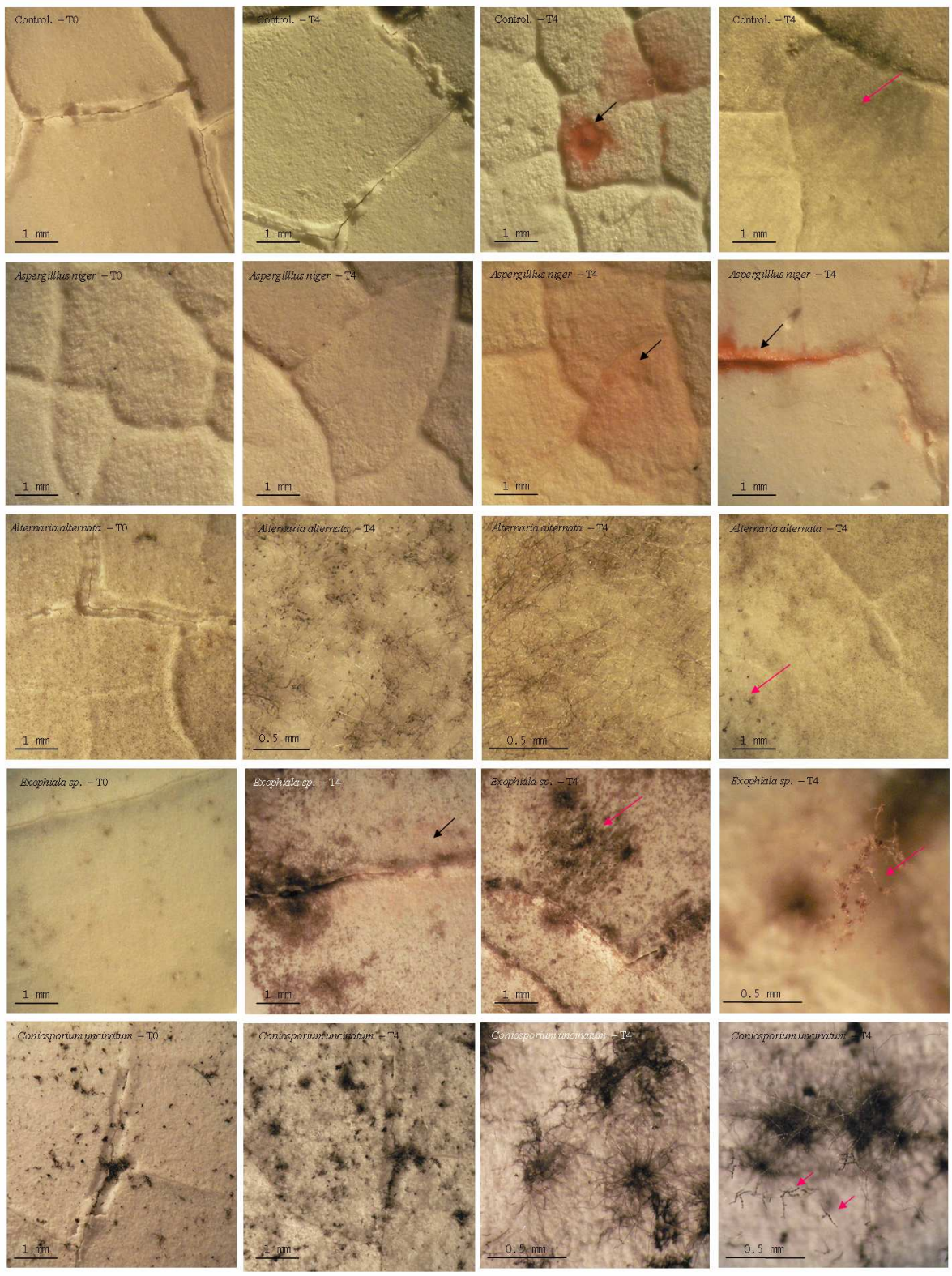


Figure 61: Stereomicroscopic observation performed on the carbonated then leached specimens – black arrows point out the assumed bacterial contamination; pink arrows the fungal one

5.1.3.3.Periodic acid Schiff staining

The staining with the Schiff's reagent permits estimation to the extent of the microbial colonisation on and within the matrix. Therefore, it completes the visual and stereomicroscopic observations which give information only on the presence of microbial growth on the specimen surface.

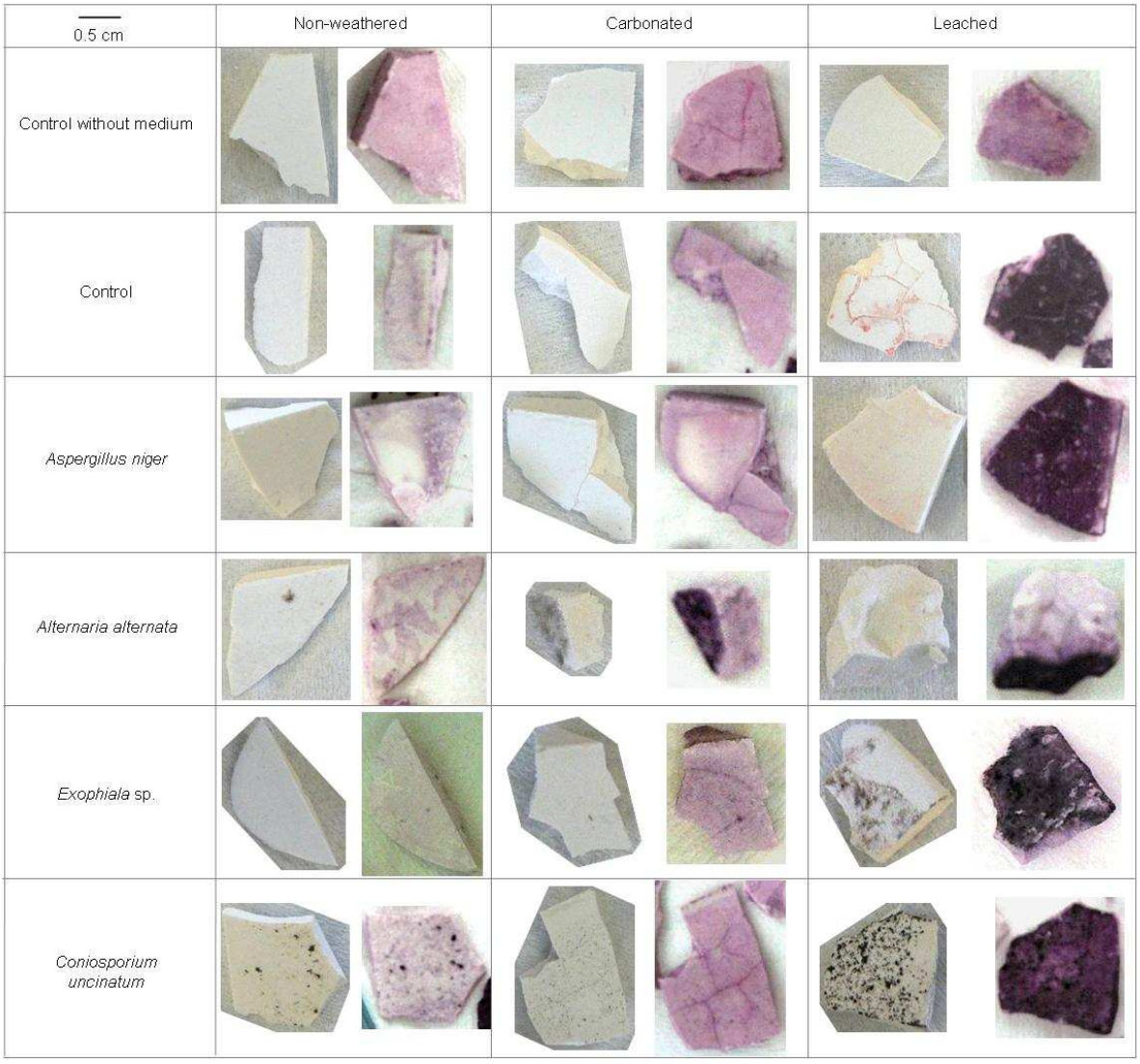


Figure 62: Observations with stereomicroscope of samples before (left pictures) and after (right picture) PAS staining

First, the PAS is performed on the non-incubated specimens. That is to say on specimens which were neither inoculated with fungi nor medium only. These specimens are named "control without medium". This stage should be performed to be sure that nutritive medium doesn't react with the Schiff's reagent, and also to confirm that the staining is independent

from weathering status of the specimen. Therefore, it might be assumed that as the weathering changes the porosity and the chemical composition of the matrix it could result in a different staining in the altered area.

