



RILEM TC 183-MIB: 'Microbial impacts on building materials – Weathering and conservation'

Techniques applied to the study of microbial impact on building materials

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ABSTRACT

This paper presents various techniques for materials characterisation in relation with the question of microbial impact. Applications examples of some of these techniques are described as well as the respective results obtained by researchers working in this field. Additional readings are also reported.

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RÉSUMÉ

Cet article présente diverses techniques de caractérisation des matériaux vues sous l'angle de l'étude des impacts microbiens. Des exemples d'application de certaines d'entre elles sont décrits ainsi que les résultats obtenus par des chercheurs travaillant dans ce domaine. Une bibliographie complémentaire est également fournie.

1 BIOLOGICAL TECHNIQUES

2 1.1 Sampling with adhesive tape (SAT)

3 In many circumstances the sampling and monitoring of a
4 building materials biological growth should be performed
5 using a technique that is not only non-contaminatory but
6 also non-destructive to the construction.

7 The use of adhesive tape, a technique borrowed from
8 clinical mycology, was first brought into the cultural
9 heritage community by Garagni [1]. Sampling is carried
10 out by gently applying a strip of adhesive tape to the
11 surface being studied, which is then removed and placed on

12 sterile glass microscope slides and kept in a sterile box until
13 arrival in the laboratory. The strips are then cut into small
14 pieces and sorted for microscopic and cultural examination.

15 Light microscopy is carried out by adding a drop of
16 sterile water or other liquid, dependant on technique you
17 wish to use, on the strip and placing a glass slide on the
18 reverse of the tape (to keep it flat during examination). The
19 drop of liquid often expands the microscopic organisms to
20 make it easier to identify each of them under low
21 magnification.

22 For SEM examination the adhesive tape is placed (face
23 up) on a stub with bioadhesive tape, the sample is then
24 dehydrated via ethanol series and covered with carbon powder.
25 After this simple preparation SEM observations can be made.

1 Cultural analysis is performed by introducing the tape into
 2 either the solid or liquid medium of your choice, although
 3 typically a nutrient rich general medium is used. After a short
 4 period of growth it becomes possible to identify many of the
 5 organisms present in the community. This is particularly
 6 useful for microorganisms (*e.g.*, algae and certain types of
 7 bacteria) which can be grown on a specific medium that does
 8 not allow the growth of organisms normally found on non-
 9 sterile adhesive tape. Unless the tape can be sterilised (a
 10 difficult process), many bacteria and fungi will grow on
 11 nutrient rich media as contaminants.

12 1.2 Cell Counts (CC)

13 It is often necessary to report on the size of a microbial
 14 community, and there are various methods for counting the
 15 size of a population using microscope methods to give the
 16 total number of cells (live + dead), or viable counting
 17 methods to enumerate cells capable of reproduction on
 18 artificial laboratory media. A couple of the commonest
 19 methods are described below. These methods may be used
 20 directly on liquid samples, but when the microorganisms
 21 are attached to a surface, they must first be removed by a
 22 technique such as scraping, swabbing, ultrasonic vibration,
 23 etc. For direct cell counts on an opaque surface, a
 24 microscopic technique such as electron microscopy or
 25 epifluorescence microscopy can be used.

26 The Helber counting chamber is a slide 2-3mm thick with
 27 an area in the centre called a platform, which is surrounded
 28 by a ditch. On the top of the slide is a 1mm² grid divided
 29 into 400 small squares each 0.0025mm² in size. When the
 30 glass cover slip is placed over the slide, it leaves a clearance
 31 of 0.02mm between itself and the grid; thus the volume over
 32 each square is 0.00005ml. The specimen is diluted and then
 33 a loopful of suspension is placed on the grid area, the cover
 34 glass is placed over the slide and this is then examined
 35 underneath a microscope. The number of organisms per
 36 square is counted until the count reaches 300-500. Dividing
 37 the total count by the number of squares examined to reach
 38 this number, multiplying by 20 000 000 and the original
 39 dilution factor, will result in the number of organisms per ml
 40 [2]. This method is more suitable for counting algae or fungal
 41 spores than for bacteria, as the X100 objective cannot be
 42 used with the thick cover slips required.

43 In the drop count method, developed by Miles and
 44 Misra, small drops (generally 10 or 50µl) of the suspension
 45 are placed on agar plates and the colonies in the inoculated
 46 areas are counted after incubation. The concentration of
 47 cells in the original sample may be calculated as colony
 48 forming units per cm³ (cfu cm⁻³). This method may also be
 49 used for arbitrary standards; *e.g.* if less than ten colonies
 50 per five drops of sample the specimen is deemed fit for use.

51 1.3 Streak tests (ST)

52 Streak tests are used for two distinct purposes, firstly to
 53 isolate pure species from an environmental sample and
 54 secondly to test the purity of the isolated organisms. Petri
 55 dishes containing a nutrient agar medium are prepared. A
 56 flame-sterilised wire loop is used to put a small spot from the
 57 sample on the plate. Sterilise the loop again and make a

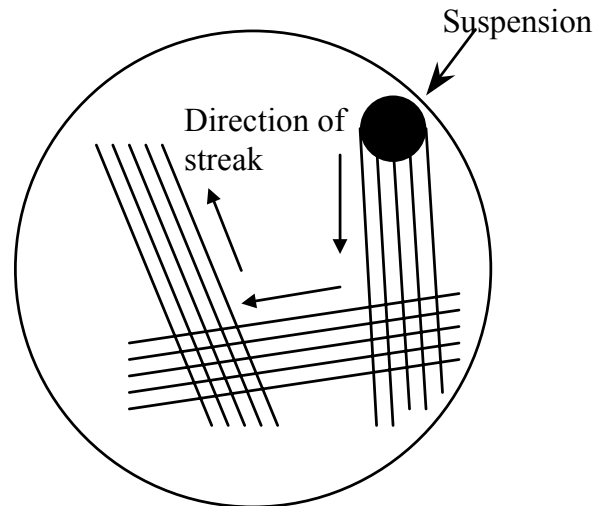


Fig. 1 - The typical streak arrangement on a streak plate.

