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1 Assessment of acid mist on mortar biodeterioration simulating the wall of Jardim da
2 Princesa, the National Museum of Rio de Janeiro, Brazil

3

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35 ABSTRACT

36 This work aimed to evaluate the effect of acid mist on microbial communities
37 developed on mortar with same composition from the wall of the National Museum in
38 Rio de Janeiro. Five autochthonous microbial groups were inoculated on coated with
39 acrylic paint and uncoated mortar surfaces and submitted to acid mist or water mist for
40 100 days. A heterogeneous biofilm was well developed in uncoated coupons under acid
41 mist. Molecular analysis revealed that the bacterial communities were structured
42 according to the incubation condition. The impact of coating on bacterial community
43 structure was more important than the acid mist effect. The eukaryotic community
44 structure was not affected by the acid mist, whereas the impact of coating by paint has
45 been observed. The alpha diversity indexes also varied according to the treatment. H'
46 and D were higher for uncoated conditions, particularly when submitted to acid mist.
47 Actinobacteria, Chloroflexi, Cyanobacteria, Firmicutes and Proteobacteria were
48 dominant. Additionally, eukaryotic community was not affected by the acid mist and
49 paint and Tremellomycetes dominated.

50

51 Keywords: National Museum of Rio de Janeiro; bioreceptibility; biodeterioration; acid
52 mist.

53

54

55

56 1 INTRODUCTION

57 Ancient and modern monuments are prone to deterioration caused by variable
58 interaction of biotic and abiotic factors. They are composed by a wide range of
59 materials of different texture and structure, features determining their ability to resist
60 weathering (Miller et al., 2012, Mapelli et al., 2012; Saddique & Chahal, 2011; Sarró et
61 al., 2006). The occurrence of biodeterioration in historical monuments is multifactorial
62 and not fully understood because it is a multifactorial phenomenon that affect
63 differently according to the type of materials, the location, and the colonization by
64 microorganisms (Mihajlovski et al., 2017; Perez-Alvaro, 2016). Anthropogenic
65 activities accelerate the biodeterioration processes by depositing organic pollutants on
66 material surfaces that stimulates microbial growth (Manso et al., 2014; Saiz-Jimenez,
67 1995). In addition, abiotic conditions, such as marine spray and acid rain, also favor the
68 microbial development increasing the deterioration of materials, including modern
69 materials such as mortar (Traversetti et al., 2018; Shirakawa et al., 2010; Mitchell &
70 Gu, 2000).

71 Bioreceptibility, concept proposed by Guillitte (1995), aims to characterize materials
72 according to their ability to be colonized by microorganisms, providing useful
73 information on the resistance of materials to biodeterioration. Several studies have
74 characterized the bioreceptibility of construction materials such as stones (Nuhoglu et
75 al., 2017), granite (Vásquez-Nion et al., 2018) or mortar (García-Esparza et al., 2017;
76 Jurado et al., 2014), including both covered and uncovered surfaces (Shirakawa et al.,
77 2011; Gaylarde et al., 2011). Cement based materials contain organic adjuvants
78 increasing bioreceptibility, i.e. favoring the primary colonization by microorganisms
79 involved in the biodeterioration process (De Muynck et al., 2009). However, further
80 studies are required in order to evaluate the parameters influencing the microbial
81 colonization and development dynamics in cements-based materials, knowledge of
82 paramount importance for the implementation of preservation strategies (Wiktor et al.,
83 2011; Reis-Menezes et al., 2011).

84 Regrettably, little attention has been devoted to the biodeterioration effects on Brazilian
85 heritage monuments. Brazil, a 520-year-old country, own many historical monuments
86 and cities that are strengthened by biodeterioration, particularly context of global
87 changes (Gaylarde et al., 2018; Shirakawa et al., 2013; Lutterbach et al., 2013). The
88 present work aimed to evaluate the biodeterioration process on material similar to that
89 found in the walls of the Jardim da Princesa, located in the National Museum of Rio de

90 Janeiro (NM). In a perspective of preservation, the study examined the effect of acid
91 mist on microbial colonization of mortar coated and uncoated coupons. We developed
92 an experimental microbial ecology approach combining elemental and chemical
93 analysis, imagery and biology tools, in order to follow microbial colonization and the
94 effects on the construction materials. The gained information will be useful for the
95 implementation of countermeasure for the preservation of Brazilian cultural heritage
96 monuments.

