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Bioreceptivity of Portland and Calcium Sulphoaluminate Cements in Urban Sewerage Networks

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1 1. Introduction

2 Cements are essential materials for construction. Those listed in the European standard NF
3 EN 197-1 [1] all contain Portland clinker, a significant source of CO₂ from the decarbonation
4 of limestone and cooking fuels. Calcium Sulphoaluminate cements (CSA) can however
5 constitute a more ecological alternative to their use because their clinker is obtained by
6 firing a powder in which the limestone is partially replaced by bauxite and calcium sulphate
7 [2; 3] at a more moderate temperature. The major constituent of CSA clinker is ye'elinite
8 Ca₄Al₆(SO₄)O₁₂ which is a variety of calcium sulphoaluminate.

9 Sewerage networks are made up of a set of underground structures built in stone, brick, cast
10 iron, ceramics, PVC and, especially, concrete. Due to their physical-chemical properties, the
11 effluents conveyed in these networks constitute favourable environments for the
12 proliferation of numerous micro-organisms which naturally interfere with the materials that
13 make up the structure whether submerged and/or emerged [4] This often results in
14 superficial bioalterations (stains, dirt), or even deeper biodeteriorations that can affect and
15 even improve the structural properties of the structure [5]. However, these pathologies
16 depend on the bioreceptivity of the concrete support, i.e. its capacity to be colonized by the
17 present microorganisms [6].

18 Many parameters condition the bioreceptivity of concretes including:

- 19 - Porosity, mainly controlled by the water dosage of the fresh mix (water/cement ratio).
20 This conditions the pore volume (the quantity of pores whose size varies from a few
21 nanometres to a few millimetres [7]) and also influences surface roughness. The porous
22 network may be more or less connected and can also be the vector for the penetration
23 of external agents such as biological elements (microorganisms, rhizoids) and
24 metabolites [4, 5];
- 25 - Surface condition, characterized in particular by roughness, topography and pH [7, 8].
26 The carbonation of cementitious materials decreases the alkalinity of the surface (from
27 a pH ≈ 13 to a pH ≈ 9.5), which favours the colonization of neutrophilic bacteria and the
28 formation of a biofilm [9], i.e. a group of microorganisms in which the cells stick
29 together;
- 30 - The chemistry and mineralogy of cement, including the nature and proportions of the
31 hydrated phases that may be conducive to the development of certain microorganisms
32 [10];
- 33 - The physical-chemistry of the surrounding environment, including temperature,
34 hygrometry, pH, oxygen, carbon dioxide, nutrients present, etc. [10; 11]. These
35 parameters can promote or inhibit the development of some varieties of
36 microorganisms depending on their neutrophilic or acidophilic character, which reflects
37 their needs and/or metabolic intolerances [5];
- 38 - Microorganisms present in contact with the material (genus, species, etc.) [12];
- 39 - Etc.

40 Moreover, in the case of concrete sewerage network, studies have shown that the most
41 severe biological degradation is linked to the sulphur cycle controlled by the presence of
42 hydrogen sulphide (H₂S) [10; 13]. The latter comes from the metabolic activity of the
43 Sulphate-Reducing Bacteria (SRB) that develop in anaerobic zones, when the flow of
44 effluents is low. This is because the liquid medium is then poor in oxygen and so the SRB, by
45 their metabolism are capable of reducing the sulphates present to hydrogen sulphide [14].

46 When the flow becomes turbulent (following a storm for example), dissolved H₂S in
 47 supersaturation is released into the atmosphere of the pipe and condenses on the emergent
 48 vault (alkaline surface) in reduced form. Here it constitutes a nutritive substrate for Sulpho-
 49 Oxidizing Bacteria (SOB) [15]. These bacteria include in their metabolism the production of
 50 sulphuric acid (H₂SO₄) which reacts locally with different hydrates of the cement matrix to
 51 form expansive degradation products such as gypsum and ettringite [12;15]. These
 52 expansive and not very cohesive phases then become the source of internal pressures within
 53 the material and generate the appearance of cracks that can lead to the disintegration of the
 54 concrete. These degradation products will cause swelling, cracks and crazing which can lead
 55 to the ruin of the structure [16].

56 The main objective of this study is to observe and compare the bioreceptivity of two
 57 varieties of Portland Cements and two varieties of Calcium Sulphoaluminate Cements placed
 58 in a real urban sewer network. The behaviour of Calcium Sulphoaluminate Cements in this
 59 type of environment is still poorly known.

60 The paper first presents the physico-chemical characterization of the cements and samples
 61 of the study. The colonization density of the samples and the evolution of the surface
 62 characteristics of the material over time (mineralogy, etc.) are then described and analysed.

63 2. Materials

64 2.1 Cements

65 Two NF EN 197-1 Portland Cements and two Calcium Sulphoaluminate Cements were used.
 66 They are designated as follows:

- 67 - OPC for a cement CEM I 52.5 N CE CP2 NF taken as reference. It contains only clinker (K)
 68 and calcium sulphate (CS). Its normal strength class (N) is 52.5 MPa. It meets the
 69 requirements of European (CE) and French (NF) regulations and can be used for the
 70 manufacture of prestressed concrete (CP2);
- 71 - PSC for a cement CEM III/A 42.5 N-LH PM-ES-CP1 containing 62% blast furnace slag (S). It
 72 is declared Low Heat of Hydration (Low Heat LH). Its strength class is 42.5 MPa with
 73 normal hardening (N). It is suitable for work at sea (PM) and in waters with high
 74 sulphate content (ES) and its sulphide content is limited (CP1);
- 75 - CSA1 for a Belite based Calcium Sulphoaluminate Cement reputed to be resistant to
 76 sulphates;
- 77 - CSA2 for a Calcium Sulphoaluminate Cement with a higher iron content, reputed to be
 78 resistant to sulphates and the marine environment (High Fe₂O₃ Calcium
 79 Sulphoaluminate Cement Clinker).

80 The composition, fineness and density of these cements and their clinker as declared in the
 81 product data sheets are given in the Tabs. I-1 and Tab. I-2.

82 Tab. I-1: Composition of Portland Cements and their clinker, fineness and density

Notation	Standardized designation	Mass content (%)			Mass composition of clinker (%)				SSB (cm ² /g)	Bulk density (g/cm ³)
		K	S	CS	C ₃ S	C ₂ S	C ₃ A	C ₄ AF		
OPC	CEM I 52.5 N CE CP2 NF	97	–	3	55.2	18.3	9.4	9.9	3874	3.17
PSC	CEM III/A 42.5 N-LH PM-ES-CP1	37	62	1					4630	2.96

83

84 Tab I-2: Composition of Calcium Sulphoaluminous Cements, fineness and density.

Cement	Mass content (%)							SSB (cm ² /g)	Bulk density (g/cm ³)
	CaO	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	SO ₃	TiO ₂	Free lime		
CSA1	45	12	20	2	20	2.5	1	5077	2.88
CSA2	44	9	25	7.5	–	–	–	5135	3

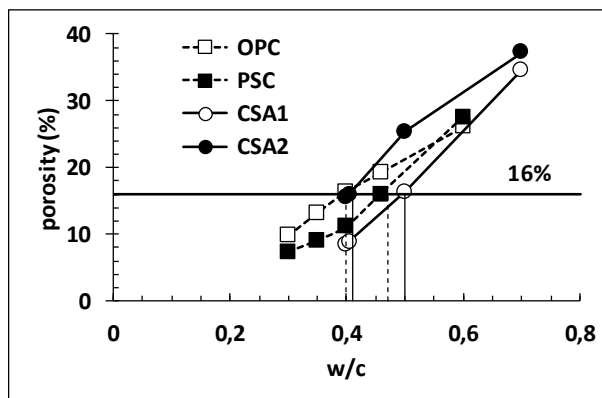
85 (K: clinker, S; blast furnace slag; CS : secondary constituents; C₃S: Alite (CaO)₃SiO₂; C₂S : Belite: (CaO)₂SiO₂; C₄AF:
86 Brownmillerite (CaO)₄Al₂O₃Fe₂O₃; C₄A₃S̄: Ye'elimite: (CaO)₄(Al₂O₃)₃SO₃, SSB: Blaine Specific Surface Area.

87 Let us briefly recall that OPC/PSC hydration gives mainly hydrated calcium silicates (C-S-H),
88 calcium hydroxide C-H (Portlandite), and tri (primary ettringite C₆A₃H₃₂¹) and/or hydrated
89 calcium mono sulphoaluminate C₄A₃H₁₂¹). Hydrates formed from Calcium Sulphoaluminate
90 Cements are mainly ettringite, hydrated calcium monosulphoaluminate, Gibbsite (AH₃¹) and
91 strätlingite (C₂ASH₈¹). C-S-H, portlandite (CH) and hydrogarnet (C₃ASH₄¹) may also be present
92 depending on their C₂S content [3; 17]. Moreover, the rise in resistance of CSAs is much
93 faster than that of OPC/PSCs [18].