58 number of parallel streaks through the sample, as shown in
 59 Fig. 1. Resterilise the loop and draw a second set of lines
 60 perpendicular to the first set, repeat for a third set
 61 perpendicular to the second set of lines. Cover and incubate
 62 the plate under suitable growth conditions for a period of 24h –
 63 28 days, depending on the growth rate of the specimen.

64 After incubation, colonies can be examined
 65 macroscopically and microscopically to check whether it is
 66 a pure sample and to begin the identification process. If
 67 further purification is necessary, individual colonies should
 68 be picked and streaked repeatedly until identical colonies
 69 containing identical cells are obtained.

70 1.4 Fluorescent cell staining (FCS)

71 Microbial cells can be stained with a variety of dyes to
 72 make them visible under the microscope. For direct
 73 examination of cells on an opaque surface, using epi-
 74 illumination, fluorescent stains, such as acridine orange and
 75 DAPI (4', 6-diamidino-2-phenylindole), may be used. This
 76 allows microbial cells to be counted, and their shapes to be
 77 distinguished, without removal from the substrate. Cells are,
 78 however, normally killed by the staining technique. A variety
 79 of fluorescent dyes may be used and some of these allow
 80 differentiation of viable, or active, from non-viable, or
 81 inactive, cells. Some microorganisms are autofluorescent and
 82 can be visualized without staining. These include
 83 photosynthetic organisms such as algae and cyanobacteria.
 84 There are also ways of making microorganisms
 85 autofluorescent, by inserting genes for fluorescence into the
 86 cells. Fluorescent green protein is the most common of these
 87 and has been used for rapid detection of inoculated
 88 organisms in experimental studies to trace the survival and
 89 spread of organisms in natural and artificial environments.

90 1.5 Detection of biomolecules (DB)

91 Cells may be detected and quantified by analysis of
 92 specific biological molecules such as proteins, phospholipids,
 93 nucleic acids, chlorophyll and enzymes. These are not
 94 necessarily destructive techniques and colour changes

1 brought about by the activity of respiratory enzymes have
 2 been used to quantify microbial biofilms on stone surfaces.
 3 However, most of the analytical methods used (for lipids and
 4 chlorophyll, for example) necessitate the destruction of the
 5 sample, use of suitable extractive chemicals and analysis by
 6 chromatographic or spectrophotometric techniques. Enzyme
 7 assays may be particularly useful, as they measure active
 8 cells, rather than merely cells capable of reproduction to give
 9 colonies on solid media.

10 1.6 DNA analytical methods (DNA-am)

11 The use of specific nucleic acid probes and the
 12 polymerase chain reaction (PCR), which allows the
 13 production of multiple copies of selected regions of the cell
 14 genome, has only recently begun to be applied to building
 15 materials. These are specialised techniques that allow
 16 microorganisms to be detected and identified either *in-situ*
 17 or *ex-situ* with a high degree of specificity and sensitivity.
 18 They are rapid, especially in comparison with culture
 19 methods, and allow the detection of microorganisms that
 20 cannot yet be grown in artificial culture media. Most
 21 methods do not allow ready quantification of the microbial
 22 population, although this is possible with FISH
 23 (fluorescence *in situ* hybridisation) techniques, which use
 24 fluorescent gene probes to visualise microorganisms under
 25 the microscope. At present, there are no standard
 26 techniques and protocols must be developed for the
 27 material under investigation, based on methods already
 28 published for clinical and environmental specimens.

29 1.7 Immunological Methods (IM)

30 Antibodies labelled with detector molecules such as
 31 fluorescent dyes or enzymes can be used to detect and
 32 quantify specific microorganisms. These methods have
 33 been long used in the medical field, but are relatively rare
 34 in building materials research. They seem to offer no
 35 advantages over the DNA-based techniques, which are
 36 becoming so popular.

37 2 TECHNIQUES APPLICATION

38 The research using fifteen of the described techniques
 39 are presented by some researchers working in the field of
 40 the microbial impact on building materials. Each example
 41 of application gives the analysed material, the objective of
 42 the study, the results obtained, the conclusion and the
 43 advantage of the use of the technique(s). Isolated
 44 employment of ten techniques and the use of an ensemble
 45 of seven techniques are presented.

46 2.1 Environmental scanning electron 47 microscope (SEM/ESEM) and energy 48 dispersive x-ray analysis (EDX) - Application 49 example

50 This technique was applied by the team of Nele De Belie
 51 at the Magnel Laboratory for Concrete Research, Dept. of

52 Structural Engineering, Ghent University, Belgium.

53 Concrete, mortar, limestone were the analysed materials.
 54 The objective of the use of these techniques was to
 55 determine the presence of micro-organismes on
 56 concrete/stone and to get a first idea about the type of
 57 micro-organismes; to visualise the formation of crystals,
 58 e.g. gypsum or ettringite formed by the action of sulphuric
 59 acid excreted by *Thiobacilli*; to visualise the effect of
 60 excreted acids on the cement paste and the transition zone
 61 with the aggregates; EDX can be used together with a study
 62 of the morphology to determine which crystals are present.

63 The following results were obtained:

- 64 • The presence of *Thiobacilli* on concrete sewer pipes was
 65 confirmed (Fig. 2)
- 66 • Lichens were observed on concrete (Fig. 3).
- 67 • SEM-EDX prove that gypsum crystals are formed on
 68 concrete due to sulphuric acid excretion by *Thiobacilli*
 69 (Figs. 4 and 5)
- 70 • The presence of ettringite crystals was detected through
 71 this technique.
- 72 • In a research on microbiologically induced calcium
 73 carbonate precipitation for repair of concrete and stone
 74 surfaces (biomineralisation), the quality of the deposited
 75 CaCO₃-layer is studied with SEM.