97

98 2 MATERIALS AND METHODS

99 2.1 Site of study

100 The National Museum (NM), located in the Quinta da Boa Vista, São Cristóvão
101 neighborhood, (22° 90 '57 "S, 43° 22' 65" W) is the oldest scientific institution in Brazil
102 and the largest museum of natural history and anthropology in Latin America. It was
103 created by King D. João, the 6th on June 6, 1818, serving as Residence of the Brazilian
104 Imperial Family until 1889. The NM is located in an urban area, surrounded by heavy
105 vehicle traffic. The place is well wooded, however, characterizing a zone that is usually
106 very humid (60-70%).

107

108 2.2 Biomass collection

109 The samples were taken from two equidistant points on the northern wall of the Jardim
110 da Princesa in the NM, by means of scraping, with a sterile scalpel (Medbisturi 23) and
111 placed in penicillin bottle with 50 ml reducing solution, composed by NaCl 0.85 g/L,
112 ascorbic acid 0.132 g/L and 4 mL of rezasurin solution 0.0025 g/L (Dias et al., 2016).

113 Quantification of seven microbial groups were carried out as follows (Table S1):
114 aerobic and facultative (not “strict anaerobe”) heterotrophic bacteria, aerobic and
115 facultative acid producing bacteria, iron-oxidizing bacteria, filamentous fungi, and
116 photosynthetic microorganisms, using spread plate and Most-Probable-Number (MPN)
117 techniques (Genhardt et al., 1994). Significant differences between conditions were
118 determined by analysis of variance (ANOVA).

119

120 2.3 Biodeterioration experiments

121 The assay was carried out in a glass reactor (Figure 1), measuring 60 cm wide x 30 cm
122 deep x 40 cm high, was fitted with an artificial lighting system with two 60W tubular
123 fluorescent lamps (Famastil). Fifty-six coupons similar to the composition of mortar

124 found in the wall of the NM were prepared with the mixture of sand (20 g), cement (30
125 g; commercial cement composed by tricalcium and dicalcium silicate, 10-70%; calcium
126 iron-aluminate, 5-15%; calcium sulfate, 2-10%; tricalcium aluminate, 1-15%; calcium
127 carbonate, 0-5%; magnesium oxide, 0-4%; calcium oxide, 0-0.2%), clay (5 g) and
128 distilled water (13 mL). The coupons were molded into rectangles (4.0 cm long x 2.0
129 cm wide x 1.0 cm high) and cylinders (1.5 cm in diameter x 1.0 cm in height). Half of
130 the coupons were coated with commercial acrylic paint (Coral, Rio de Janeiro, Brazil).
131 Biofilm development on the coupons was investigated in triplicate over 100 days
132 ($28^{\circ}\text{C}\pm 3^{\circ}\text{C}$ and humidity ranged 30-50%), under 8 conditions (Table S2, Condition 1-
133 CUW: Absence of microorganism, uncoated coupons and water mist; Condition 2-
134 CCW: Absence of microorganism, coated coupons and water mist; Condition 3-CUA:
135 Absence of microorganism, uncoated coupons and acid mist; Condition 4-CCA:
136 Absence of microorganism, coated coupons and acid mist; Condition 5-MUW: Presence
137 of microorganism, uncoated coupons and water mist; Condition 6-MCW: Presence of
138 microorganism, coated coupons and water mist; Condition 7-MUA: Presence of
139 microorganism, uncoated coupons and acid mist; Condition 8-MCA: Presence of
140 microorganism, coated coupons and acid mist).

141 The inocula (50 mL) corresponded of a consortium prepared by mixing equal volumes
142 of suspensions of five isolated groups from the wall samples: aerobic heterotrophic
143 bacteria (10^{10} CFU/mL), anaerobic heterotrophic bacteria (10^8 cells/mL), iron-oxidizing
144 bacteria (10^8 CFU/mL), total fungi (10^6 CFU/mL) and photosynthetic organisms (10^5
145 Cells/mL). The consortium was inoculated on the coupon surface at a rate of 0.25
146 mL/cm² in 3 applications at day 0, 7 and 14.

147 In order to monitor the climatic conditions of the system, a hygrometer (INCOTERM®)
148 was used, and a humidifier (Multitoc Health) coupled with a thermostat (ATMAN, AT-
149 100) were used to humidify the system. In order to simulate the composition of rainfall
150 in the city of Rio de Janeiro, an acid mist was prepared containing: NaNO₃ (1607
151 µg/L), CaSO₄.2H₂O (5281 µg/L), K₂SO₄ (1570 µg/L), NH₄Cl (1118 µg/L), NaCl (3680
152 µg/L), NaOH (212 µg/L), pH 5.12 (Hernández et al., 2010). The control mist was made
153 with sterilized distilled water, pH = $6,0 \pm 0,5$.