The staining with Periodic Acid Schiff's reagent provides interesting results. They are presented on Figure 62. The staining performed on control without medium shows the coloration of samples without microbial growth, and without eventual interactions with nutritive medium. In this case, all samples (non-weathered, carbonated, carbonated then leached) appear pale purple coloured (Figure 62). Now, comparing these results with those obtained for control (with medium) and the non-weathered specimens, the same pale purple colour is noticed.

For the carbonated specimens, deep purple coloration is observed only on the sample surface exposed to *Alternaria alternata*.

For carbonated then leached specimens, all samples are coloured in deep purple in homogeneous way all over the exposed surfaces. This confirms previous observations, namely that leached specimens were subjected to microbial colonisation. The PAS staining allows a better visualization of microbial development extent: from stereomicroscopical observations for control and specimens inoculated with *Aspergillus niger*, pinkish contamination only on restricted areas on the specimen surface was noted. Whereas, the PAS staining reveals that the micro-organisms grew on the whole surface. Moreover, observations of cross section (Figure 63, Figure 64) show that microbial colonisation also penetrates within the first micrometers of the matrix.

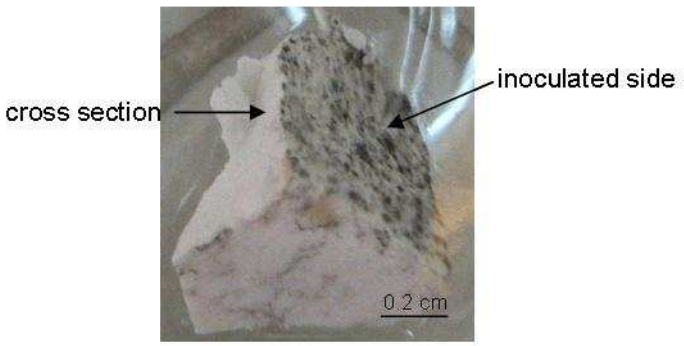


Figure 63: Location on the specimen of the different side observed

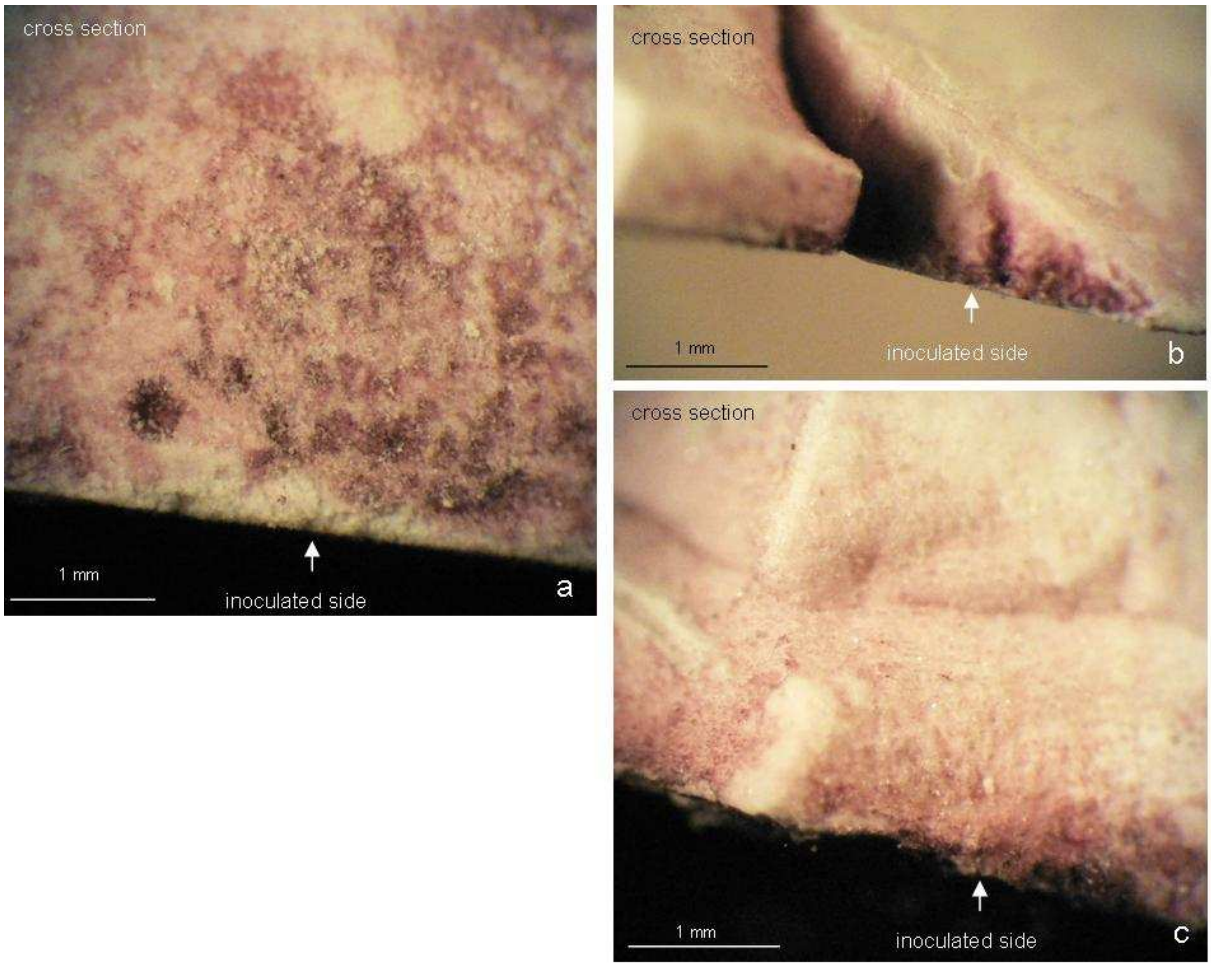


Figure 64: Observations with stereomicroscope of cross-sections details of carbonated then leached specimen after the PAS staining – (a) inoculated with *Aspergillus niger*, (b) control, (c) inoculated with *Coniosporium uncinatum*

5.1.3.4. Scanning Electron Microscopic (SEM) analyses

The SEM observations of the surface and cross section of specimens were performed in order to study interactions between microbes and the matrix. The pictures corresponding to the specimens for whom a microbial development was previously noticed are presented, except for the control without medium. Briefly, specimens are fixed in glutaraldehyde solution and then dehydrated in alcohol.

- **Controls without medium**

Controls without medium are also sampling and prepared for the SEM. Therefore, the fixation of samples for SEM analyses involves chemical reactions to fix the biological material.

During this fixation step, the matrix itself may react and be modified by the chemical solution. Figure 65 presents pictures of the surface of the controls without medium.