76 Details on this research can be obtained in some

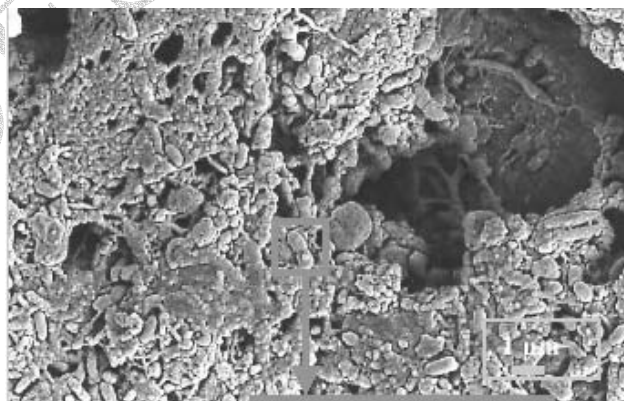


Fig. 2 - *Thiobacillus* on concrete.

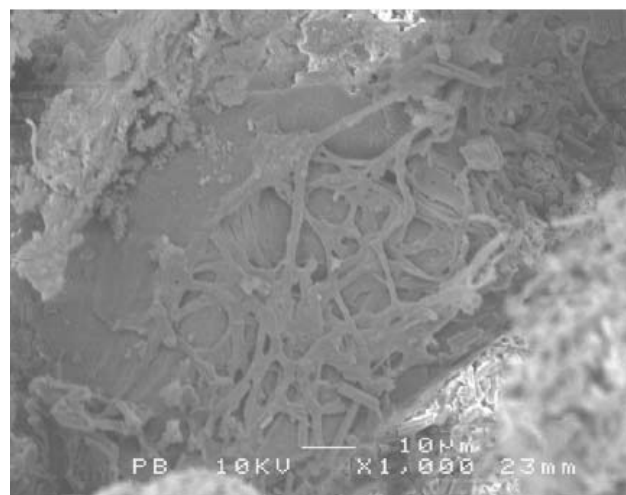


Fig. 3 - Lichens on concrete.

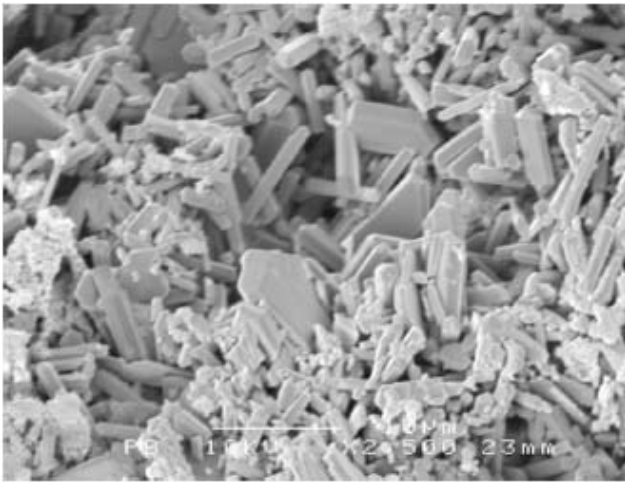


Fig. 4 - Gypsum crystals formed on concrete due to sulphuric acid excretion by *Thiobacillus*.

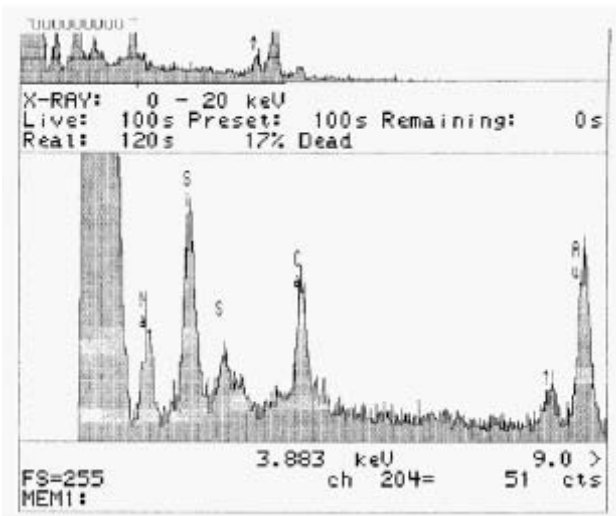


Fig. 5 - EDX confirms the presence of gypsum crystals (S-peak).

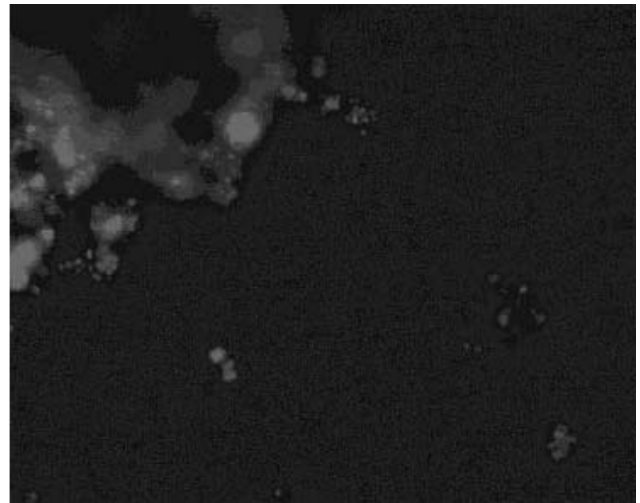


Fig. 6 - Lichens in a fouled concrete surface (red stains). There is no indication of dead bacteria.