154 Microbial quantification was carried out in the one hundred day of incubation. Aerobic
155 heterotrophic bacteria, iron-oxidizing bacteria and total fungi were determined using
156 spread plate technique and photosynthetic microorganisms, facultative heterotrophic

157 bacteria, aerobic and facultative acid producing bacteria were estimated using MPN
158 technique.

159

160 2.4 Composition and structure of materials by X-Ray Fluorescence (XRF) and
161 Scanning Electron Microscopy (SEM)

162 The oxides composition of the wall and coupons were determined by X-ray
163 fluorescence (XRF) spectrophotometry (WDS, AXIOS Panalytical). Both samples, from
164 wall and coupons were prepared in a VANEON automatic press (20 mm mold, P = 20
165 ton and t = 30s), using boric acid 1:0.5 (2 g of the sample to 1.0 g of boric acid) as
166 binder. The results are semi-quantitative expressed in %, calculated as 100%
167 standardized oxides. The determination of the loss by calcination (LBC) of the samples
168 was done in Leco TGA-701 equipment. First heating ramp 10°C/min at 25-107°C (for
169 moisture removal), second ramp 40°C/min at 107-1000°C (for LBC determination). The
170 test was completed after 3 identical weighing sequences. The measurement was
171 performed under normal air conditions.

172 The images of the coupons were performed by Scanning Electron Microscopy (SEM,
173 TM3030 PLUS). The samples had been previously metallized with gold/carbon with
174 argon as the entrainment gas, using BAL-TEC (SCD 005, Sputter Coater), with a 30
175 mA current for 150 seconds under pressure between 10¹-10² Bar.

176

177 2.5 Microbial molecular analysis

178 2.5.1 DNA extraction

179 Biofilm samples for molecular analysis were collected from coupons, using sterile
180 scalpel, and suspended in 50 mL sterile reducing solution (NaCl 0.85 g/L, ascorbic acid
181 0.132 g/L and 4 mL/L of rezasurin solution at 0.0025 g/L) in Penicillin bottles. 10 mL
182 of solution was filtered through a membrane (0.2 µm Supor® Membrane, Microfunnel
183 Filter Unit, Life Sciences). Membranes were cut before DNA extraction using
184 UltraClean Soil DNA Isolation Kit (MOBIO) according to the manufacturer's
185 instructions.

186

187 2.5.2 DNA amplification, purification and Terminal Restriction Fragment Length
188 Polymorphism (T-RFLP) analyses

189 16S rRNA genes were amplified using Bacteria primers 63F (5'-
190 CAGGCCTAACACATGCAAGTC-3') and 1387R (5'-GGGCGGWGTGTACAAGGC-

191 3') (Marchesi, et al., 1998). 18S rRNA genes were amplified using universal eukaryotic
192 primers Euk1 (5'-CTGGTTGATCCTGCCAG-3') and Euk516R (5'-
193 ACCAGACTTGCCCTCC-3') (Sogin & Gunderson, 1987). The reaction mix (25 μ L
194 final volume) contained 12.5 μ L of AmpliTaq Gold 360 Master Mix 2x (Life
195 Technologies), 0.2 μ M of each primer and 5 μ L of DNA template. PCR conditions were
196 as follows: initial denaturation (95°C for 10 min) followed by 35 cycles of denaturation
197 (95°C for 30 s), annealing (58°C for 45 s for Bacteria 16S rRNA; 56°C for 45 s for 18S
198 rRNA gene) and extension (72°C for 1 min). PCR products were purified using an
199 Illustra GFX 96 PCR Purification Kit (GE Healthcare) and digested with 3 U of Alu1
200 and Rsa1 (New England Biolabs) for 16S rRNA and with 3 U of Alu1 and HaeIII (New
201 England Biolabs) for 18S rRNA, in a total volume of 10 μ L at 37 °C for 3 h. T-RFLP
202 analysis was performed as previously described (Stauffert et al., 2009). Datasets were
203 constructed using a minimum peak height of 100 fluorescence units. T-RFLP profiles
204 were aligned by identifying and grouping homologous fragments and normalized by
205 calculating relative abundances of each terminal restriction fragment (T-RFs) from
206 height fluorescence intensity, using T-REX software (Culman et al., 2009).
207 In order to compare bacterial and eukaryotic communities (T-RFLP patterns data)
208 according to treatment and time, two-dimension nonmetric multidimensional scaling
209 ordination (NMDS) based on Bray–Curtis distances was performed using Primer 6
210 software (Primer E, Plymouth, UK software). The significant differences between
211 groups were tested with an analysis of similarities (ANOSIM) and similarity
212 percentages test (SIMPER) performed with Primer 6.