Observations for the carbonated then leached specimens are presented. Those corresponding to the non weathered and the carbonated only specimens were also performed and lead to the same conclusions.

Crystals are noticed on the sample surface. They look like very thin plates clustered in rosettes, fairly crystalline. This precipitation certainly occurred during the sample preparation.

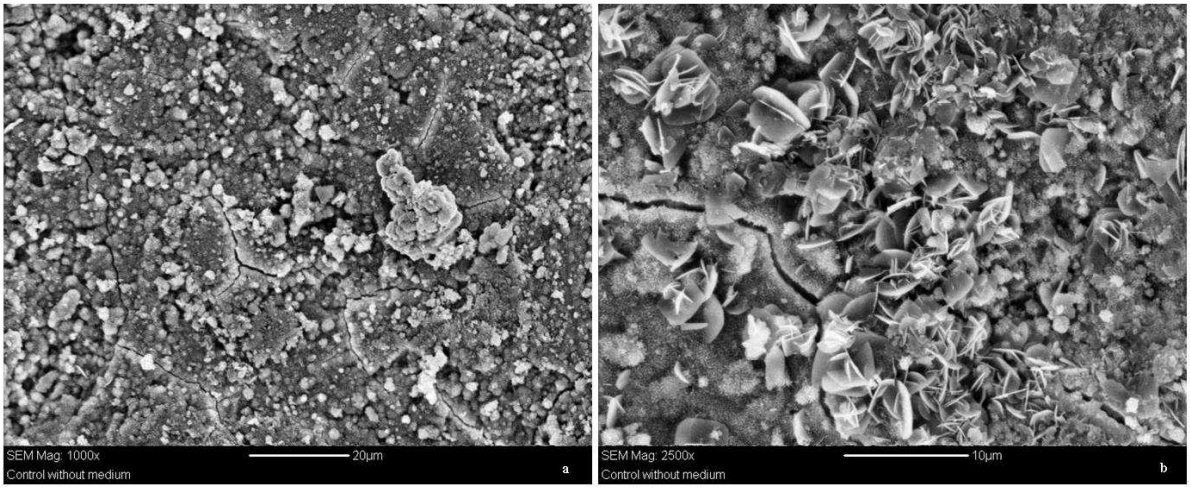


Figure 65: SEM observations of the carbonated then leached control without medium – (a) and (b) surface observations

- **Controls**

Examination of the sample surface does not give any information about possible microbial growth (Figure 66a). All compounds observed seemed to belong to the matrix.

Characteristic shapes of bacteria are noticed on the examinations of the cross-sections. They are indicated by the pink arrows on pictures (Figure 66b). Nevertheless, observations with higher magnification and with FEG show that these long cylindrical shapes seemed to be covered by numerous little grains (Figure 66c, Figure 66d). In addition, the chemical analyse shows the presence of Ca, Si, and Al. It can be assumed that these shapes could belong to the matrix, namely ettringite covered by aragonite layer. Hence, ettringite crystals are usually elongated particles, sometimes roughly circular in cross section, and sometimes bounded by plane surfaces, which are hollow or partly hollow (de Moraes Pinheiro et al., 2003).

On the other hand, the chemical analyse performed directly on mycelium of one sample for which fungal development was observed shows the presence of Ca, Si, Al, Cl, Ag and Cu,

and not of C. The question that comes to mind is if the chemical analyse is enough precise to examine sample as punctually as we except.

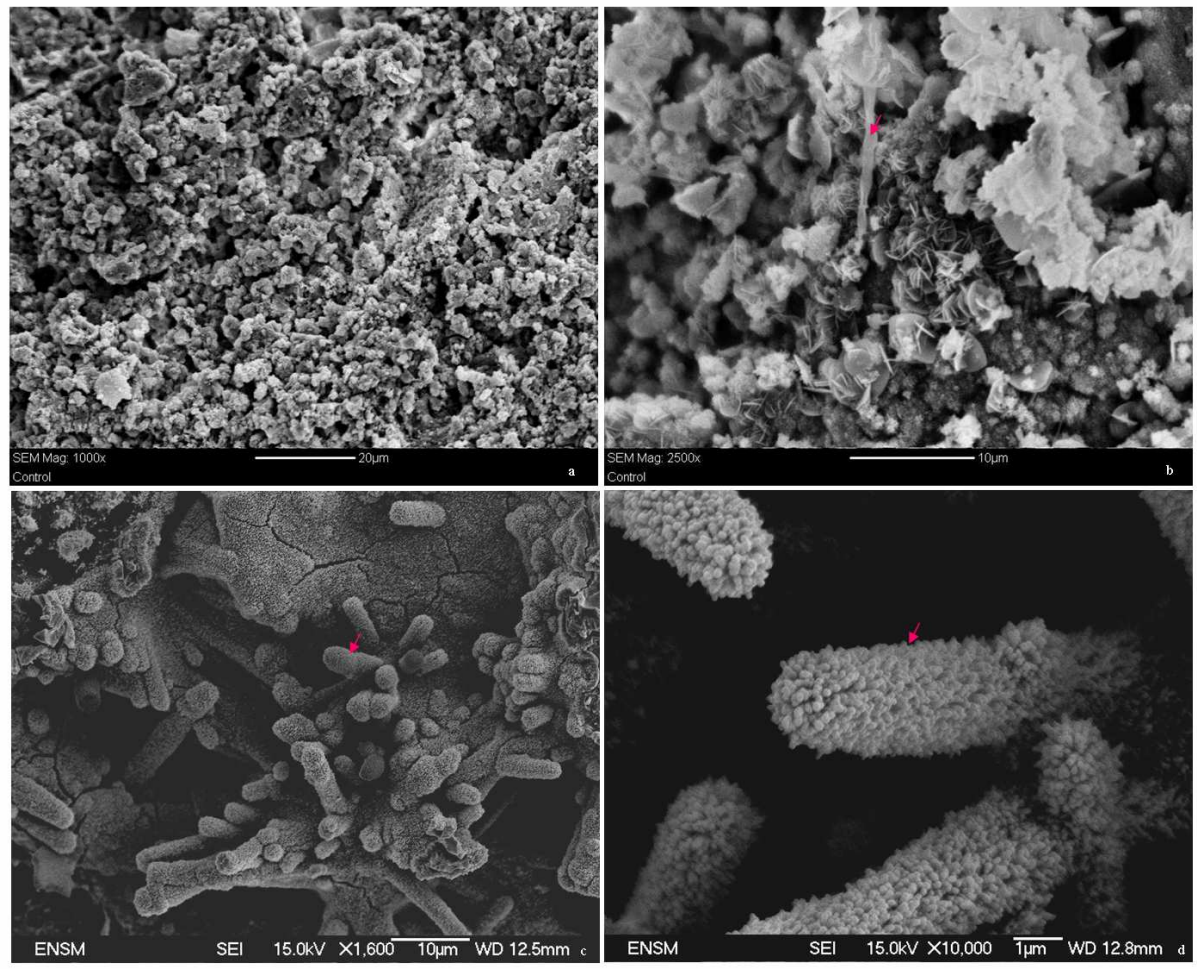


Figure 66: SEM observations of the carbonated then leached controls – (a) surface observation; (b), (c) and (d) observation of the cross-section

The presence of bacteria inside the matrix may also confirm the microbial development pointed out with the PAS staining. According to the information available, it seems difficult to conclude about the nature of the observed shapes, both assumptions could be possible.

Moreover, Figure 66b and Figure 66 c, Figure 66d were performed on the same specimen but not the same day and not with the same SEM. Close examination of these pictures lead to think that shapes are very similar but not correspond to the same area on the specimen and so not refer necessary to the same element. In the first case, Figure 66b, it is probably bacteria. But, on Figure 66c, Figure 66d the shapes seem to be slightly taller, and so could rather belong to the matrix element.