94 2.2 Hardened cement paste specimens

95 The materials used in this study are hardened cement pastes. Considering the various factors
96 influencing bioreceptivity (see § 1), we decided that they all have approximately the same
97 "initial" porosity at 28 days, which guarantees a comparable *a priori* physical surface state.
98 For this purpose, cement pastes with different water/cement mass ratios (w/c) were first
99 poured into plastic tubes 16 cm long and 4 cm in diameter with plugs. These pastes were
100 made with a mixer standardized to NF EN 196-1 [19]. The w/c ratios were chosen in order to
101 obtain a range of consistency from firm to very fluid. The firmest pastes were poured
102 progressively under vibration (evacuation of the occluded air). The paste specimens of
103 normal and more fluid consistency were subjected to a slow rotation for 24 hours after
104 vibration to avoid segregation before setting. The specimens were removed from the moulds
105 after 24 hours and stored for 28 days in a moist chamber (20°C ± 1°C, RH > 90%). They were
106 then cut into several samples on which the accessible water porosity was measured
107 according to the method described below (see § 3.2, porosity accessible to water). The
108 results obtained are shown in Fig. 1 and meant that the same "initial" water-accessible
109 porosity of 16% could be chosen for all the cement pastes in the study. The corresponding
110 w/c ratios are 0.4 and 0.47 for OPC and PSC, and 0.5 and 0.41 for CSA1 and CSA2,
111 respectively. The choice of this porosity made it possible to produce pastes of non-fluid
112 consistency, which could be poured into static moulds without proven segregation. It should
113 be noted, however, that the complete hydration of cementitious hydrates is not fully
114 ensured with these w/c ratios, especially for calcium sulfoaluminate cements for which the
115 stoichiometric ratio of ye'elimite is about 0.78 [3].

¹ Cement notation



116

117 Fig. 1: Evolution of the accessible water porosity as a function of the water/cement ratio of
 118 the different cement pastes.

119 Tab. II: Compositions of the different cement pastes having the same accessible water
 120 porosity from 16% to 28 days.

Cementitious grade	OPC	PSC	CSA1	CSA2
Cement (g)	450	450	450	450
Water (g)	180	211,5	225	184,5
w/c	0,40	0,47	0,50	0,41
Porosity at 28 days (%)	16.42	16	16.34	16

121

122 Based on these results, the cement pastes for bioreceptivity tests were manufactured
 123 following the protocol described above. Their composition is given in Tab. II. They were
 124 poured under vibration into specific rectangular moulds made of “bakelized” wood with
 125 dimensions 300×45×20 mm³, in the middle of which two nylon wires with a diameter of 0.55
 126 mm had previously been stretched. The specimens were removed from the moulds at 24
 127 hours and stored for 28 days under standard curing conditions (20°C ± 1°C, RH ≥ 90%). The
 128 nylon wires were then removed and the specimens were cut with a diamond saw into
 129 45×20×20 mm³ plates. These plates were all polished with grade 180 sandpaper to make
 130 their surface finish uniform. The effective porosity of a few plates, measured at 28 days,
 131 completes Tab. II (average of 5 measurements). They were then grouped together in batches
 132 corresponding to one measurement date and attached separately, without contact, by two
 133 nylon wires of 0.25 mm diameter passing through the previous reservations, before being
 134 suspended on appropriate supports subsequently placed in the exhibition sites (Fig. 2).

135 Each batch consisted of 12 plates, i.e. three plates per cementitious grade and four
 136 cementitious grades. This gave a total of nine batches for nine measurement deadlines set at
 137 intervals of about two months, i.e. 18 months of exposure. They were placed on four sites of
 138 the urban network selected for the study (see § 4.1), plus on a control site in the laboratory
 139 (conservation in a closed tank above a tap water supply). In total, some 540 plates were
 140 produced in this way.



141

142 Fig. 2: Conditioning of samples before exposure on real sites. Case of a manhole cover.

143 3. Methods of study

144 The specimens described above were used to perform a bacterial count at each observation
145 date, accompanied by microscopic and mineralogical analyses (SEM/EDS and DRX). The
146 protocol for collecting the samples on site was as follows:

- 147 - Removal of the support of a batch of specimens (reminder: a batch is made up of 12
148 plates), by handling only the attachment wires;
- 149 - Individual removal of each specimen, by severing the wires, in a sterile 100 mL pot
150 containing distilled water with 9.5 g/L NaCl added (to adjust the osmotic pressure of the
151 fresh water).

152 3.1 Bacterial enumeration

153 The bacteria most implicated in the degradation processes encountered in wastewater
154 systems are Sulpho-Oxidizing Bacteria (SOB) [5; 16; 17]. This is why two microbial groups
155 were considered: the "general" flora, which considers all bacterial flora, cultivated from a
156 culture medium composed of yeast extract and tryptone, and the sulpho-oxidizing flora
157 (SOB) cultivated using Gübner's specific culture medium [20]. The composition of these two
158 media is recalled in the appendix. The cultures were carried out on a solid support agar in
159 Petri dishes [21]. At each sampling time, the following protocol was applied in the
160 laboratory, for each cement grade:

- 161 - Ultrasonic agitation (sonication - 40 kHz) of one of the 100 mL pots containing one of the
162 plates, for 5 minutes, to detach the biofilm on the surface;
- 163 - Inoculation of three to five Petri dishes with a 10 μ L suspension taken from the pot using
164 an inoculating loop;
- 165 - After 3 days of incubation at 37 °C, we counted the number of colonies on each of the
166 Petri dishes and calculate the average in Colony Forming Units (CFU/cm²). This value is
167 defined as the average number of bacteria counted, multiplied by the ratio of the
168 volume of the dilution to the volume collected, all in relation to the total outer surface
169 area of the specimen.

170 It should be noted that bacterial enumeration was not always possible after incubation,
171 especially for the general medium, because the cultures formed a dense cluster that
172 prevented the distinction of individual Colony Forming Units. In this case, the corresponding
173 measures were not considered in the analysis of the results.

174 3.2 Accessible porosity water

175 The porosity of cementitious materials is a factor that influences bioreceptivity and
176 biodeterioration [6]. It represents the voids (pores) of a material which can be filled with
177 water under ordinary pressure and temperature conditions. It was measured using the
178 method of "three weighings" [22] of each cementitious grade aged 28 days on 5 plates,
179 before exposure of the samples on the different sites (initial porosity, see Tab. II). Given the
180 presence of hydrates, the samples were dried (dry mass) at room temperature by successive
181 immersion in two organic solvents (isopropanol, then diethyl ether) [23].

182 3.3 Analysis by Scanning Electron Microscopy (SEM)

183 The upper surface of one of the three cement paste plates was observed as soon as it was
184 removed, using a Scanning Electron Microscope under controlled pressure (SEM-PC- Quanta
185 650 FEG from FEI) coupled with an Energy Dispersion Spectroscopy (EDS from Bruker),
186 without prior metallization of the surface. The observations were carried out under partial
187 water vapour pressure of 100 Pa and with an acceleration voltage of 15 kV allowing the
188 observation of microorganisms.

189 3.4 X-Ray Diffraction Analysis (XRD)