213

214 2.5.3 High-throughput sequencing and data analysis

215 The bacterial and eukaryotic diversity of 36 samples was analyzed by Illumina MiSeq
216 sequencing. MiSeq sequencing was performed at the GeT platform (Toulouse, France).
217 The primers used were 104F (GGCGVACGGGTGAGTAA) (Wang & Qian, 2009) and
218 530R (CCGCNGCNGCTGGCAC) (Weisburg et al., 1991) for amplification of 16S
219 rRNA gene, and EUK1A (CTGGTTGATCCTGCCAG) and 516R
220 (ACCAGACTTGCCCTCC) (Sogin & Gunderson, 1987) for amplification of 18S
221 rRNA gene. PCR conditions included 10-min heating step at 95 °C followed by 30
222 cycles of 95°C - 30 s, 56°C - 30 s, 72°C - 45 s, and final extension at 72°C for 10 min
223 for Bacteria and 10 min heating step at 95°C followed by 35 Cycles of 95°C - 45 s,
224 56°C - 45 s, 72°C - 1 min, and final extension at 72°C for 10 min for Eukaryote.

225 QIIME2 software (Quantitative Insights Into Microbial Ecology) was used to analyze
226 gene sequence reading of 16S rRNA and 18S rRNA (Bolyen et al., 2018). The
227 taxonomy assignment (97% similarity) was performed comparing reference sequences
228 to SILVA (v132) database of known 16S and 18S rRNA genes (Quast et al., 2013). The
229 alpha-diversity analysis (Shannon-Weaver, Simpson, Pielou's evenness and Good's
230 coverage) was performed on rarefied count data using the apha_diversity.py script in
231 Qiime2. Differential analysis and barplots were performed with SHAMAN (SHiny
232 application for Metagenomic Analysis, shaman.c3bi.pasteur.fr) (Quereda et al., 2013).
233 The complete dataset was deposited in the NCBI Sequence Read Archive (SRA)
234 database (SUB7892718). It is available under the Bioproject ID PRJNA655939.

235

236 3 RESULTS

237 3.1 Characterization of coupons and wall

238 The composition of coupons was similar to that of the National museum (NM) wall,
239 with the same composition of nine oxides (Table S3), with only a difference in the
240 percentage of Al₂O₃.

241

242 3.2 Characterization of biodeterioration

243 The impact of biodeterioration on the surface of the coupons and the formed biofilm
244 were characterized by SEM observations after 100 days of incubation (Figure 2). The
245 coated surfaces (conditions 2-CCW, and 4-CCA) showed smoother surfaces than
246 uncoated conditions (conditions 1-CUW and 3-CUA), which presented roughness and
247 deposits of inorganic and organic matter, dust and other debris (Figure 2). Noteworthy,
248 hyphae were observed in uncoated condition treated with acid mist (condition 7-MUA)
249 associated with the greenish and orange color as shown in Figure 3 and observed by
250 optical microscopy (data not shown), probably due to the development of
251 photosynthetic microorganisms and by the activity of iron-oxidizing bacteria,
252 respectively.

253 Biofilms of coated conditions were less dense compared to the uncoated coupons
254 (conditions 5-MUW and 7-MUA). In addition, the coupons in condition 5 (MUW)
255 showed the bulkier structure and less adherent than coupons in condition 7 (MUA). The
256 presence of microorganisms altered the porosity of the material, as revealed by grooves
257 observed in the surface of coupons. The coated coupons submitted to water mist
258 (condition 6-MCW) presented depigmentation, despite the lower microbial

259 development. Cracks were observed in the surface of coated coupons (conditions 6-
260 MCW and 8-MCA), suggesting microbial activity.

261 After scraping off the biofilms, coated coupons presented more visual alterations than
262 uncoated coupons, with numerous spots over all the surface. In addition, whitening was
263 observed in the coupons exposed to acid mist (condition 8-MCA), suggesting growth of
264 fungal mycelium and/or presence of iron-oxidizing bacteria. On the other hand, the
265 color of the paint changed to bright yellow, quite different from the coupons not
266 exposed to the acid mist (condition 6-MCW).