- **Specimens inoculated with *Aspergillus niger***

Cylindrical shapes typical of those of bacteria are observed on the sample surface (Figure 67a, Figure 67c, Figure 67d). Contrary to observations of the controls, these cylindrical shapes don't seem to be covered by any layer. Moreover, examination of the surface shows areas for which no microbial development is apparent (Figure 67b).

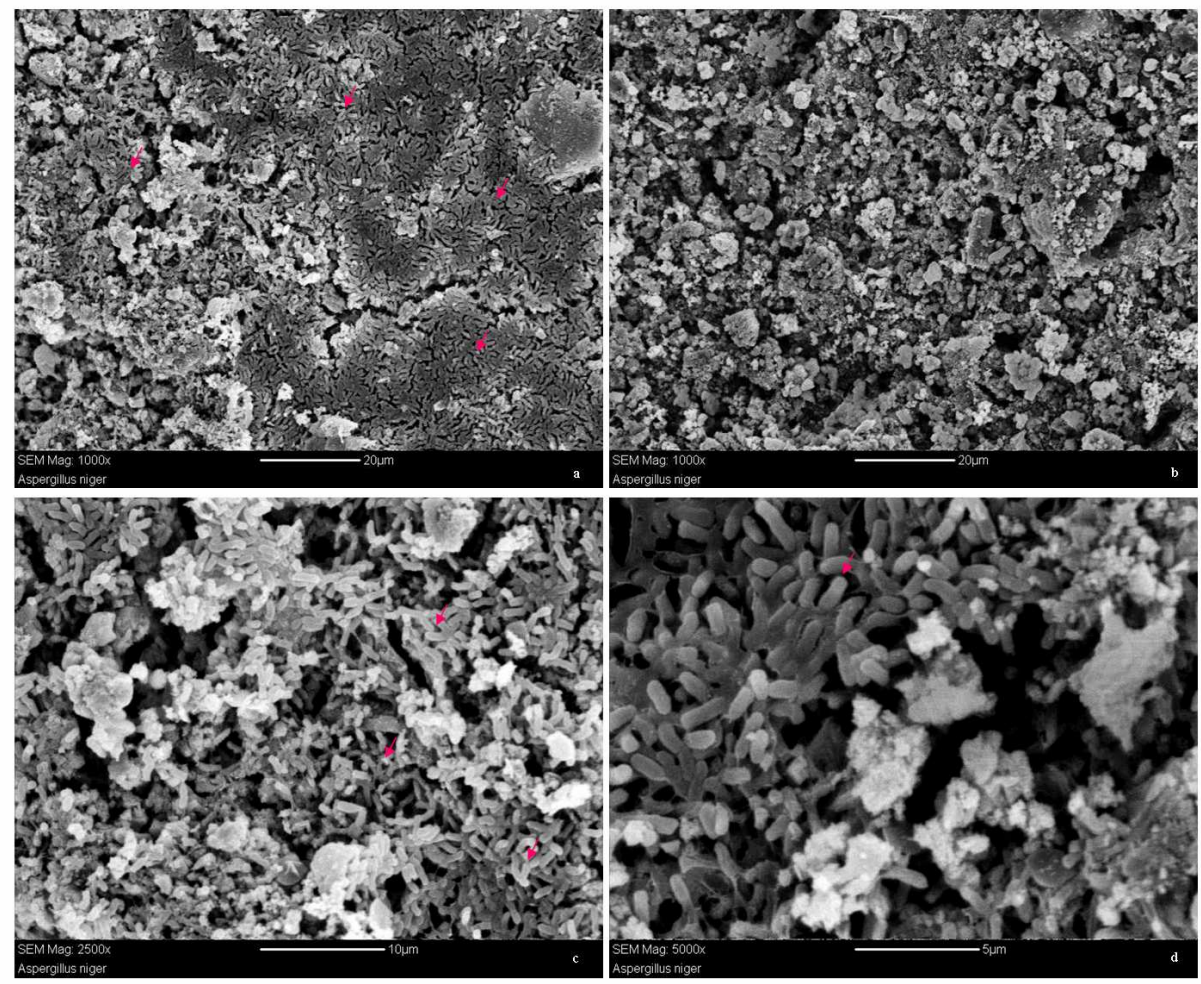


Figure 67: SEM observations of the carbonated then leached specimens inoculated with *Aspergillus niger* - (a), (b), (c) and (d) surface observation

The development seems to occur on various areas on the surface exposed, which may confirm stereomicroscopic observations and PAS staining. Nevertheless, no bacteria shapes are observed on the cross-section.

- **Specimens inoculated with *Alternaria alternata***

For both samples, carbonated then leached and carbonated only, numerous hyphae are noticed on the surface (Figure 68a, Figure 68b). Crystals, plates like, resulting probably from the sample preparation, are noted on the specimen surface.