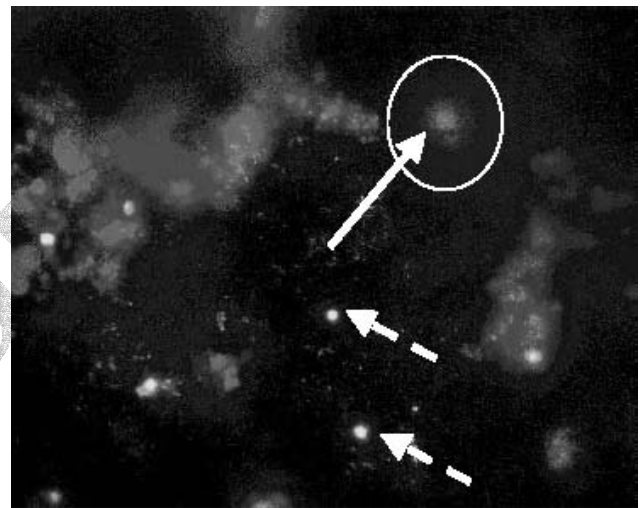


Fig. 7 - The same surface as Fig. 6, with the dead lichens at the same location. In between the fouling, green and yellow dots and vails are clearly visible (white arrow and circle. These are bacteria and biofilms. The big yellow stains are living lichens (white dashed arrows).

1 references [3-5].

2 An advantage of this technique is that although a rather
3 small area of the concrete/stone surface is studied, a
4 qualitative impression can be obtained about the presence
5 of micro-organisms and their effect on the substrate.

6 2.2 Fluorescent cell staining (FCS) - 7 Application example

8 The team of Nele De Belie carried out this research at
9 the Magnel Laboratory for Concrete Research, Dept. of
10 Structural Engineering and the Laboratory for
11 Microbial Ecology and Technology, Ghent University,
12 Belgium

13 The analysed materials are concrete and stone.

14 The objective of the study is to determine presence and
15 viability of micro-organisms in the material.

16 Viability staining was performed using commercial
17 live/dead stain (L-13152, Molecular Probes, Leiden, The
18 Netherlands). This stain allows fluorescence microscopy to
19 distinguish between organisms with intact cell membranes

20 (stained green and scored alive) and organisms with
21 damaged cell membranes (stained red and scored dead).
22 25 μl stain was put directly on 1 cm^2 of mortar surface and
23 was incubated for 10 min in the dark and examined by
24 standard epifluorescence microscopy on a Zeiss Axioskop
25 II microscope (Carl Zeiss, Jena, Germany). The microscope
26 was equipped with a Peltier cooled single chip digital
27 colour CCD camera (Hamamatsu Orca IIIIm, Hamamatsu,
28 Massy Cedex, France) and connected to a PC to obtain
29 digital images. For each treatment, two preparations were
30 examined under fluorescent microscopy.

31 Staining of untreated concrete cubes polluted with
32 organic fouling, resulted in a red staining of the dead lichen
33 cells, illustrated in Fig. 6. Clusters of live cells could be
34 detected on the mortar cubes treated with the cleaning Thio-
35 S culture, that were not seen on untreated specimens. These
36 cells were present as groups of attached individual cells
37 (Fig. 7) and also as organised in biofilm structures. It was

1 proposed that these cells represented active *Thiobacillus sp.*
 2 cells from the Thio-S culture. The results are similar for
 3 portland and blast furnace slag cement samples. These
 4 results prove that the micro-organisms are using the
 5 concrete as a substrate and that they can locally produce
 6 sulphuric acid, having an active cleaning effect.
 7 The advantage on using this technique is to allow the
 8 determination of the presence and viability of micro-
 9 organisms. Details on this research can be found in [3].

10 2.3 Mössbauer spectrometry - Application 11 example

12 The authors of this study are Prof. Liz Karen Herrera and
 13 X. Anleo, both from the University of Medellin, Colombia.
 14 Peridotite, source and weathered rocks of the church of
 15 Veracruz in Medellin Colombia were studied in this
 16 research.

17 Mössbauer analysis was performed to complete the
 18 identification of the oxidations products, already made by
 19 using FT-IR and XRD [6-9].

20 The room temperature Mössbauer spectra (MS) were
 21 obtained in transmission mode using a constant acceleration
 22 drive and triangular reference signal. A 57A Co/Rh source
 23 with initial activity of 25mCi was used. The spectra were
 24 fitted using a program called MOSF which is based on a
 25 non-linear least squares fitting procedure which assumes
 26 Mössbauer lines of Lorentzian shape standard hematite was
 27 used to calibrate regularly the spectrometer and the
 28 calibration line width was about 0.28 ± 0.02 mm/s.

29 Comparing the Mössbauer spectrum of source rock
 30 (Fig. 8) with that of weathered rock (Fig. 9), the two
 31 doublets in Fig. 8 should correspond to olivines whereas the
 32 doublet of weathered rock in Fig. 9 should be assigned to
 33 the present of source rock, (the major one) and the to a
 34 mixture of goethite and lepidocrocite (as a result of
 35 weathering processes).

36 MS provides the complete, *in situ* measurement, non-
 37 destructive, three-dimensional identification of corrosion
 38 products and is the only technique able to accurately
 39 measure the fraction of each oxide in a corrosion product
 40 layer. Thus, it can be used to study the electric, magnetic
 41 and structural characteristics of metals, alloys, soils and
 42 minerals. MS can be considered as a “fingerprint”
 43 technique of varying degrees of sophistication in
 44 mineralogical and geo-chemical studies. More fingerprint
 45 applications are characterization of oxidation state iron
 46 (e.g., Fe^{2+} or Fe^{3+}), electronic configuration of iron (e.g.
 47 high or low spin) coordination symmetry about the iron
 48 atom (e.g. octahedral or tetrahedral) and site distortion from
 49 either octahedral or tetrahedral symmetry.