267 The description of deterioration aspects and the quantification of microbial groups are
268 summarized in Table 1. Under all inoculated tested conditions, heterotrophic bacteria
269 were dominant, with a prevalence of aerobic communities. Also, the numbers of aerobic
270 acid producing bacteria were similar under all conditions, with values around 10^2
271 cells/cm². The largest biomass was observed on the uncoated coupons (conditions 5-
272 MUW and 7-MUA). The paint was not inhibiting microbial growth, except that of the
273 facultative acid-producing and photosynthetic microorganisms (conditions 6-MCW and
274 8-MCA) for which the number of cells/cm² was reduced by at least 10^3 units (cells/cm²)
275 in comparison to the uncoated coupons (ANOVA, p-value < 0.001) irrespective of the
276 atmospheric treatment (Table 1, conditions 5-MUW and 7-MUA). The presence of
277 coating (condition 6-MCW) inhibited also the development of the iron-oxidizing
278 bacteria (10^3 cells/cm², Table 1) (ANOVA, p-value < 0.001) and the total fungi (10^3
279 CFU/cm²) (ANOVA, p-value < 0.001) but the inhibition effect was suppressed in
280 presence of acid mist (condition 8-MCA).

281

282 3.3 Microbial communities structure and composition

283 Bacterial and eukaryotic community structures were monitored by T-RFLP based on
284 16S and 18S rRNA genes analyses for all incubation conditions at the end of the
285 experiment (Figure 4). The T-RFLP analysis revealed that the bacterial communities
286 were structured according to the incubation condition after 100 days of incubation
287 (Figure 4A), the impact of coating (ANOSIM R= 0.833) on bacterial community
288 structure was more important than the acid mist effect (R=0.289). The eukaryotic
289 community structure (Figure 4B) was not affected by the acid mist (R=0.130), whereas
290 the impact of coating by paint (R=0.389) has been observed. These results suggest that
291 paint coating contributed to the selection of specific microbial species resulting in the
292 dominance of some microbial groups.

293 In order to determine the impact of coating and acid mist on the microbial assemblages
294 in the biofilms developed in coupons, bacterial and fungal communities composition
295 were characterized by high throughput sequencing of 16S and 18S rRNA genes
296 respectively. For bacterial community analysis, a total of 485567 16S rRNA gene reads
297 were obtained for the 12 samples. The obtained valid reads after preprocess filtering and
298 normalization were reduced to 6983 sequences for each sample, clustered into 126
299 amplicon sequence variants (ASVs). For eukaryotic community, 267103 18S rRNA
300 gene reads were obtained. After filtering and normalization, and focusing only in Fungi,
301 4599 sequences were obtained for each sample, clustered into 23 ASVs. Good's
302 coverage values, nearly 100% in each sample, indicated that the main bacterial and
303 fungal diversity were assessed.

304 The alpha diversity indexes [Shannon (H'), Simpson (D) and equitability] varied
305 according to the treatment for bacterial and fungal communities (Table 4). H' and D
306 were higher for uncoated conditions, particularly when submitted to acid mist.
307 Furthermore, microbial communities from coated conditions were dominated by some
308 microorganisms, as indicated by equitability index (Pielou's evenness).

309 The dataset consisted of 5 distinct bacterial phyla: Actinobacteria, Chloroflexi,
310 Cyanobacteria, Firmicutes and Proteobacteria. Among the most abundant genus in
311 uncoated coupons with water mist (condition 5-MUW, Figure 5A), *Microbacterium* was
312 the most abundant (comprising 40.1 % of the classified community), followed by
313 *Brevundimonas* (34.9 %), *Roseomonas* (10.6 %), *Skermanella* (6.5 %) and
314 *Methylobacterium* (4.5 %). In uncoated coupons submitted to acid mist (condition 7-
315 MUA) a modification of the bacterial community composition was observed (Fig. 5A)
316 showing the dominance of Cyanobacteria belonging to *Leptolyngbya* (38.8 %) and
317 *Nostoc* (5.4 %) genus. A decrease of the abundance of *Microbacterium* (6.7 %) and the
318 detection of *Micromonospora* (7.6 %), both belonging to Actinobacteria, was also
319 observed. Proteobacteria dominated the bacterial communities of coated coupons
320 (conditions 7-MUA and 8-MCA), with *Methylobacterium* (Fig. 5A) as the most
321 abundant genus in both water and acid mist conditions (conditions 7-MUA and 8-MCA,
322 81.2 % and 91.1 % respectively).