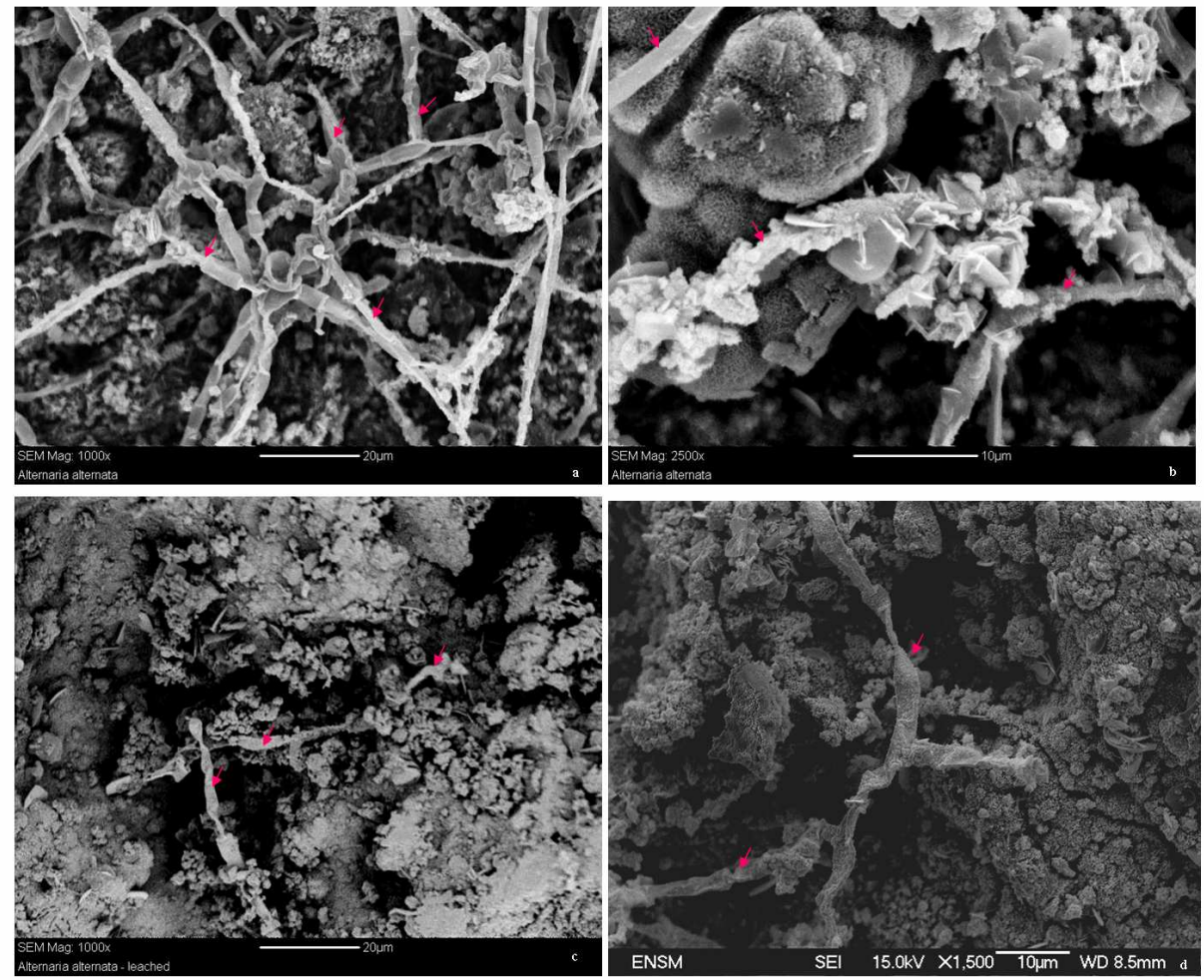


Figure 68: SEM observations of specimens inoculated with *Alternaria alternata* – (a), (b) surface observation of the carbonated specimen; (c) observation of the cross section of the carbonated then leached specimen, (d) observation of the cross-section of the carbonated specimen

Observations of the cross-section show also hyphae but in a more dispersed way (Figure 68c, Figure 68d). It is explained by the fact that fungi can penetrate inside the matrix only by the open porosity, provided the pore diameter is large enough, or through the existing cracks. Therefore, the space available for the fungal growth inside the matrix does not permit extensive development. Nevertheless, the resulting biodeterioration could be more intense inside the exposed material than on the surface.

- **Specimens inoculated with *Exophiala* sp.**

Hyphae are observed on the specimen surface (Figure 69a, Figure 69b). They are not homogeneously spread all over the surface, but grouped in clusters. They seem to be closely linked to the matrix. Moreover, some spherical shapes are observed very close to hyphae (Figure 69b), which lead us to think that it could be spores. It could be in this case, a fungal contamination. In addition, cylindrical shapes are also observed near these hyphae and spores, which could reflect a bacterial contamination. These assumptions are consistent with previous stereomicroscopic observations.

Zones on the sample surface don't seem to be colonised neither by bacteria nor fungi (Figure 69c)

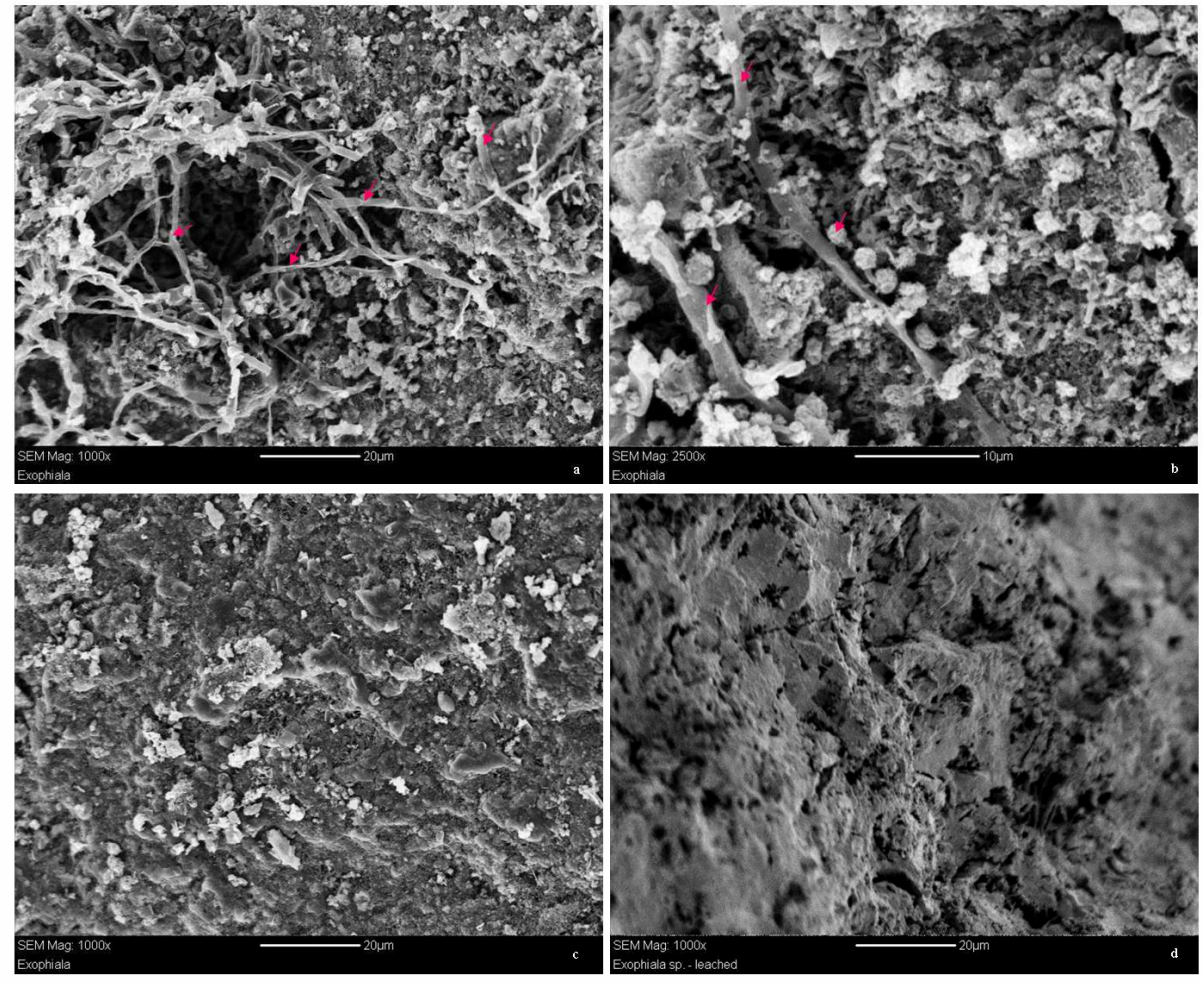


Figure 69: SEM observations of the carbonated then leached specimens inoculated with *Exophiala* sp. – (a), (b), (d) surface observation; (d) observation of the cross-section

Nevertheless, no microbial element is observed on the examination of the cross-sections (Figure 69d). This observation is a little bit surprising. Hence, from the surface examination it looks like the fungus doesn't develop by spreading all over the surface, but agglomerates in spots. It could be thinking that the fungus wants to penetrate deeper into the matrix to search nutrients and develop inside it.

- **Specimens inoculated with *Coniosporium uncinatum***

SEM pictures of the carbonated then leached specimens inoculated with *Coniosporium uncinatum* provide interesting information. The surface observations exhibit the hyphae spread on all over the surface (Figure 70a, Figure 70b). Moreover, hyphae are closely linked to the matrix, and even seem to penetrate inside it.