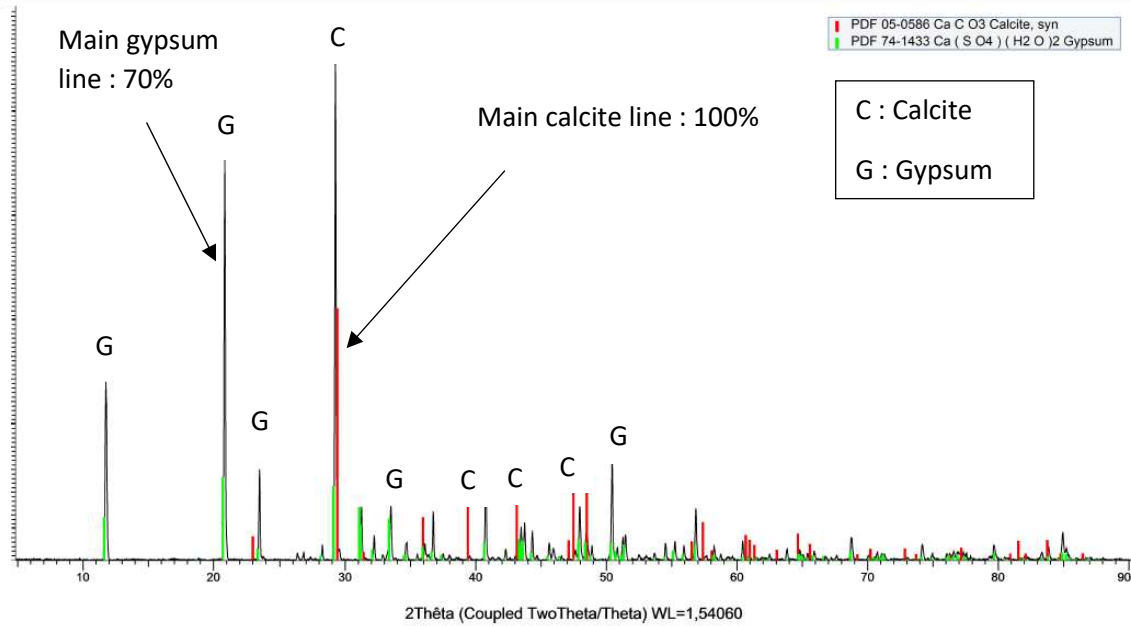
190 The upper surface mineralogical analysis of another of the three plates was carried out by
191 DRX to detect and identify the crystalline phases present and their possible evolution over
192 time. The apparatus used is a Bruker D8 Advance diffractometer CuK α equipped with a
193 germanium monochromator. The sample did not undergo any preliminary preparation to
194 avoid destabilizing the crystallized phases present on the surface. The measuring parameters
195 used were as follows: i) angular range 5 to 90°, ii) angular measuring step of 0.021°, iii)
196 counting time in 158 s steps. The quantification of the phases present, by the Rietveld
197 method in particular, was not considered relevant, given the data acquisition conditions
198 (preferential orientations, representativeness of the investigated volume, choice of relevant
199 interpretation parameters, mineralogical complexity, etc.). However, failing this, a "relative
200 quantification" was carried out based on the height of the main line of the mineral phases
201 detected on the diffractograms. In concrete terms, on each diffractogram, the highest main
202 line was normalized to 1 (100%) and the height of the other main lines was relativized with
203 respect to this standard (Fig.3). Thus, a relative quantification could be established of the
204 phases present over time. Tab. III recalls the value in 2 θ of the main line of the main mineral
205 phases detected in this study.

206 Table III: Value in 2 θ of the main line of the mineral phases observed in this study

Mineral phase	2 θ
Calcite CaCO ₃	29.406
Vaterite CaCO ₃	33.027
Gypsum CaSO ₄ .2H ₂ O	20.722
Bassanite CaSO ₄ .0.5H ₂ O	29.757
Ettringite 3CaO.Al ₂ O ₃ .3CaSO ₄ .32H ₂ O	35.023
Katoite 3CaO.Al ₂ O ₃ .6H ₂ O	33.533
Gibbsite Al(OH) ₃	18.266
strätlingite 2CaO.Al ₂ O ₃ .SiO ₂ H ₂ O	21.239

207

(Coupled TwoTheta/Theta)



208
209 Fig. 3: Diffractogram of the CSA1 at the S2u site at 10 months.

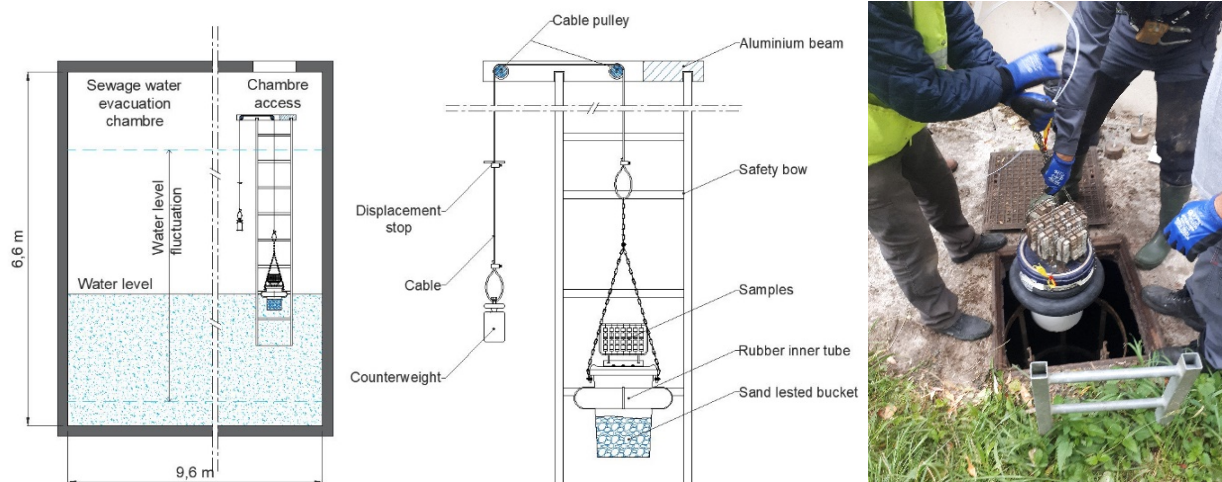
210 4. Study environments

211 4.1 Exhibition sites

212 The exhibition of the samples in a real environment was carried out in partnership with the
213 Greater Nancy Metropolitan Area (Meurthe-et-Moselle, France) which authorized access to
214 different parts of its sewer network. Four sites with different environmental conditions were
215 selected. These sites were chosen because they offer a diversity of effluent types
216 (stormwater, hospital wastewater, domestic, etc.). They also differ in the size of the pipes
217 and the flows that pass through them, ranging from a few litres to several cubic metres of
218 effluent per minute. Sites supplied by rainwater are subject to strong surges during rainy or
219 stormy episodes. Finally, the position of the samples in relation to the effluent is also a
220 significant difference. The samples were placed in such a way that they would not come into
221 contact with any other material, nor with the effluents. A summary description of the sites
222 and exposure devices is given below:

223 - Site S1: a large lifting/pumping chamber (approximately 700 m³) designed to collect and
224 store effluents from a sector of the city (wastewater and rainwater), before their
225 periodic discharge to the treatment plant. This site is characterized by periods without
226 significant fluctuations in level (balanced supply and discharge flow) and sudden events
227 (rain, storm) causing the level in the chamber to rise rapidly. This context constitutes a
228 probable source of H₂S production and release (stable period followed by a turbulent
229 regime) which can however be disrupted by monthly maintenance cleaning. In order to
230 keep the samples close to the surface of the liquid at all times, while following its
231 fluctuations, a floating support surrounded by a protective air chamber was constructed
232 (see diagram and photo Fig. 4). It was fixed in the centre of a crinoline providing access
233 to the chamber, by means of a cable connected to a counterweight passing through two
234 pulleys attached to a removable crossbar anchored in the top of the crinoline. In this

235 way, the samples were always located at the same distance from the effluent, whatever
236 the context and the hydraulic regime in the chamber;



237

238 Fig. 4: Description of the sample installation at site S1.

- 239 - Site S2: It is located at the exit of a large hospital centre equipped with a separation
240 network. The wastewater network (S2u) carries black water and domestic water with a
241 moderate constant flow. The air circulating in this network has the characteristic smell
242 of medical products. Although recent, the concrete, steel bars and the underside of the
243 cast iron manholes are corroded. The rainwater network (S2p) only carries water from
244 the roofs of buildings and car parks. Both networks are connected to the same unitary
245 network downstream (~ 500 meters) so that the odours from the sewer network are fed
246 back into the rainwater network, but with a low intensity. The samples were placed on
247 metal frames with feet (see Fig. 2) resting on the manhole gutters;
- 248 - Site S3: this is a large collector-interceptor (\varnothing 1500 mm) which transports the effluents
249 brought by several secondary networks to the treatment plant. The flows in this
250 collector-interceptor are always abundant, and increase in rainy periods. The samples
251 were placed on a metal frame hung on the first bar of one of the access manholes, at a
252 distance of at least three meters from the usual liquid level;
- 253 - Site S4: This is a standard unitary network in the city centre. As before, the samples
254 were placed on metal frames resting on the manhole gutter (Fig.2).

255 In addition, a series of "control" samples, prepared under the same conditions as before,
256 were kept in the laboratory. The plates were placed in a closed tank partially filled with tap
257 water maintained at 20°C, on a grid fixed above the water level. Similarly, a sample of each
258 cement paste was immersed for 24 hours in 4 solutions of sulphuric acid (of chemical origin)
259 of 200mL, with concentrations of 0.1, 0.5, 1 and 5 mol/l respectively, in order to identify (by
260 DRX as previously) the products that appeared and were potential markers of an attack.