323 Fungal communities were represented by two phyla, Ascomycota and Basidiomycota.
324 Uncoated coupons (conditions 5-MUW and 7-MUA) were dominated by the class
325 Tremellomycetes (68.8 % and 69.8 % respectively) followed by Sordariomycetes (31.2
326 % and 30.1 % respectively) irrespective of the mist condition (Figure 5B). Coated

327 coupons (conditions 6-MCW and 8-MCA, Figure 5B) were dominated by
328 Sordariomycetes (97.3 % and 89.4 % respectively). At the ASVs level, for the two
329 fungal dominant classes (Figure 5C), data analysis showed that conditions (coat and
330 mist) had an impact on the distribution of ASVs abundances but not on the composition.

331

332 4 DISCUSSION

333 4.1 Biodeterioration of mortar

334 The composition of coupons was overall similar to that of the National museum (NM)
335 wall supporting that coupons can be used to simulate NM wall. However, a difference
336 in the aluminium oxide content was observed. Aluminium toxic effects on
337 microorganisms has been showed (Piña & Cerventes, 1996) as well as tolerance
338 capacities (Piotrowska-Seget et al, 2005; Ahmed et al, 2020). The concentration of
339 Al₂O₃ observed in the NM wall (400 ppm) can be considered as high and inhibitory for
340 microbial growth (Piña & Cerventes, 1996). This suggests that microorganisms
341 colonizing the wall are tolerant and able to be adapted and grow in the coupons, result
342 obtained in the present study support this hypothesis. Moreover, calcination loss showed
343 differences, probably due to the presence of organic matter on the wall from the paint,
344 and/or microorganisms, exopolysaccharides and other particles deposited by wind and
345 rain (Hartmann et al., 2014).

346 The impact of biodeterioration of mortar samples was greater on the surface in uncoated
347 coupons than on the coated coupons. The uncoated coupons were more susceptible to
348 bioreceptibility due to their structural features that favor microbial development as
349 previously described (Silva et al., 2019; Viana et al., 2017).

350 In addition, the uncoated coupons also showed thicker biofilm and microbial
351 morphological diversity, showing heterogeneous development, compared to the coated
352 coupons, suggesting the influence of paint on microbial growth and biofilm
353 development, without complete inhibition. These observations are in agreement with
354 several studies showing that external painted surfaces support a diverse microbial
355 population (for review, see Gaylarde et al., 2011). Furthermore, a previous study
356 showed that microbial activity had promoted material surface deterioration, resulting in
357 an increase in the porosity and a decrease in its resistance and subsequent crumbling
358 (Papida et al., 2000), as well as desquamation was reported as result of volume change,
359 matrix penetration and release of microbial metabolites such as organic and inorganic
360 acids (Coutinho et al., 2013).

361 The positive effect of acid mist on the biofilm adhesion has been observed irrespective
362 of the presence of paint, suggesting that acid mist promote microbial growth and
363 consequently, material biodeterioration. These observations corroborate the hypothesis
364 that acid mist causes more damage to the material than humidity caused by the water
365 mist, because it keeps the surface moister beyond the acids act on surface deterioration.
366 These observations are in accordance with a study that demonstrated the impact of
367 atmospheric conditions on the degree of material deterioration (Gil et al., 2010).
368 Additionally, microbial influence was greater than that of the atmosphere in the process
369 of surface deterioration, due to production of acids and other metabolites (Pitzurra et al.,
370 2003).

371 The results of microbial quantification are consistent with the SEM observations that
372 showed a larger biofilm development on uncoated coupons. It has been reported that the
373 paint on materials (coated) impact in microbial growth (Gaylarde et al., 2011). Most of
374 microorganisms in our study have not been totally inhibited, in the exception of
375 photosynthetic microorganisms (condition 6-MCW). This observation was in
376 contradiction with several studies that reported the presence of Cyanobacteria on
377 painted surface, particularly in tropical and subtropical countries (Shirakawa et al.,
378 2011; Gaylarde & Gaylarde, 1999). It was also reported that fungi constitute the major
379 biomass on painted surfaces in Latin America (Gaylarde & Gaylarde, 2005).

380 It is important to highlight that the presence of acrylic paint (condition 6-MCW) also
381 inhibited the development of two groups: iron-oxidizing bacteria and the total fungi and
382 the inhibition effect was suppressed in the presence of acid mist (condition 8-MCA).
383 Overall, the acid mist has favored microbial development, especially on the coated
384 coupons (Table 1, condition 5-MUW vs condition 7-MUA and condition 6-MCW vs
385 condition 8-MCA). The same observation was previously demonstrated by some studies
386 (Flemming & Wingender (2001), Costerton et al. (1995).