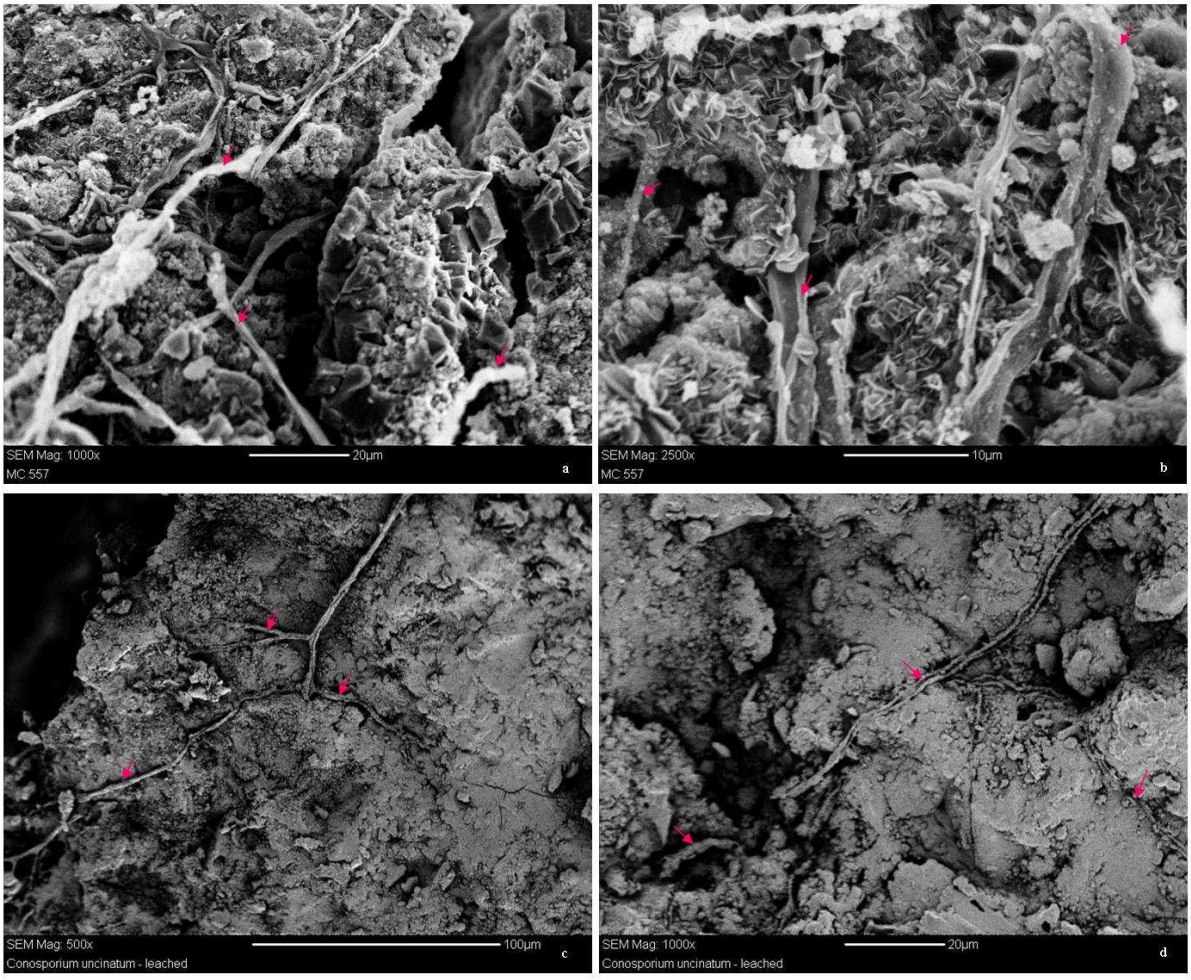


Figure 70: SEM observations of the carbonated then leached specimens inoculated with *Coniosporium uncinatum* – (a), (b) surface observation; (c) and (d) observation of the cross-section

Examinations of the cross-section clearly show hyphal penetration into the matrix through cracks. It points out the capacity of the fungi to develop not only superficially but also inside the matrix, via structural default, accentuating the physical deterioration.

5.1.4. Discussion

Results about microbial development according to the weathering of the matrix are summarized in Table 7.

Table 7: Observations and evolution of microbial growth on specimen surfaces during 4 weeks of accelerated biodeterioration test

		unweathered				
		control	*Asp. niger*	*Exophiala*	*Alt. alt*	*C. uncinatum*
fungal growth	1st week	-	-	-	-	-
	2nd week	-	-	-	-	-
	3rd week	-	-	-	-	-
	4th week	-	-	-	-	-
contamination		-	-	-	-	-

		carbonated				
		control	*Asp. niger*	*Exophiala*	*Alt. alt*	*C. uncinatum*
fungal growth	1st week	-	-	-	+	-
	2nd week	-	-	-	++	+
	3rd week	-	-	+	+++	++
	4th week	-	-	+	++++	+++
contamination		-	-	+	+	+

		carbonated and leached				
		control	*Asp. niger*	*Exophiala*	*Alt. alt*	*C. uncinatum*
fungal growth	1st week	-	-	+	++	+
	2nd week	-	-	++	+++	++
	3rd week	-	-	+++	++++	+++
	4th week	-	-	+++	+++++	++++
contamination		++	+	+	++	++

Asp. niger = *Aspergillus niger* *Alt. alt* = *Alternaria alternata*

Exophiala = *Exophiala* sp. *C. uncinatum* = *Coniosporium uncinatum*

The results point out that in the present study the microbial growth is promoted with the accelerated weathering of the matrix. It plays a major role. No microbial growth is noticed on non-weathered specimens (Table 7). These observations were also made by Shirakawa et al. (2003). Decrease of surface pH increases considerably matrix bioreciptivity. The microbial colonisation is observed on some carbonated specimens and on all carbonated then leached specimens. Carbonation is the most common chemical reaction influencing cement-based materials in natural environmental scenarios (Macias et al., 1997; Gervais et al., 2004). This is the reason why accelerated weathering of matrix is generally performed by carbonation (Dubosc, 2000, Shirakawa et al., 2003; de Moraes Pinheiros et al., 2003). But cement based materials are also exposed to the elements (humidity, acidic rain, snow…) which leads to cement compounds leaching (Barbieri Albert, 2002). In our case, carbonation is followed by leaching operation. This allows to obtain a surface pH about 8.8 after 30 days, but also to have two ways of weathering involved in natural weathering of materials. Beside the pH decrease, the accelerated weathering results in the fissure formation all over the surface. This allows the fungi to penetrate and develop inside the matrix.
Physical weathering of a mineral substrate acts to enhance or accelerate rates of chemical, biomechanical and biochemical weathering and *vice versa*. The development of cracks, fissures and weathering rinds in rocks accelerates biological weathering by providing a niche that can easily be exploited by opportunistic micro-organisms (Burford et al., 2003).

The presence of *Alternaria alternata* and *Coniosporium uncinatum*, two melanin producers, was noted on the SEM observations of the cross-sections. Melanin pigmentation of rock-inhabiting fungi confers extra-mechanical strength to the hyphae that are then better able to grow into crevices (Dornieden et al., 1997; Sterflinger and Krumbein, 1997; Gorbushina, 2007). Increased penetration augments contact of fungal hyphae and their metabolites with the rock, amplifying their geochemical influence on the mineral substrate. Expansion of hyphal growth into deeper rock layers also helps to protect the cells from the UV radiation (Gorbushina, 2007). One characteristic of meristematic fungi is the ability to form filamentous hyphae that develop from clump-like colonies to penetrate deep into rocks thus protecting themselves from environmental stresses. In this sense, the visible portion of melanized micro colonial fungi is like the tip-of-the-iceberg, because the hyphae can rapidly penetrate several mm to cm into hard rocks in search of more protected environments (Gorbushina, 2007). The fungi form explorative hyphae to seek nutrients on the surface, or mycelium during the growth. Under natural conditions, nutrient availability, even if small,

enhances the chance of fungal units (single cells, conidia, or hyphae fragments) surviving and starting the colonization (Urzì et al., 2001a, Urzì and De Leo, 2007).

The presence of contaminations is noticed on all the leached specimens. This underlines the importance of matrix sterilisation for the test. Sterilisation by exposition to UV is not a sufficient way, the better solution for future test is to sterilise specimens by ionisation with γ-radiations.