50 2.4 Optical microscopy - Thin section 51 petrography - Application example

52 Nele De Belie, from the Magnel Laboratory for Concrete
 53 Research, Dept. of Structural Engineering, Ghent University,
 54 Belgium, developed a study using this technique.

55 Concrete, mortar and limestone were analysed.

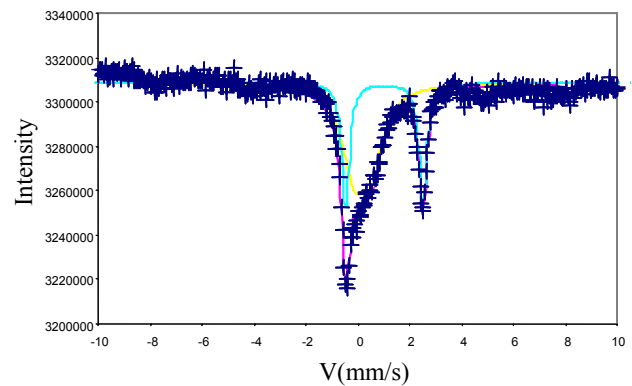


Fig. 8 - Mössbauer spectrum of peridotite (source rock). Source rock of the church of Veracruz in Medellin Colombia.

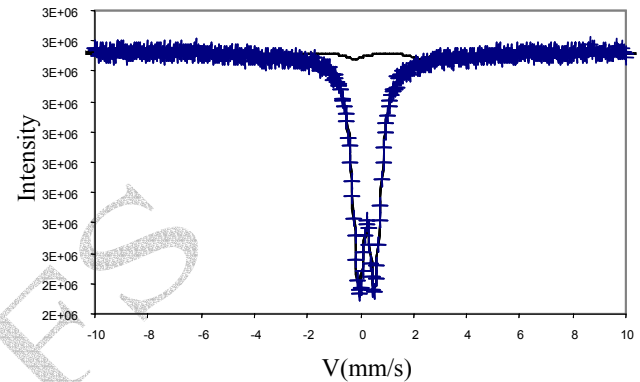


Fig. 9 - Mössbauer spectrum of peridotite (weathered rock). Weathered rock of the church of Veracruz in Medellin Colombia.

56 The aim of the study was to determine the degradation
 57 depth of the specimens; to visualise to which extent the
 58 material is influenced for instance by acids excreted by
 59 bacteria; to visualise concrete carbonation; to study the
 60 presence of porosities or microcracking, suggesting the
 61 action of acids or expansive salts, respectively. For the
 62 study of pores and cracks, the fluorescence mode can be
 63 used besides the classical ordinary and polarised light
 64 modes. To allow use of fluorescence microscopy, the
 65 samples should be impregnated beforehand with an epoxy
 66 resin containing a fluorescent dye.

67 See for instance Fig. 10: the same thin section is
 68 photographed at ordinary light (top), polarised light
 69 (middle) and fluorescent light (bottom). The presence of a
 70 more porous zone at the edge of the specimen is clearly
 71 visible (brown coloration at polarised light and more
 72 intense fluorescence at fluorescent light). This zone has
 73 been degraded by lactic acid, excreted by bacteria.

74 From the border to the core of concrete samples degraded
 75 by lactic acid, three zones could be distinguished with
 76 different stage of degradation. The samples with blast furnace
 77 slag cement had a denser structure than the samples with
 78 ordinary Portland cement (OPC), with less air voids and a
 79 smaller capillary porosity. Also test specimens with limestone
 80 aggregates seemed to have a smaller capillary porosity than
 81 the corresponding specimens with gravel aggregates. In the
 82 outer concrete layer, the transition zone between cement paste

1 and aggregates had been highly degraded, especially for the
2 concretes with OPC.

3 More details on this research can be found in [10]. These
4 techniques are very useful to quantify how deep the effect of
5 the bacteria is penetrating into the concrete or stone specimens.

6 2.5 Mercury intrusion porosimetry (MIP) - 7 Application example

8 This technique has been used by Sylva Modrý from the
9 Czech Technical University, Klokner Institute, Prague,
10 Czech Republic.

11 Cementitious materials - pastes, mortars, concrete, rocks
12 (also weathered), ceramics, catalysts supports, wood, paper,
13 insulations, sintered powder iron and corroded concretes
14 were analysed [11].

15 Mercury is intruded into pore structure of materials
16 under high pressure. From the dependence of intruded
17 volume of mercury on the pressure applied pore structural
18 characteristics can be assessed.

19 Specimens for MIP measurement are usually prepared as
20 granules (*e.g.* of 3-5 mm in diameter). All liquids must be
21 removed from the specimens before mercury penetration. In
22 the case of hardened binders the mode of drying plays
23 extremely important role, as was proved by D. Winslow
24 and S. Diamond [12].

25 As relatively high pressures are used in MIP it is of utmost
26 importance to know whether and to what extent the samples
27 under investigation are affected. Permanent changes, *i.e.*
28 plastic deformations and/or deterioration can be pronounced in
29 some materials that corrections have to be introduced with
30 respect to compressibility. In some cases the method cannot be
31 used at all, *e.g.* some coals or sandstones may serve as an
32 example. From the results given in the literature follows that
33 resp. distortion of samples is dependent on mechanical
34 properties of the solid phase itself, and at the same time on the
35 shape and size of the pores and on the mode of their mutual
36 connection. In general, materials with narrow pore entries
37 leading to large cavities are less resistant and, consequently,
38 easier to deform [13]. The used value of the pressure plays a
39 great role. For example pressures up to 98 MPa do not cause
40 measurable alteration in the sample of hardened cement [14].