387

388 4.2 Microbial communities structure and composition

389 The culture-based approach used in the present study allow to obtain diverse microbial
390 communities in the different conditions. The composition of these microbial
391 communities is representative of the microbial community observed *in situ* (data not
392 shown). Indeed, the *in situ* community was mainly dominated by Cyanobacteria (46%,
393 relative abundance), Proteobacteria (20%, relative abundance) and Actinobacteria (17%,
394 relative abundance), also observed as main bacterial phyla in coupons from the

395 biodeterioration experiment. The fungal *in situ* community was dominated by the
396 phylum Basidiomycota, as observed in coated coupons. The comparison of
397 environmental and culture-derived microbial communities showed that the diversity of
398 culture-derived communities reflected selectivity of the culture media and/or the culture
399 condition, as previously described (Pédrón et al., 2020).

400 The five phyla observed in the present study have been found dominant in recent works
401 evaluating the biodeterioration of cultural heritage monuments (Lepinay et al., 2018;
402 Adamiak et al., 2018; Chimienti et al., 2016). Among these phyla, Actinobacteria have
403 been identified as dominant in biofilms of historic monuments (Adamiak et al., 2018;
404 Mihajlovski et al., 2017). It is well known that they inhabit more effectively stone and
405 mural paintings, by their ability to use various nitrogen and carbon sources (Sterflinger
406 et al., 2013). Surprisingly, in the present study these microorganisms were impacted by
407 the acid mist. In fact, these microorganisms are known to play a crucial role in the
408 decomposition of organic compounds and environmental pollutants, and thus expected
409 to resist and adapt to the acid mist. It is a premise that the atmospheric condition inhibits
410 both the size and diversity of the biofilm microbial community (Mitchell & Gu, 2000).

411 The acid mist promoted the development of Cyanobacteria on uncoated coupons.
412 Cyanobacteria are known to support the growth of heterotrophic bacteria and fungi by
413 being a nutrient source, thus constituting a key element in the biofilm formation and
414 maintenance. Cyanobacteria have been described to be involved in biodeterioration of
415 cultural heritage monuments (Dyda et al., 2018; Herrera & Videla, 2004) and to be
416 dominant in biofilms in different climatic sites (Gaylarde & Gaylarde, 2005).
417 Additionally, Proteobacteria dominated the bacterial communities of coated coupons,
418 indicating their resistance to coating and supporting their implication on material
419 biodeterioration (Lepinay et al., 2018; Mihajlovski et al., 2017; Jurado et al., 2014).

420 Concerning fungal diversity, the uncoated coupons were dominated by
421 Tremellomycetes and Sordariomycetes, whereas coated coupons were dominated by
422 Sordariomycetes. These observations suggested that fungi affiliated to this class are
423 adapted to the presence of paint. Our results also support the impact of paint and acid
424 mist in the fungal community composition (e.g. TRFLP analysis, Figure 4B) showing
425 stronger impact of paint than acid mist in the fungal community composition. Fungi
426 affiliated to the Sordariomycetes class have been identified as common colonizers of
427 paint biofilms in Brazil (Shirakawa et al., 2010; Shirakawa et al., 2002) and recently

428 identified in mortar coatings of a historical building (Guerra et al., 2019). Furthermore,
429 they have been described as involved in concrete deterioration (Wei et al., 2013).

430 The use of paint reduced the fungal population. Some antimicrobial additives found in
431 paints, such as titanium dioxide (Amorim et al., 2020), zinc pyrithione (Reeder et al.,
432 2011), ammonium-aqueous solution, ethane 1,2-diol and kaolin (Chukwujike and Igwe,
433 2019) act to prevent fungal adsorption and consequently avoid the establishment of
434 biofilm. The paint used in the present work, one of the most sold in Brazil, is composed
435 by antifungal, antialgal and antibacterial additives, according to manufacturers.

436

437 5 CONCLUSIONS

438 Our results indicated that microbial deterioration will always occur, even at different
439 degrees, irrespective of the coating and moisture condition. The experimental approach
440 applied in the present work is proposed as a strategy to address bioreceptibility. This
441 approach has the advantage to be less time-consuming than *in situ* studies, not
442 destructive, using lab controlled systems with the possibility of extrapolation to the real
443 ecosystem.

444

445

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772 Table 1. Deterioration and microbial density found in coupons after 100 days, in the *in situ* and in the inoculum samples. The values are
 773 presented as mean \pm SD (n=3).