Aspergillus niger doesn't develop as it was expected. It must be noticed that only this strain didn't come from site sampling. It underlines a major point: presence of a strain on a monument doesn't obligatory mean it is source of the deterioration. Although *Aspergillus niger* has been identified from many site sampling, its presence on sample may appear after a first microbial colonisation.

Results obtained with *Coniosporium uncinatum* are encouraging as we notice a good development all over incubation time. It could be thinking that the fungal growth is really accelerated because generally meristematic fungi grow slowly.

6. Discussion

These results underline two essential points:
Firstly, relating to the methodology itself, the various test performed permits to point out the key parameters. It appears that the inoculation step and the accelerated weathering of the matrix are crucial points. Table 8 presents an overview of the results relating to the development of the methodology for the biodeterioration test. Therefore, for the time of experiment studied (4 weeks) the fungal development is faster on the carbonated then leached specimens and inexistent for on the non weathered one.
Moreover, nutrients should be brought in restricted quantities, to avoid creating interface between the matrix and fungi.
The fungal growth is accelerated by two ways (except the pH decrease):

- Fungal units' suspension is used as fungi can develop from hyphae fragment and not only from spores.
- An important quantity of fungal units' suspension is inoculated, greater than in test developed to study microbial growth on building materials (Shirakawa

et al., 2003; de Moraes Pinheiros, 2003; Nielsen et al., 2004; Urzì and De Leo, 2007).

Secondly, from a biodeterioration point of view, the test developed permits to observe and to points out aesthetical and physical biodeterioration mainly. Table 9 sums up the results in terms of fungal development for the carbonated then leached specimens inoculated in the test E. Microbial development was really observed for this test only, so the biodeterioration can be studied from these results essentially.

Results point out the necessity to have not only microscopic observations to study the microbial development and colonisation. From stereomicroscopical observations we notice microbial growth only on the specimen surface. While this shows the aesthetical biodeterioration of specimens, it underestimates the real extent of the microbial colonization. The PAS staining reveals microbial growth on and within the matrix. Hence, for all the carbonated then leached specimens, microbial development is observed all over the surface and penetrates into the matrix. The SEM observations permit to identify characteristic bacterial shapes on sample inoculated with *Aspergillus niger* and on controls. Observations of cross section show penetration of micro-organisms inside the matrix. These analytical methods appeared very complementary and essential for the study of biodeterioration. They permit to access to the physical biodeterioration, which is certainly the most critical aspect of biodeterioration to study. Moreover, bacterial development is observed inside the matrix. It can be assumed that their growth results in the release of acid metabolites which can interact with the matrix, leading to the chemical biodeterioration. Nevertheless, this remains only presumptions as no analytical methods were developed in this study to follow the chemical biodeterioration specifically.

Ergosterol and protein assays were developed in order to quantify the biomass during the biodeterioration test. Nevertheless, these assays couldn’t be performed in the inoculated specimens. Experiments were carried out in another laboratory and experimental device weren’t available for the assays. Besides, ergosterol and protein assays should be included in the biodeterioration test.

Table 8: Overview of the results obtained for the development of the biodeterioration test

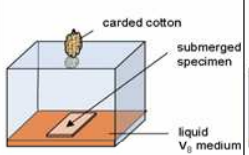
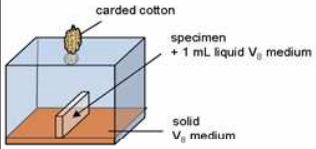
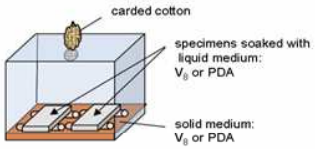
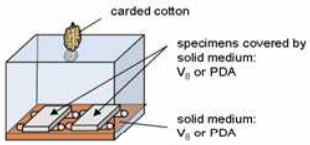
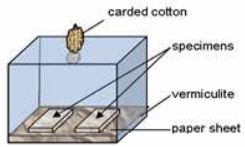
	Test A	Test B	Test C	Test D	Test E
Strain inoculated	*A. alternata*	*A. alternata*	*A. alternata* *A. niger*	*A. alternata* *A. niger*	*A. alternata* *A. niger* *Exophiala sp.* *C. uncinatum*
Specimens inoculated	non weathered carbonated	non weathered carbonated	non weathered carbonated carbonated + leached	non weathered carbonated carbonated + leached	non weathered carbonated carbonated + leached
Nutrients brought	-	1 ml V_8 liq / week	1,5 ml V_8 liq / week	1,5 ml V_8 liq / week	-
Experimental device					
Observations	Liquid medium became gel like	Fungal development on the solid medium only Spores remains agglomerated on the specimen surface	Fungal development on the solid medium only	**Non weahtered specimens:** --> no fungal growth **Carbonated specimens:** –> fungal growth only for 1/3 specimens inoculated with *A.niger* **Carbonated the leached specimens** --> fungal development on all the specimens The growth occurs on the thin layer of solid medium only and doesn't interact with the matrix	**Non weahtered specimens:** --> no fungal growth **Carbonated specimens:** --> fungal growth of *A. alternaria* only **Carbonated the leached specimens** –> fungal development on all the specimens except for A. niger Microbial contamination on all the specimens included the controls
Conclusion	The supply of nutrient should be changed	The vertically disposition of the specimen should be changed	Inoculation step should be improved	Inoculation step should be improved	Validation of the experimental part of the biodeterioration test

Table 9: Overview of the results obtained for the carbonated then leached specimens inoculated in the Test E, from a fungal growth point of view

	Specimens inoculated with				
	Controls	***Aspergillus niger***	***Exophiala* sp.**	***Alternaria alternata***	***Coniosporium uncinatum***
Direct and stereomicroscopic obervations	Bacterial contamination	Bacterial contamination	Bacterial contamination Fungal contamination Fungal growth	Fungal contamination Fungal growth	Fungal contamination Fungal growth
PAS staining	Microbial colonisation spread on the whole inoculated surface and also in the matrix volume on the first micrometers				
MEB observations	Bacterial contamination on the surface and probably inside the matrix	Bacterial contamination	Hyphae only on the surface	Hyphae on the surface AND inside the matrix	Hyphae on the surface AND inside the matrix
Aestetical biodeter.	+	+ (bacteria)	++	++++	+++++
Chemical biodeter.	assumed	assumed	not controled	not controled	not controled
Physical biodeter.	+	-	-	++	++++

biodeter. = biodeterioration

7. Conclusion

The accelerated laboratory test developed permits to obtain a rapid fungal growth on cement specimens. Accelerated conditions chosen are:

- Accelerated weathering of matrix by carbonation (2 days) followed by leaching operation (28 days).

- Acceleration of fungal growth by inoculation of concentrated fungal cells suspension.

To avoid contamination during test, specimen sterilization should be performed by ionisation. Analyses used to study microbial growth during test are complementary methods:

- Stereomicroscopical observations permit a direct observation of specimens without sample preparation.

- PAS staining allows visualization of microbial colonisation extent on/within matrix.

- SEM observations provide information on interactions between matrix and fungi, and also to recognize some characteristic microbial shapes.

- Ergosterol and protein assays for the biomass quantification.

Relating to the fungal strains studied, the results pointed out that *Exophiala* sp., *Alternaria alternata* and *Coniosporium uncinatum* are obviously responsible of an aesthetical biodeterioration. *Alternaria alternata* and *Coniosporium uncinatum* were observed inside the matrix, hence they could be responsible of physical biodeterioration.