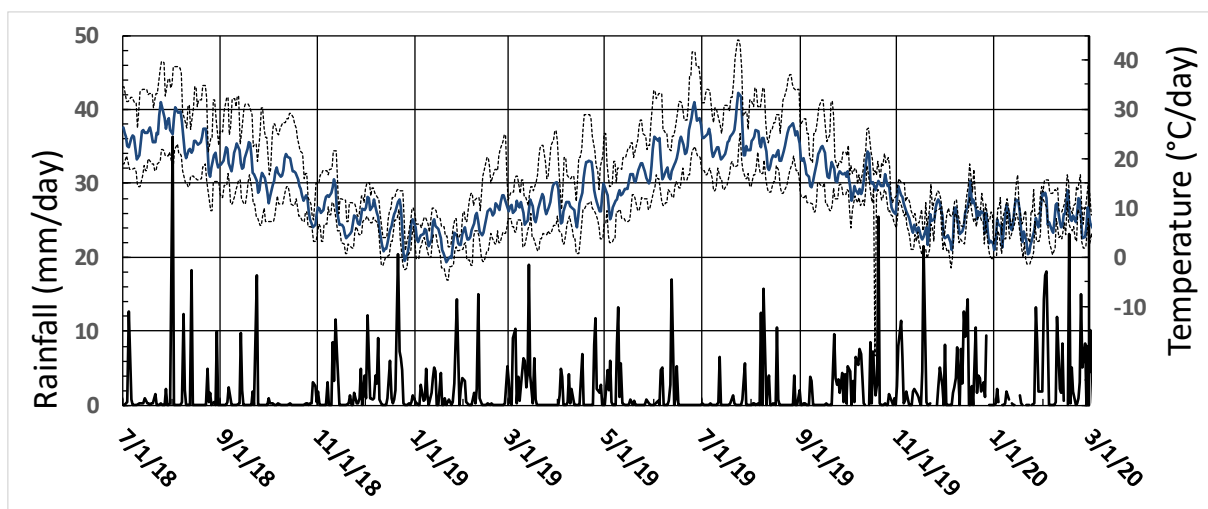
261 4.2 Environmental conditions

262 Owing to the lack of technical means available, the exposition sites could not be equipped
263 with recorders allowing the punctual or continuous measurement of the prevailing
264 environmental conditions within them (temperature, humidity, pH, H₂S content, etc.).
265 However, these parameters did not appear to be indispensable because:

- 266 - There is a great diversity of contexts, each with its own configuration: the
- 267 measurements carried out on (only) four sites would therefore not have been truly
- 268 exhaustive;
- 269 - The aim was to compare the bioreceptivity of the cement grades among themselves, in
- 270 different real contexts subject to numerous random fluctuations over a long period of
- 271 time;
- 272 - The real interdependence between the numerous environmental parameters that can
- 273 have an influence, and the bioreceptivity of materials, is not yet (well) known;
- 274 - Samples are placed in the system without ever being in contact with the effluent.
- 275

276 However, some qualitative information could be collected, in particular concerning the
 277 presence of H₂S on site S1. Indeed, the agents who regularly maintain this station are
 278 equipped with a detector for this gas, in accordance with safety regulations. They stated that
 279 there were variable levels of H₂S, mainly at the bottom of the structure, without however
 280 having recording the levels. This presence thus requires the complete ventilation of the unit
 281 before entering it.

282 Finally, the detailed temperature and precipitation records for the city of Nancy over the
 283 study period were retrieved. The result of their processing, in terms of maximum, minimum
 284 and average daily temperature and cumulative daily rainfall, is shown in Fig. 5. These two
 285 parameters influence the study environments as a whole, since it can be assumed that there
 286 is a direct dependence between rainfall and flow rates (at least for the combined sewerage
 287 and stormwater networks) and the temperatures outside and inside the networks.



288
 289 Fig. 5: Daily temperature and precipitation records during the study period.

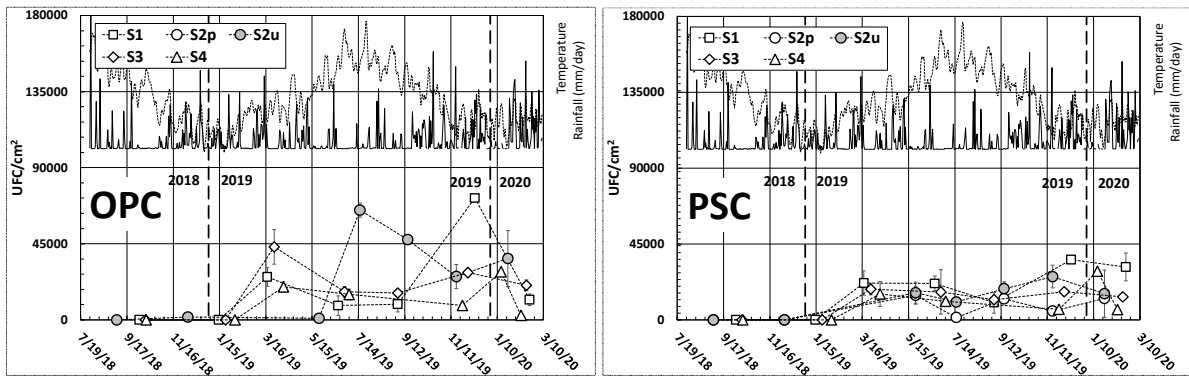
290 The samples were exhibited at the beginning of September 2018. The following two months
 291 were characterised by very low rainfall and rather high temperatures which are
 292 characteristic parameters for the end of the summer period in Nancy. The period between
 293 the end of October and the beginning of February was marked by numerous and sometimes
 294 intense rainy episodes with temperatures generally below 10°C. The period from February to
 295 mid-May had lower rainfall, with a few (long) periods without rain and temperatures rising
 296 gradually. From mid-May to the end of August, rainfall was low, with the exception of a few
 297 thundery episodes, and the temperature reached high values that occasionally exceeded
 298 40°C. These evolutions are typical of the overall climatological cycle of the region.

299 **5. Results**

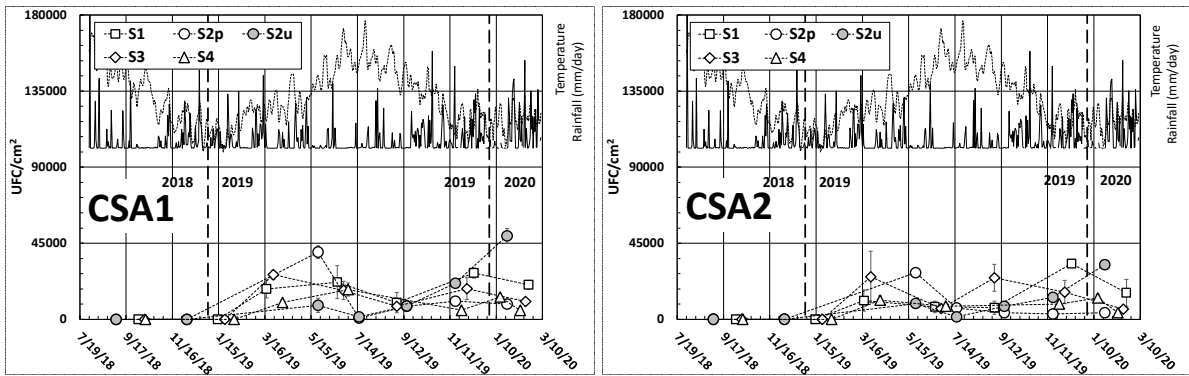
300 **5.1 Bacterial colonization**

301 The evolution of the bacterial population density over time, for the different cements and at
302 different sites, in Colony Forming Units per cm² (CFU/cm²), is shown in Fig. 6 for the general
303 microbial flora, and Fig. 7 for the SOB. These figures also include the meteorological
304 chronicles described above (mean daily temperature, cumulative daily rainfall) in order to
305 establish possible correlations. The enumeration of the general flora on the surface of the
306 samples is an indicator of interest of the bioreceptivity of the support material, without
307 necessarily leading to biodegradations. The enumeration of BSO is an indicator of possible
308 degradations of the support material, considering their metabolism involving the production
309 of sulphuric acid. Note that the (thin) lines connecting the various points only serve to
310 improve the reading of the graphs. They in no way indicate the existence of any linear
311 evolution of bacterial colonization between two consecutive observations, nor that the
312 bacterial population could not have evolved in this period of time above or below the point
313 values measured. Furthermore, the standard deviation associated with the measurements (5
314 Petri dishes) sometimes shows a rather strong dispersion around the mean (points),
315 indicating the difficulty of the precise characterization of this parameter (bacterial
316 colonization). Finally, it is important to note that no bacterial colonization by SOB was
317 detected on the samples stored in the laboratory (see § 4.1), whatever the age of storage
318 and the cement grade. This result is logical because there is no H₂S present and therefore no
319 presence of sulphur phases necessary for the development of these bacteria. On the other
320 hand, as the samples and the medium are non-sterile, colonization by the general medium
321 was indeed detected as early as 6 months, but its enumeration was not always possible
322 (dense cluster, cf. § 3.1). These two pieces of information have not been included in the
323 graphs.