41 The Washburn model is used to convert mercury intrusion
42 data under two assumptions: 1) the pores are cylindrical and 2)
43 that they are accessible to the outer surface of specimens. It is
44 obvious that the pore shapes in majority of real natural or
45 artificial materials are quite different from cylindrical pores.
46 This fact influences the output of MIP pore size distribution
47 measurements. The obtained sizes of pores are much smaller
48 than their actual sizes. This can be proved by comparison of
49 results of an indirect MIP method with a direct microscopical
50 observation [15].

51 Due to the problems listed above, Diamond [16]
52 recommends to use the threshold diameter and the total
53 volume of intruded mercury only as parameters for
54 comparison with pore structure of other cement pastes or
55 cement mortars, instead of plotting MIP size distribution
56 according to the Washburn model, which does not reflex the
57 actual distribution of pore sizes.

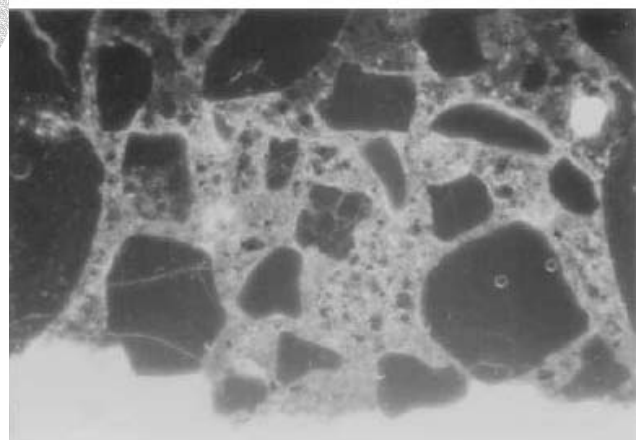
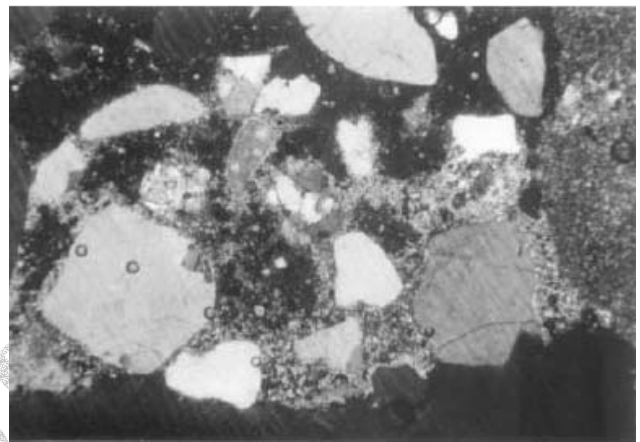
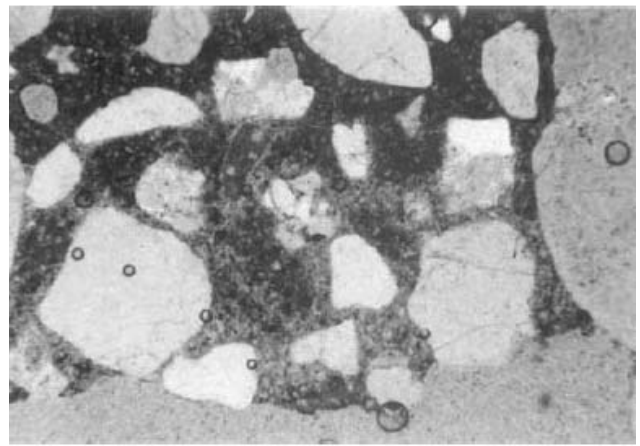


Fig. 10 - Thin sections photographed at ordinary light (top),
polarised light (middle) and fluorescent light (bottom).

58 The grade of complexity of the pore system can be
59 qualitatively assessed with the aid of retention coefficient,
60 which shows what the volume of the mercury trapped in the
61 pore system is. The mercury penetration and retraction curves
62 for hardened cement pastes with different ratio of cement to
63 water can be seen in the Fig. 11 [17]. The dependence of the
64 retention coefficient on water to cement ratio and also on
65 porosity can be seen.

66 The advantage of MIP method is that it is very simple
67 and fast.

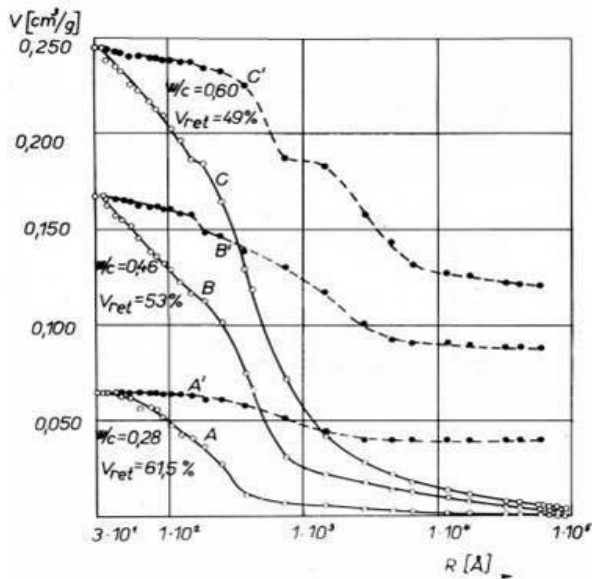


Fig. 11 - Penetration (A, B, C) and retraction (A', B', C') curves of dependence of forced in mercury volume V on pore radius R .

1 Main disadvantage is the application of high pressure. It
2 is necessary to find what the behaviour of material tested
3 under high pressure is.

4 2.6 Water uptake - Application example

5 The team of Nele De Belie used this technique at the
6 Magnel Laboratory for Concrete Research, Dept. of Structural
7 Engineering, Ghent University, Belgium in collaboration with
8 the Laboratory for Microbial Ecology and Technology, Ghent
9 University.