CONCLUSION GÉNÉRALE

Le développement des micro-organismes (bactéries, cyanobactéries, algues, champignons) sur les matériaux de constructions conduit à l'altération des propriétés du matériau. Une meilleure compréhension des mécanismes impliqués dans la biodétérioration permettra de mieux lutter contre les dommages engendrés et à terme de prévenir le développement de ces micro-organismes. Le but de cette étude était de développer un test accéléré de laboratoire pour étudier la biodétérioration d'une matrice cimentaire par des champignons.

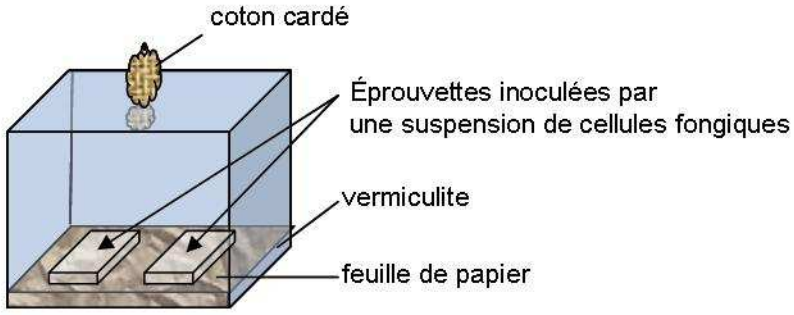
L'étude de la biodétérioration nécessite une approche pluridisciplinaire. Dans un premier temps, ce travail a été abordé d'un point de vue purement « micro-organismes ». Cela a permis d'identifier et de définir les paramètres optimums de culture et croissance fongique. Dans un second temps, l'attention s'est portée sur la préparation d'une matrice cimentaire compatible avec le développement fongique. Enfin, les deux approches ont été combinées permettant l'étude de la croissance des micro-organismes sur le matériau. Ce travail a conduit au développement d'un test accéléré permettant une croissance fongique rapide (< 4 semaines) sur une matrice cimentaire.

Concernant les micro-organismes, notre intérêt s'est porté sur l'étude des champignons microscopiques (micromycètes). Ceci nous permet d'aborder les différents aspects de la biodétérioration : esthétique, chimique mais aussi physique. L'étude bibliographique montre que les micromycètes colonisateurs des matériaux de construction peuvent se regrouper en quatre grandes catégories. Ainsi, nous avons sélectionné une souche représentative de chaque catégorie : (i) *Alternaria alternata* pour représenter un hyphomycète producteur de mélanine, (ii) *Aspergillus niger* pour un hyphomycète acidogénique, (iii) *Exophiala* sp. pour un champignon lévuriforme, et (iv) *Conosporium unicinatum* pour les champignons méristématiques.

D'un point de vue matériau, le vieillissement accéléré de la matrice s'est rapidement imposé ; le pH basique des matrices cimentaires fraîchement préparées ne favorisant pas la croissance

fongique. Les éprouvettes en ciment ont été dans un premier temps carbonatées, puis lixiviées. Ce traitement a permis d'abaisser le pH de surface de 12 à 8,8. Le vieillissement a également conduit à une modification importante de l'aspect de surface et de la composition chimique de la matrice. Ces changements ont été caractérisés à l'aide de différentes techniques analytiques : DRX, IR-TF, porosimétrie mercure, pycnométrie hélium, et observations au MEB. Le vieillissement de la matrice se traduit par l'apparition de fissures, une diminution de la porosité et une composition chimique de surface proche de celle d'une roche calcaire. La surface est progressivement recouverte d'une couche de $CaCO_3$. Ce phénomène est d'autant plus prononcé que le vieillissement accéléré est important.

A propos du test de biodétérioration, une partie importante de la mise au point concerne l'optimisation de l'inoculation. En effet, cinq configurations ont été testées avant de déterminer les conditions optimales. Le dispositif expérimental retenu est le suivant :



Ce travail a abouti au développement d'une méthodologie permettant d'une part d'obtenir une croissance fongique rapide sur la matrice cimentaire, et d'autre part de disposer d'un panel de techniques analytiques très complémentaires pour l'étude de la biodétérioration.
En cours de test, le développement fongique est suivi toutes les semaines par observations directes et au stéréomicroscope. L'étendue de la colonisation microbienne est estimée par coloration des échantillons au réactif de Schiff (PAS) en fin de test. L'interaction entre les micro-organismes et la matrice a été observée au microscope électronique à balayage (MEB). Enfin, les dosages de l'ergostérol et des protéines ont été développés au sein du laboratoire. Ces dosages pourront être utilisés pour quantifier la biomasse.

Les résultats obtenus ont notamment mis en évidence le rôle fondamental joué par le pH de surface sur le développement microbien, confirmant ainsi les observations faites par

Shirakawa et al. (2003). Les travaux ont montré que l'analyse des échantillons au stéréomicroscope sous-estime l'étendue réelle de la colonisation microbienne. L'analyse doit donc être complétée par d'autres méthodes. Ainsi, la coloration au réactif de Schiff a mis en évidence que la colonisation microbienne n'est pas seulement en surface mais intervient également dans le volume. De plus, des hyphes d'*Alternaria alternata* et de *Coniosporium uncinatum* ont été observés au MEB à l'intérieur de la matrice, soulignant ainsi leur potentiel de biodétérioration physique.

La pénétration des micro-organismes à l'intérieur de la matrice est rendue possible et facilitée par l'existence des fissures. De plus, la respiration des bactéries et des champignons augmente la concentration en CO_2 localement. Cela conduit à la formation de H_2CO_3 et à la diminution du pH du matériau en contact avec ces micro-organismes (Gorbushina, 2007). La biodétérioration est un domaine complexe pour lequel l'impact biologique ne peut être dissocié des conditions environnementales jouant sur le vieillissement naturel du matériau. Tous ces facteurs sont étroitement liés et agissent en interaction. Les résultats montrent qu'il ne peut y avoir de développement microbien sans un vieillissement préalable du matériau. Le même scenario est rencontré en environnement naturel. La carbonatation et la lixiviation sont deux phénomènes qui interviennent dans le vieillissement naturel du matériau.

Le test développé dans cette étude nécessite trois mois d'expérimentation pour obtenir des résultats significatifs en terme de développement fongique. Ces trois mois sont répartis de la façon suivante : un mois de conservation des éprouvettes, un mois de vieillissement accéléré, un mois d'incubation. En ce sens, ce test se distingue des tests de laboratoires existants qui nécessitent des durées de vieillissement ou d'incubation, de quatre à quinze mois (Dubosc, 2000 ; Shirakawa et al., 2003 ; De Moraes Pinheiro et al., 2003 ; Nielsen et al., 2004 ; Urzì et De Leo, 2007).
Les conditions expérimentales retenues sont proches de celles utilisées par Escadeillas et al. (2007). Les mortiers étudiés par les auteurs subissent également un vieillissement accéléré composé d'une carbonatation suivie d'une lixiviation, pour une durée totale de deux à cinq mois. Néanmoins, ces deux tests se distinguent par la durée de chaque opération. Escadeillas et al. réalisent une carbonatation totale des échantillons puis une lixiviation rapide (7 jours), alors que dans notre cas la carbonatation superficielle des éprouvettes est suivie d'une lixiviation importante de la matrice. D'autre part, la différence fondamentale entre ces deux

tests porte sur la nature des micro-organismes étudiés ; Escadeillas et al. (2007) étudient les algues, alors que notre travail porte sur les micromycètes.

L'originalité de ce test tient d'une part dans la complémentarité des souches fongiques étudiées : l'étude de leur développement permet de travailler sur les différents aspects de la biodétérioration : esthétique, chimique et physique pour un même matériau.
D'autre part, une méthodologie expérimentale et analytique a été développée pour suivre le développement fongique sur une matrice cimentaire et caractériser la biodétérioration selon ses différents aspects.