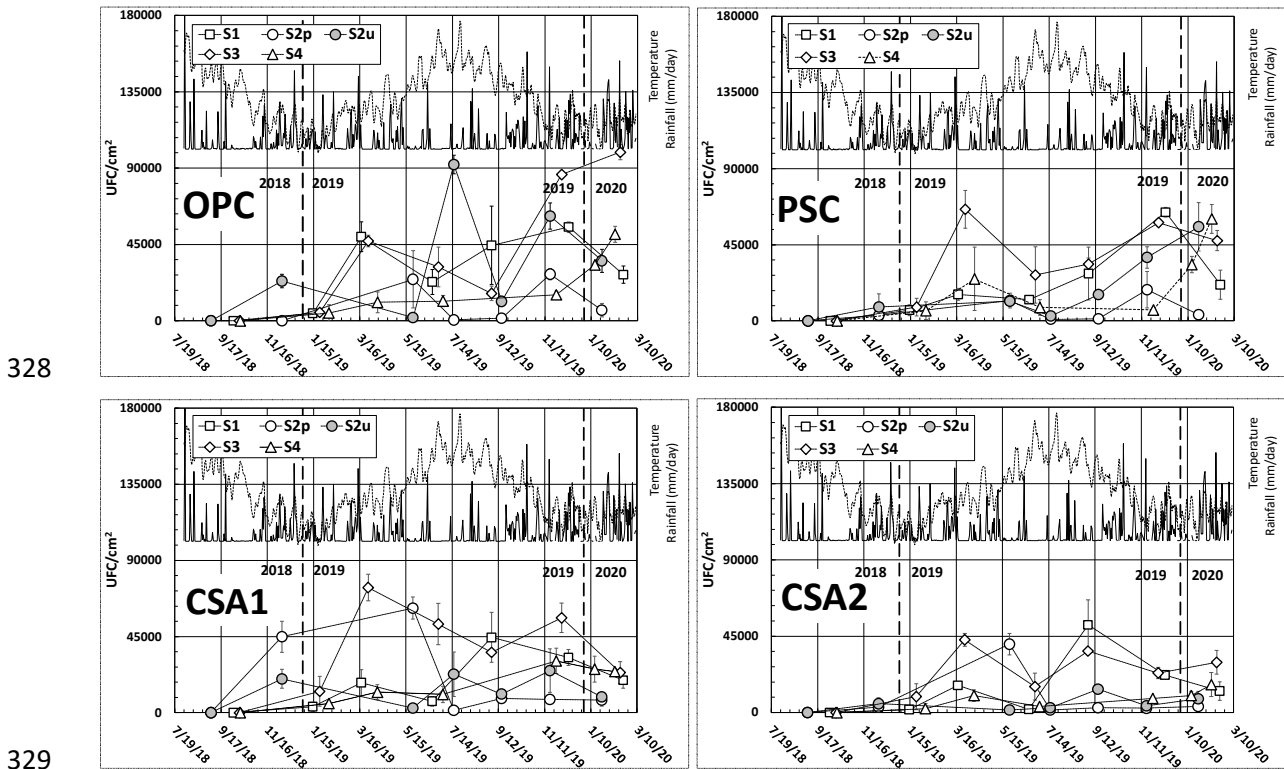
324



325



326 Fig. 6: Cultivable bacterial population in Ordinary General Medium (agar medium), in CFU
 327 (Colony Forming Units), for the four cement grades and the five exposure sites.



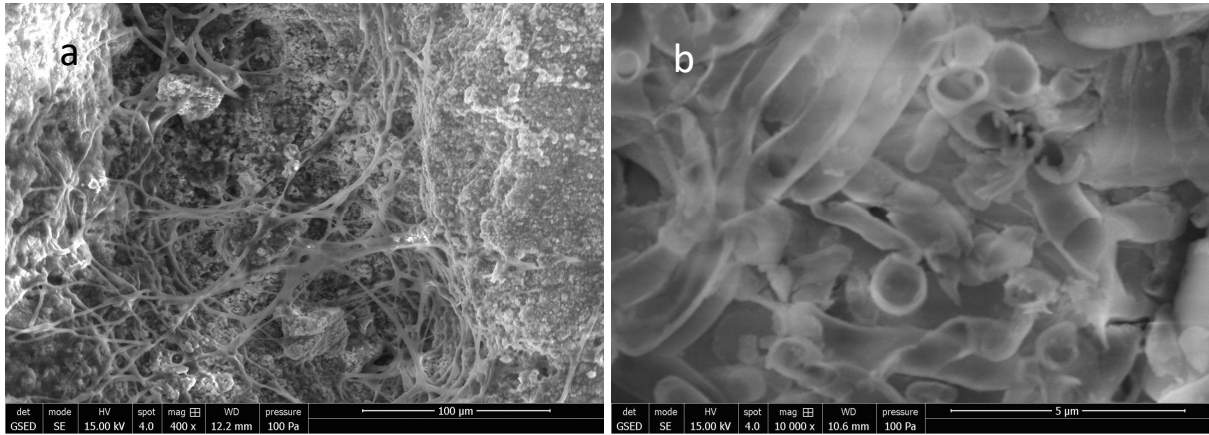
330 Fig. 7: Cultivable SOB bacterial population, in CFU (Colony Forming Units), for the four
 331 cement grades and the five exposure sites.

332 5.2 Microscopic analyses (SEM - PC)

333 As indicated previously, at each deadline the upper surface of one of the plates of each
 334 cement grade taken from the different sites was observed without prior preparation with a
 335 Controlled Pressure Scanning Electron Microscope (see § 3.3). The images (and the
 336 associated EDS spectra) provided essentially qualitative data, which could complete the DRX
 337 analyses. Above all, they made it possible to observe bacterial colonization, which was
 338 detected very often. These colonies can be scattered or more concentrated, at the scales of
 339 observation.

340 Thus, before 4 months no bacteria are visible and only traces of biofilm are locally present,
 341 particularly on OPC pastes. However, the results of the bacterial cultures (Figs. 6 and 7) show
 342 the presence of SOB as early as 2 months, at least on certain cement grades and on certain
 343 sites. This "contradiction" can be attributed to the complexity of the surface topography and
 344 the presence of accumulated (observed) secondary layers which can create a "masking
 345 effect" for the (rare) bacteria present at this stage.

346 After 4 months of exposure, the biofilm is often well developed and bacteria are observed
 347 more frequently. By way of example, for this period, Fig. 8a shows the biofilm observed on
 348 the OPC cement paste at site S1. Fig. 8b shows stick and shell bacteria observed on the CSA1
 349 paste at site S2u.



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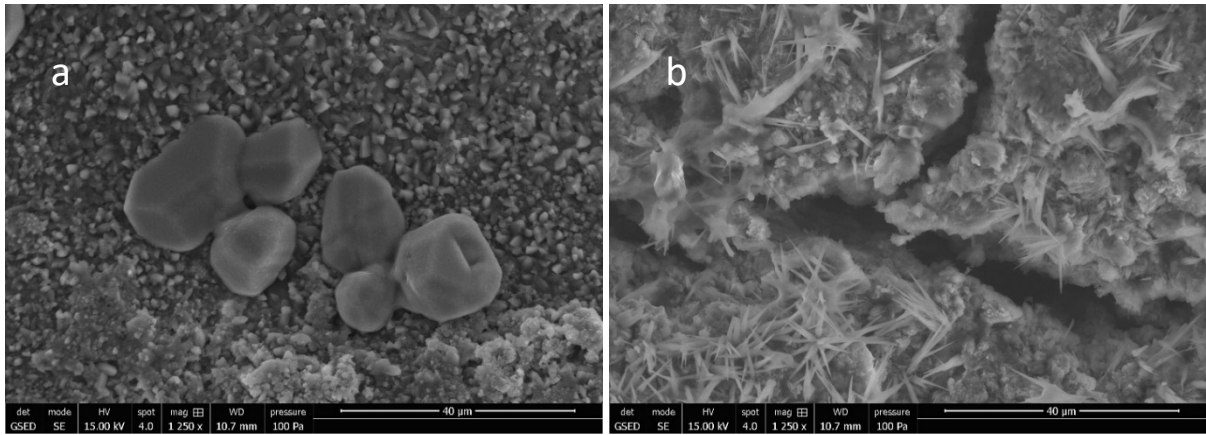
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Figure 8: SEM-PC image, 4-month term.

a. Dehydrated Biofilm on the OPC paste. Site S1.

b. Bacteria in sticks and shells on CSA1 paste. Site S2u.