10 Water absorption is the process whereby fluid is drawn into
11 a porous unsaturated material under the action of capillary
12 forces. The capillary suction depends on the pore volume and
13 geometry, and the saturation level of the stone. Water
14 absorption is an important transport mechanism near the
15 surface, and can therefore be related to the durability of the
16 surface layer.

17 A modified version of the sorptivity test based on the
18 Belgian standard NBN B 05-201 was used to determine the
19 effect of a microbiologically produced calcium carbonate
20 layer on the water absorption of concrete or stone. The
21 specimens are coated at the four edges adjacent to the side
22 treated with the microbiological suspension, to ensure
23 unidirectional absorption through the treaded side.

24 Capillary water absorption is compared to full saturation
25 under vacuum.

26 The effect of a microbiologically induced calcium
27 carbonate layer on the water absorption could be monitored.
28 This allowed to select specific strains of *Bacillus sphaericus*
29 that were effective to precipitate a regular calcite layer and to
30 decrease capillary water uptake. The effect of the precipitated
31 layer on Euville limestone was concentrated on the first 2 days
32 of capillary water uptake. After 2 days all the tested samples
33 reached more or less the same saturation level [4, 5].

34 2.7 Weight loss - Application example

35 The use of this technique was applied by Nele De Belie
36 from the Magnel Laboratory for Concrete Research, Dept.
37 of Structural Engineering, Ghent University, Belgium

38 The objective of the research was the measurement of
39 weight loss of concrete or stone samples in a laboratory test
40 on microbial (or chemical or mechanical) deterioration is a
41 very easy and direct method to quantify the deterioration.

42 The bioreceptivity of different concrete types or stone
43 specimens can be monitored. For instance the degradation
44 of concrete types with different aggregates
45 (limestone/gravel), production methods (immediate form
46 removal/hardening in the formwork/centrifugation) or
47 cement types (portland cement/blastfurnace slag cement) by
48 biogenic sulfuric acid corrosion was obtained [18].

49 The results of the higher mentioned study are:

50 Concrete with limestone aggregates showed a smaller
51 degradation depth and weight loss than concrete with inert
52 aggregates. The limestone aggregates locally created a
53 buffering environment protecting the cement paste. This
54 was confirmed by microscopic analysis of the eroded
55 surfaces. The production method of concrete pipes
56 influenced durability through its effect on W/C ratio and
57 water absorption values. In the microbiological tests, HSR
58 Portland cement concrete performed slightly better than
59 slag cement concrete. A possible explanation can be a more
60 rapid colonisation by micro-organisms of the surface of
61 slag cement samples.

62 The advantage of the method is that it is a very simple
63 and direct measurement method.

64 2.8 X-ray diffraction analysis - Application 65 example

66 Nele De Belie applied this technique on studies carried
67 out at the Magnel Laboratory for Concrete Research, Dept.
68 of Structural Engineering, Ghent University, Belgium.

69 The analysed materials were concrete, mortar, stone.

70 The aim of the study was to determine the composition
71 of crystalline compounds formed through microbial action.

72 E.g. Fig. 12 shows crystals that were formed on concrete



Fig. 12 - Crystals formed on concrete through action of
bacterially produced organic acids (lactic and acetic acids).

1 specimens through interaction with bacterially produced
 2 organic acids (lactic and acetic acid). XRD revealed that
 3 these crystals contained calcium acetate, different calcium
 4 acetate hydrates and calcium lactate hydrate.
 5 Also calcium carbonate crystals formed through
 6 microbiological precipitation by *Bacillus sphaericus* were
 7 investigated with XRD: the precipitated calcium carbonate
 8 appeared to be calcite and vaterite crystals [5, 19].
 9 The technique allows obtaining the mineralogical
 10 composition of degradation products.

11 2.9 Set of six techniques (CA, TG, DTA, XRD, 12 SEM and MC) - Application example

13 Research carried out by Moema Ribas Silva (from the
 14 PPGEC, Federal University of Espírito Santo - Vitória, Brazil)
 15 and her group, at the University of Brasília. They used six
 16 different techniques in order to analyse concrete samples taken
 17 from structures submitted to three different climates.

18 The following techniques were applied together for
 19 studying the biodeterioration of concrete: Chemical
 20 Analysis (CA), Thermogravimetry (TG), Differential
 21 thermal analysis (DTA), X-rays diffraction (XRD),
 22 Scanning electron microscopy/energy dispersive analysis
 23 (SEM/EDX) and Mineralogical calculation (MC).

24 These techniques give useful information on the material
 25 condition concerning its deterioration (biodeterioration).
 26 DTA and XRD identify the amorphous and crystallised
 27 compounds, including the deterioration products. SEM /
 28 EDX allows observing, among others, the presence of these
 29 compounds, the texture of the material as well as detecting
 30 the presence of microorganisms and characterising
 31 morphologically these microorganisms. With the aid of the
 32 MC (which uses the results of CA and TG as data), it is
 33 possible to identify the deterioration (or biodeterioration)
 34 mechanisms what allows to indicate a method for stopping
 35 or avoiding it. A study on the influence of three different
 36 environmental conditions on the concrete biodeterioration
 37 was carried out.

38 Samples were collected on damaged concrete structures
 39 submitted to three different environmental conditions



Fig. 13 - Solubilised calcium carbonates observed in a concrete where *Cladosporium* and protozoa were also present.



Fig. 14 - Diatom algae found in the same sample as Fig. 9 taken from a concrete structure placed in a hot and dry environment.