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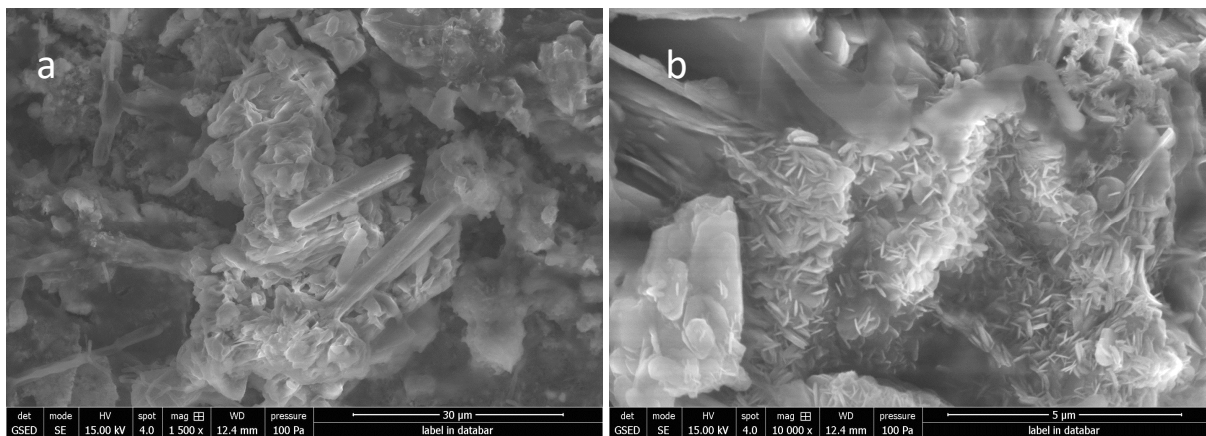
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Figure 9: SEM-PC image. S2u site at 4 months.

a. Orthorhombic sulphur crystals observed on the PSC.

b. Needles of ettringite on CSA2 paste.



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Figure 10: SEM-PC image. S2u site at 18 months.

a. Gypsum crystals observed on CSA2.

b. Bacteria in shells embedded in micrometric gypsum crystals on CSA2.

363 Other mineral phases are also observed. As an example, and still after 4 months of exposure
364 at the S2u site, Fig. 9a shows orthorhombic sulphur single crystals (confirmed by EDS)
365 appearing on the surface of the PSC paste. It should be remembered that the PSC cement
366 initially contains a low content of sulphur contributed by the setting regulator ($S_2^- \leq 0,7\%$
367 according to the data sheet), and that this sulphur is theoretically mobilised in the afm
368 phases. The sulphur detected probably comes from the surface crystallisation of the
369 dissolved H_2S discharged into the aerial part of the pipe, following an increase in its
370 concentration in the effluents (from 20 ppm to 50 ppm for example), caused by the action of
371 Sulphate-Reducing Bacteria (SRB) [24]. EDS analyses associated with SEM analysis of other
372 cement grades from the same site and from other sites, for all timeframes from 4 months
373 onwards, often reveal the presence of sulphur, sometimes in significant quantities.

374 However, the processing of these data was not considered relevant because they are only
375 one-off analyses that do not reflect the "reality" of the whole surface. These indications can
376 therefore only be considered qualitative. They do however show the presence of this
377 characteristic element of the action of SRB.

378 Fig. 9b shows ettringite needles appearing on the surface of the CSA2 paste. As there is little
379 work in the literature describing the products associated with the action of sulphuric acid
380 generated by the metabolism of SOB for CSAs, a direct sulphuric acid attack at different
381 concentrations (0.1; 0.5; 1 and 5 mol/L) was carried out on the different hardened cement
382 pulps in the study. After 24 hours of immersion, SEM observations revealed, on all the
383 pastes, the presence of gypsum on the surface, although in smaller quantities for OPC and
384 CSA2, compared to PSC and CSA1. This difference therefore reflects these two cements'
385 better resistance to sulphuric acid. It should be noted, however, that OPC presents
386 significant dissolutions, especially at low concentrations (0.1 and 0.5 mol/L) [17].

387 Figs.10a and b show gypsum crystals of different sizes, associated with bacteria (b), observed
388 on CSA2 at the S2U site at 18 months.

389 Similar SEM analyses were performed at all other times in the study and on all samples.
390 Qualitatively, a fairly good correlation was observed between surface bacterial activity
391 (biofilm, bacteria, sulphur, ettringite) and bacterial counts, i.e. more biofilms and visible
392 bacteria in spring and autumn than in summer and winter.

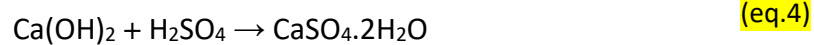
393 5.3 Mineralogical analysis (DRX)

394 The upper surface of the samples was analysed by DRX at 28 days of age and then at each
395 observation date. The diffractograms (not presented here) were interpreted according to the
396 approach presented in § 3.4.

397 The initial measurements (28 days) show that calcite is (already) the predominant mineral at
398 the surface, for all four cement grades. It shows the rapid action of carbonation, a factor that
399 causes a decrease in surface pH favourable to the colonization of micro-organisms
400 (neutrophilic bacteria $6 \leq pH \leq 8$) [23]. For OPC and PSC, hydrated phases characteristic of
401 these cements were also identified, notably portlandite (CH), CSH gels (composition close to
402 that of crystallised phases such as tobermorite ($C_5S_6H_{11}$) and jennite ($C_9S_6H_{11}$)). For CSA1 and
403 CSA2, anhydrous phases were also detected, notably ye'elimite ($Ca_3Al_6O_{12}CaSO_4$) and
404 mayenite ($Ca_{12}Al_{14}O_{33}$). Subsequently, these phases were no longer observed.

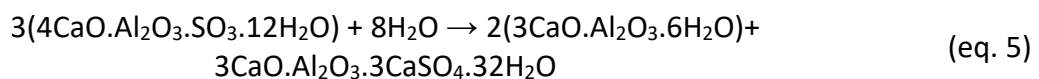
405 Fig. 11 shows the mineralogical changes observed between 2 months and 18 months of
 406 exposure, for the four cement grades and for the five sites:

407 - For OPC, calcite is almost the only mineral detected at all deadlines. Gypsum, however,
 408 appears after several months of exposure, at least at some sites (12 months for S4, 16
 409 months for S2u, Fig. 10a and b). For S3, this phase appears around 8 months and then
 410 develops until it becomes preponderant around 18 months. Its appearance is attributed
 411 to the probable reaction between the sulphuric acid produced by the bacteria (SRB) and
 412 the calcite present on the surface (eq. 1; 2; 3) or the Portlandite present below the
 413 surface (eq. 4);



414

415 - For PSC, calcite is also the predominant mineral, except for S3 at 18 months, where, as
 416 for OPC, gypsum becomes the majority on the surface. This phase (and sometimes semi-
 417 hydrate or Bassanite) also appeared on the other sites, in variable and evolving
 418 proportions, except for S4 where it is absent. On site S2p, ettringite was detected after 1
 419 year of exposure. The gypsum necessary for its formation is well present (detected) and
 420 hydrated calcium aluminate is in principle present in this type of cement (C_3AH_6 ,
 421 C_4AH_{12}). Vaterite was also detected on this cement paste and on all sites. The
 422 appearance of this unstable calcium carbonate, often associated with organic activity, is
 423 perhaps related, here too, to bacterial activity [17]. Finally, katoite ($3\text{CaO} \cdot \text{Al}_2\text{O}_3 \cdot 6\text{H}_2\text{O}$)
 424 appeared at around 16 months on the S2p site. It could result from the decomposition
 425 of monosulfoaluminate into katoite and ettringite (eq. 5) [3];



426

427 - For CSA1, calcite (and vaterite) is also the predominant mineral, except for S3 at 18
 428 months, where gypsum is again the predominant surface mineral. Contrary to Portland
 429 cements (OPC and PSC), gypsum and other hydrated phases appear earlier and in
 430 significant (relative) quantities. These are gibbsite ($\text{Al}(\text{OH})_3$), ettringite, bassanite and
 431 even also strätlingite ($2\text{CaO} \cdot \text{Al}_2\text{O}_3 \cdot \text{SiO}_2 \cdot 8\text{H}_2\text{O}$), the origin of which is to be compared with
 432 the mineralogical composition of this belitic sulphate-aluminous cement, manufactured
 433 with a quantity of water well below the stoichiometric value. It can be assumed here
 434 that these hydrates developed on the surface over time, depending on the more or less
 435 favourable hygrometry prevailing within the networks. The presence of gypsum on the
 436 surface (resulting from the indirect action of SOB bacteria) would also participate in
 437 these reactions;
 438 - For CSA2, a comparable scenario was found, but with a lower intensity and the absence
 439 of strätlingite.