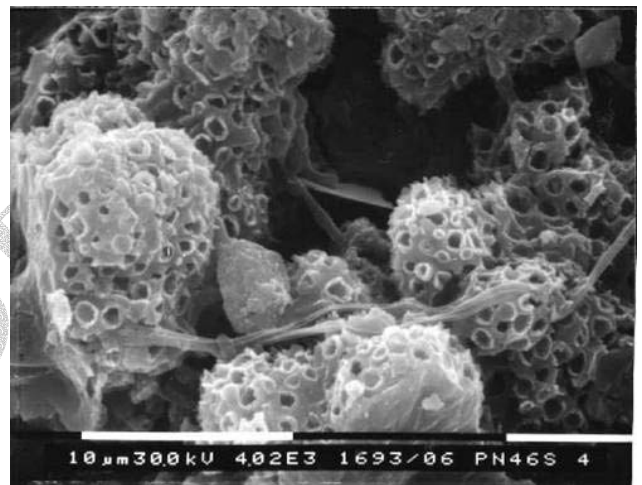


Fig. 15 - Unidentified morphology close to fungus hyphae. Organic matter was detected by differential thermal analysis in this sample.

40 (temperature from -5°C to 35°C and relative humidity from
 41 8 to 100%), aged of 7 to 30 years [20-22]. Even if these
 42 conditions were quite different, microorganisms were
 43 present in the concrete submitted to the three studied
 44 climates. The same concrete compounds, among others
 45 micas, feldspars, quartz, calcic compounds (Fig. 13),
 46 appeared solubilised in the concrete from the three analysed
 47 climates. Besides the same kind of microorganisms, such as
 48 diatom algae (Fig. 14), Actinomycete, *Thiobacillus*,
 49 *Cladosporium*, Protozoa (Fig. 15) etc., some different
 50 unidentified morphologies (Fig. 16) were observed in the
 51 studied concrete structures.

52 Through mineralogical calculation it was possible to
 53 verify that part of the silica was missed when diatom algae
 54 (which uses silica as a nutrient) was present in the concrete.

55 Deterioration products, such as ettringite (Fig. 17) and
 56 gypsum (among other sulphates) were detected in the
 57 analysed concrete structures but the sulphur source could
 58 not be detected.

59 This study leads to the following conclusions:

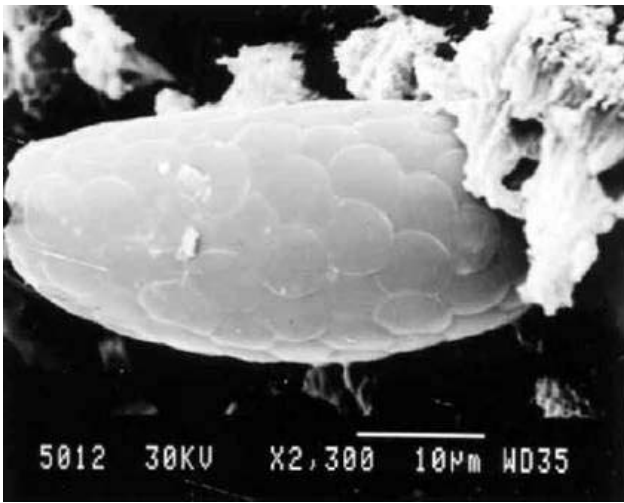


Fig. 16 - Protozoan found in the concrete submitted to a cold and humid climate.



Fig. 17 - Ettringite observed in a concrete apparently sound but where it was observed deteriorated micaceous solubilised calcium carbonates and portlandite.

- 1 • The concrete biodeterioration can be developed under
- 2 different normal climates conditions (extreme
- 3 temperature and relative humidity were not considered)
- 4 independently from the environmental conditions. So
- 5 they are not a very important factor on the development
- 6 of microorganisms.
- 7 • Some of the morphologically characterised
- 8 microorganisms were the same in the concrete under the
- 9 three analysed environmental conditions, however those
- 10 uncharacterised forms are quite different.
- 11 • The mentioned set of techniques is very useful, when
- 12 used together, on the study of concrete biodeterioration,
- 13 however they should be completed with the use of
- 14 microbiological tests in order to identify
- 15 microorganisms present in the concrete and the
- 16 corresponding metabolisms, even if these techniques
- 17 allow detecting some of the biodeterioration
- 18 mechanisms. The most frequently observed mechanisms
- 19 were the silica solubilisation by diatom algae and the
- 20 calcium compounds solubilisation by acids produced by
- 21 certain microorganisms such as fungi and bacteria.

- 22 • SEM/EDX was considered the main technique of this
- 23 study but it is necessary to use other techniques in order
- 24 to get all the necessary results for taking a coherent
- 25 conclusion for completing the study.

26 2.10 DNA analytical methods

27 Vincke and his team used conventional as well as
 28 molecular techniques to determine the microbial
 29 communities present on the concrete walls of sewer pipes
 30 [23]. The genetic fingerprint of the microbiota on corroded
 31 concrete sewer pipes was obtained by means of denaturing
 32 gradient gel electrophoresis (DGGE) of 16S rRNA gene
 33 fragments. The DGGE profiles of the bacterial communities
 34 present on the concrete surface changed as observed by
 35 shifts occurring at the level of the dominance of bands from
 36 non-corroded places to the most severely corroded places.
 37 By means of statistical tools, it was possible to distinguish
 38 two different groups, corresponding to the microbial
 39 communities on corroded and non-corroded surfaces,
 40 respectively. Characterization of the microbial communities
 41 indicated that the sequences of typical bands showed the
 42 highest level of identity to sequences from the bacterial
 43 strains *Thiobacillus thiooxidans*, *Acidithiobacillus* sp.,
 44 *Mycobacterium* sp. and different heterotrophs belonging to
 45 the α -, β - and γ - *Proteobacteria*, *Acidobacteria* and
 46 *Actinobacteria*. In addition, the presence of *N*-acyl-
 47 homoserine lactone signal molecules was shown by two
 48 bio-assays of the biofilm on the concrete under the water
 49 level and at the most severely corroded places on the
 50 concrete surface of the sewer pipe.

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71 Appendix: ADDITIONAL READINGS

72 a) Sampling with adhesive tape

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