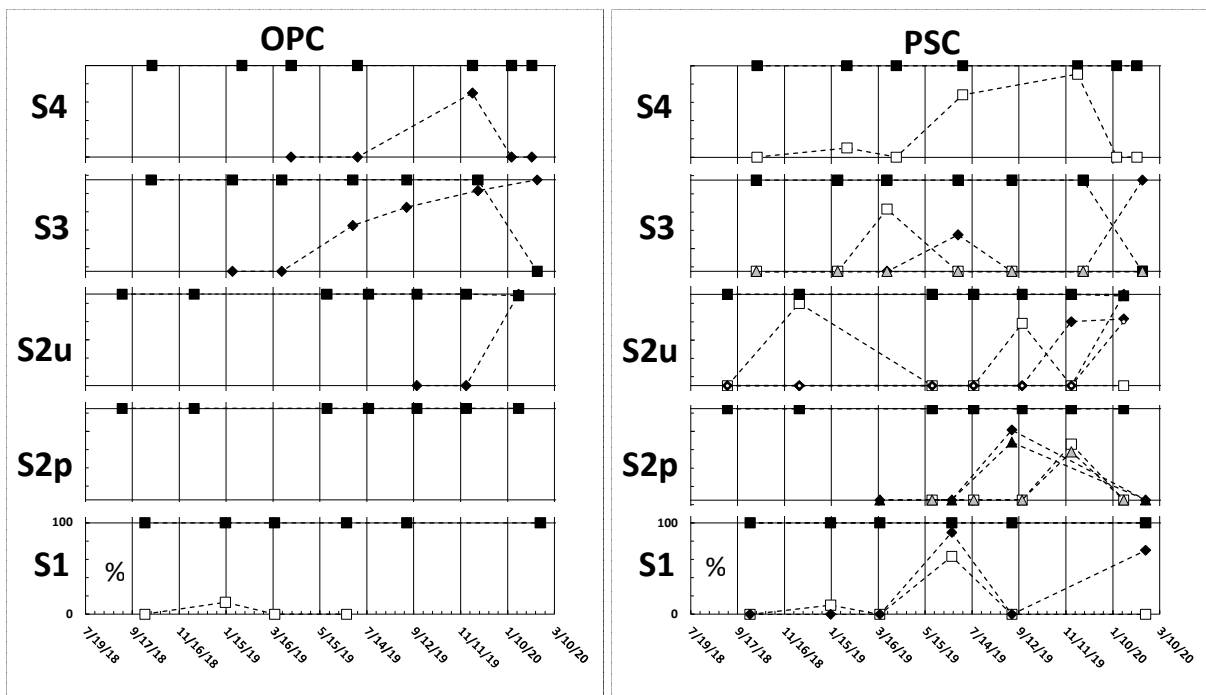
440 Finally, it should be noted that the evolution over time does not follow a regular progression
 441 (for CSA cements in particular), but is instead cyclical, in accordance with the seasons, i.e.
 442 with the intensity of the bacterial activity.

443 Here we should recall that the samples that underwent the direct sulphuric acid attack
 444 showed the abundant presence and accumulation of gypsum on their surface (confirmation
 445 of the reaction between the sulphuric acid and the carbonates and hydrates of the cement
 446 matrix).

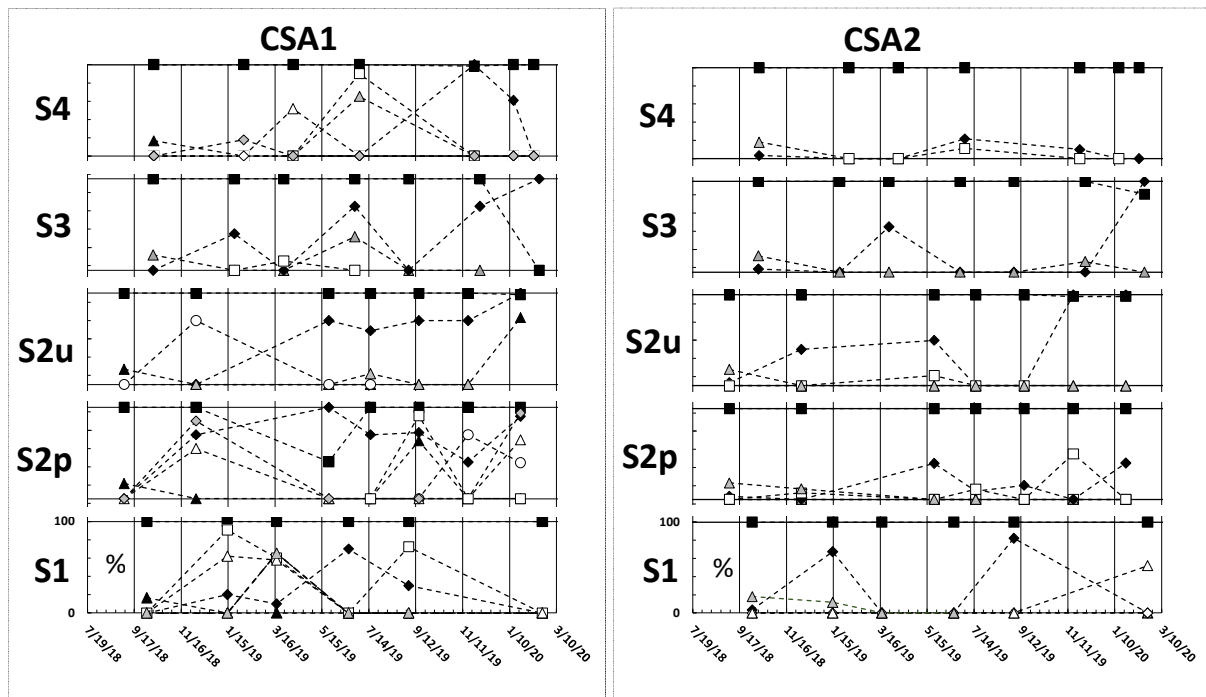
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450

451 Fig. 11: Exploitation of DRX analyses of the 4 cement grades at the 5 exposure sites for 18
 452 months.

453 6. Discussion

454 The evolution of the biocolonization (linked to bioreceptivity) of the surface of the different
 455 cement grades during the 18 months of observation, described by bacterial counts, SEM
 456 images and surface mineralogical analyses, can be compared with the evolution of the
 457 environmental and climatic conditions surrounding the selected experimental hydraulic
 458 structures, and also with the mineralogy of the supports. Over a climatic cycle of 18 months,
 459 starting with the placement of the specimens in September, relations between the action of
 460 the SRB and SOB and the mineralogical evolution of the colonized surfaces can thus be
 461 established. These observations also refer to the work mentioned earlier in the paper which
 462 described the relations between environmental conditions and bacterial colonization but in
 463 different experimental contexts. The parameters concerned are:

464 - Flow variations within the networks, between dry periods and rainy episodes (storms,
 465 rainfall,) leading to turbulent flows following stable regimes. Their consequences would
 466 be the following:

467 o Between September and November, a slow hydraulic regime with low rainfall. This
 468 regime is favourable to the metabolization of H_2S by the SRB;

469 o Between November and February, sustained rainfall leading to an often turbulent
 470 flow regime favourable to the release of H_2S , deposition of sulphur on the support
 471 materials. This nutritive substrate of the SOB leads to an increase in colonization
 472 densities on all sites for all cement grades;

473 o Between February and April, more scattered rainfall and dry periods, leading to a
 474 stabilization of the colonization density;

475 o Between April and June, heavier rainfall increased the moisture content, which led to
476 an increase in colonization, probably also due to the release of H₂S metabolised by
477 the SRBs in the previous period;

478 o Between June and September, the density of colonization decreased overall, given
479 the absence of rainfall over long periods. Occasional thunderstorms can temporarily
480 reactivate bacterial activity.

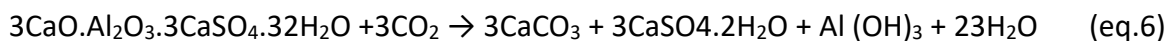
481 - The temperature/humidity couple:

482 o Temperature acts as a catalyst to accelerate reaction speeds and temperature
483 variations influence the rhythm of bacterial development. Herisson [17] reports that
484 an increase of 10°C doubles the bacterial activity. Thus, for example, the increase in
485 the density of colonization observed between March and May can also be attributed
486 to the increase in average temperature over this period, from 7.5°C to around 12.5°C;

487 o Humidity is essential for the development of bacteria [5]. Sites S1 and S3 generally
488 have constant humidity. In the other sites, as the temperature increases and relative
489 humidity decreases, the density of colonization also decreases. This is because the
490 production by the bacteria and therefore the concentration of H₂S is influenced by
491 the outside temperature.

492 - The mineralogical composition of the carrier materials:

493 o The cement pastes studied, formulated with w/c ratios that do not allow complete
494 hydration, initially present on the surface the expected hydrated or even anhydrous
495 phases: portlandite, C-S-H, etc. for Portland cements; ettringite, gibbsite, bassanite,
496 strätlingite, ye'elimite, mayenite for CSA. The rapid carbonation of these hydrates,
497 especially portlandite and ettringite, results in calcite (the dominant mineral from the
498 first analyses), gypsum and gibbsite covering the surface and causing a decrease in
499 pH. This evolution then allows colonization by neutrophilic micro-organisms. The eq.
500 6 recalls the reaction between ettringite and atmospheric CO₂ [3; 25]:



501

502 o This then explains the presence of gypsum on CSAs. However this is also explained by
503 the action of the sulphuric acid produced by the SOBs (a reaction verified by
504 immersing the cementitious pastes in baths of dilute sulphuric acid). This was
505 observed with the appearance of gypsum on OPC (50% with a colonization density of
506 40,000 UFC/cm²) and PSC (40% with a colonization density of 25,000 UFC/cm²), from
507 the 6th month of exposure on the S3 site, and later for the other sites (consistent
508 counting from this time onwards with a quantity of gypsum that increased
509 concomitantly - up to 100% with a colonization density of 100,000 UFC/cm² for OPC).
510 For CSA, the quantity of gypsum was found to increase from this time onwards.
511 However, the samples composed of CSA2 were still found to have the lowest
512 bacterial population density. This behaviour can be related to the higher alumina
513 (and iron) content of this cement (Tab. II), which would lower the surface pH to low
514 values (3 or 4), thus inhibiting the development of bacteria [10].

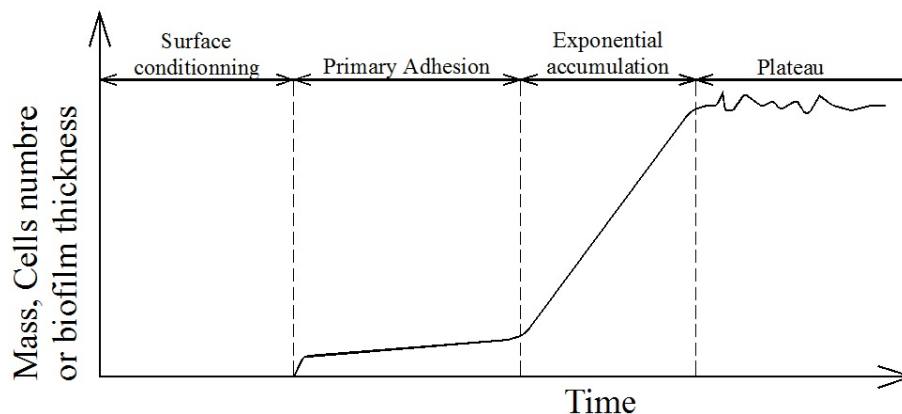
515 - The typology of the exhibition site.

516 o Site S1 has *a priori* the most favourable conditions for the development of SRB and
 517 the production of sulphuric acid (proven presence of SO₂). However, few, if any (OPC)
 518 secondary products were detected. This can be explained by the regular and
 519 intensive cleaning and dredging of the site, which probably interrupts the
 520 metabolisation of H₂S by the SRB, and thus the process of SOB development (Fig. 7).
 521 The gypsum, gibbsite and katoite detected on CSA cements (or even on PSC) then
 522 essentially come from the mineralogical evolution of the hydrates characterising this
 523 cement (eq. 1, 2, 3, 4);

524 o The S2p site reveals strong disparities between cements. Thus, OPC is characterised
 525 only by the presence of calcite and PSC by the additional and late appearance of
 526 gypsum, ettringite, and even katoite. On the other hand, the surface of CSA1 hosts
 527 early phases in relatively large quantities that are associated with the nature of this
 528 cement (gypsum, gibbsite, ettringite, strätlingite). CSA2 shows a similar "reactivity",
 529 but with a more moderate relative intensity. The most influential parameter is
 530 therefore the environmental conditions which are not very favourable to bacterial
 531 development on this site (rainwater);

532 o The S2u site displayed rather similar behaviour, with a very late appearance of
 533 gypsum for Portland cements, and earlier for CSA1 and CSA2, with a higher relative
 534 quantity for CSA1. The other phases are in smaller relative quantities, indicating that
 535 the gypsum present on CSA1 is partly due to bacterial activity linked to the
 536 favourable conditions of the site (varied chemistry of the effluents, flow,
 537 temperature, H₂S). The presence of gibbsite on CSA2 could lead to a decrease in the
 538 density of colonization by inhibiting or delaying the development of biofilm and the
 539 proliferation of bacteria on the surface of these materials [26].

540 o Sites S3 and S4 correspond to more regular configurations of sewer networks and
 541 displayed quite similar responses, with a rather late and progressive appearance of
 542 gypsum associated with the activity of SOB. The context at these sites is probably less
 543 favourable for the formation and development of biofilm (surface conditioning phase
 544 of the materials), and thus for the primary adhesion of the SOB, which precedes their
 545 exponential development, as shown in Fig. 12 [5; 6].



546
 547 Fig. 12 Formation and evolution of biofilm over time [5].

548 Finally, the results of these analyses show that, in sewer systems, the cementitious nature of
 549 the cement between Portland and CSA does not strongly discriminate against the
 550 bioreceptivity of the pastes made with these materials after 18 months exposure. As soon as

551 the pastes are carbonated and the environmental conditions (rainfall, temperature) allow
552 the SRB bacteria to produce hydrogen sulphide, the development and activity of the sulpho-
553 oxidising BSO bacteria takes place. The sulphuric acid produced then combines with the
554 carbonated or aluminous phases present to give gypsum and secondary ettringite in
555 particular. Therefore environmental conditions control the kinetics and intensity of the
556 reactions more than the intrinsic nature of the cements.

557 Nonetheless, the effect of the secondary phases formed and their accumulation over time
558 on the physical properties of the cements and concretes (porosity, capillarity, permeability,
559 resistance, etc.) remains to be assessed.

560 7. Conclusion

561 This work focuses on the bioreceptivity of cementitious materials in sewerage networks. To
562 this end, hardened cement pastes of the same porosity, made with two Portland cements
563 (OPC and PSC) and two Calcium Sulpho-Aluminate Cements, were placed on five different
564 sites of a real urban network for 18 months. Bacterial counts (general environment and
565 Sulpho-Oxidizing Bacteria), surface mineralogy and SEM observations were carried out at
566 fairly regular intervals (about 2 months). These measurements show that these different
567 cements are bioreceptive from two months of exposure, at levels that are fairly comparable
568 and variable over time. The rapid carbonation of the surfaces clearly allows bacteria to
569 establish themselves (biofilm). Subsequently, secondary mineral phases are
570 detected/observed on the surface and these are mainly gypsum and ettringite. They
571 probably result from the reaction between sulphuric acid generated by sulphate-oxidising
572 bacteria and calcite and other anhydrous or hydrated cementitious phases present. Analysis
573 of the results obtained finally shows that the quantity of phases thus formed after 18
574 months exposure depends more on environmental conditions (temperature, rainfall,
575 sometimes human intervention) - which control the production of sulphur linked to the
576 activity of the Sulphate-Reducing Bacteria living in the effluents - than on the very nature of
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656

657 Appendix

658

Appendice 1: General bacterial culture medium

Product	Quantity
Tryptone	10 g
Meat extract	5 g
Agar-agar	15 g
Sodium chloride: NaCl	9.5 g
Demineralised water	1000 mL

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Appendice 2: Gübner's SOB-specific growing medium

Product	Quantity	
Sodium thiosulphate pentahydrate: Na ₂ S ₂ O ₃ .5H ₂ O	5.00 g	
Calcium chloride: CaCl ₂ .2H ₂ O	0.13 g	
Ammonium chloride: NH ₄ Cl	1.00 g	
Manganese sulphate monohydrate: MgSO ₄ .7H ₂ O	1.02 g	
Potassium dihydrogen phosphate: KH ₂ PO ₄	0.40 g	
Potassium monohydrogen phosphate: K ₂ HPO ₄	0.60 g	
Ethylene-Diamine-Tetra-Acetic Acid: EDTA	0.003 g	
Biotin – 10 mL at 0.22 µL	0.0244 g	
Trace element solution	5 mL	
Demineralised water	985 mL	
Sodium chloride: NaCl	9.5 g	
Agar-agar	15 g	
Water	1000 mL	
Trace element solution		
Product	Quantity	
Disodium Ethylene-Diamine-Tetra-Acetic Acid: Na ₂ EDTA	50.0 g	5 mL
Zinc sulphate heptahydrate: ZnSO ₄ .7H ₂ O	2.2 g	
Calcium chloride dihydrate: CaCl ₂ . 2H ₂ O	7.34 g	
Manganese (II) chloride tetrahydrate: MnCl ₂ .4H ₂ O	5.06 g	
Iron (II) sulphate heptahydrate: FeSO ₄ .7H ₂ O	5.0 g	
Ammonium molybdenum: (NH ₄) ₆ .Mo ₇ O ₂₄	1.1 g	
Copper (II) sulphate pentahydrate: CuSO ₄ .5H ₂ O	1.57 g	
Cobalt (II) chloride hexahydrate: CoCl ₂ .6H ₂ O	1.61 g	
Sterilised distilled water - pH-adjusted with KOH	1000 mL	

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