Conditions/Pathology		Ah*	Ap-a [†]	Ib*	Ah-f [†]	Ap-f [†]	F*	Ph [†]
<i>In situ</i>	-	2.0 \pm 0.3 x 10 ⁴	1.0 \pm 0.1 x 10 ³	1.0 \pm 0.2 x 10 ³	5.0 \pm 0.1 x 10 ⁴	5.0 \pm 0.1 x 10 ²	5.0 \pm 0.2 x 10 ³	1.0 \pm 0.1 x 10 ³
<i>Inoculum</i>	-	1.0 \pm 0.1 x 10 ¹⁰	-	9.0 \pm 0.2 x 10 ⁷	1.0 \pm 0.1 x 10 ⁸	-	2.0 \pm 0.1 x 10 ⁶	6.0 \pm 0.1 x 10 ⁴
1-CUW	Not significant	---	---	---	---	---	---	---
2-CCW	Not significant	---	---	---	---	---	---	---
3-CUA	Not significant	---	---	---	---	---	---	---
4-CCA	Not significant	---	---	---	---	---	---	---
5-MUW	Soft bulkier structure and robust biofilm	5.7 \pm 0.2 x 10 ⁶	1.8 \pm 0.1 x 10 ²	6.0 \pm 0.3 x 10 ⁴	1.9 \pm 0.1 x 10 ⁶	1.4 \pm 0.1 x 10 ⁴	1.6 \pm 0.1 x 10 ⁶	9.4 \pm 0.5 x 10 ⁴
6-MCW	Presence of cracks on surface coating, less biofilm and depigmentation	4.8 \pm 0.2 x 10 ⁴	7.5 \pm 0.4 x 10 ¹	3.5 \pm 0.3 x 10 ³	2.5 \pm 0.1 x 10 ³	5.0 \pm 0.1 x 10 ⁻¹	1.9 \pm 0.1 x 10 ³	NG
7-MUA	Adherent bulkier structure, large number of hyphae. Robust biofilm. Greenish and orange colonies.	1.2 \pm 0.1 x 10 ⁷	1.8 \pm 0.1 x 10 ²	1.0 \pm 0.1 x 10 ⁵	5.6 \pm 0.2 x 10 ⁶	5.6 \pm 0.2 x 10 ³	7.0 \pm 0.4 x 10 ⁴	5.6 \pm 0.2 x 10 ⁵
8-MCA	Presence of cracks on surface coating and paint changed to bright yellow, less biofilm and whitening. Greenish and orange colonies.	3.6 \pm 0.2 x 10 ⁶	1.3 \pm 0.1 x 10 ²	5.1 \pm 0.2 x 10 ⁴	1.4 \pm 0.1 x 10 ⁶	3.1 \pm 0.05 x 10 ⁰	3.6 \pm 0.2 x 10 ⁵	3.1 \pm 0.1 x 10 ⁰

774 Ah: Aerobic heterotrophic bacteria; Ap-a: aerobic acid-producing bacteria; Ib: Iron-oxidizing bacteria; Ah-f: facultative heterotrophic bacteria;

775 Ap-f: facultative acid-producing bacteria; F: Total fungi; Ph: Photosynthetic microorganisms. *(CFU/cm²). [†](MPN cells/cm²); NG: no-growth.

776 Condition 1-CUW: Absence of microorganism, uncoated coupons and water mist; Condition 2-CCW: Absence of microorganism, coated
777 coupons and water mist; Condition 3-CUA: Absence of microorganism, uncoated coupons and acid mist; Condition 4-CCA: Absence of
778 microorganism, coated coupons and acid mist; Condition 5-MUW: Presence of microorganism, uncoated coupons and water mist; Condition 6-
779 MCW: Presence of microorganism, coated coupons and water mist; Condition 7-MUA: Presence of microorganism, uncoated coupons and acid
780 mist; Condition 8-MCA: Presence of microorganism, coated coupons and acid mist

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783 Table 2. Alpha-diversity indexes of bacterial and fungal communities from the cultured
 784 mortar treated with water mist (conditions 5 and 6) and acid mist (conditions 7 and 8)

Microbial group	Condition	# of ASVs	H'	D	Equitability
<i>Bacteria</i>	5 (uncoated)	21.00±1.70	2.68±0.60	0.75±0.14	0.61±0.15
	6 (coated)	13.00±3.60	1.13±0.25	0.36±0.09	0.31±0.05
	7 (uncoated)	42.30±9.10	3.28±0.27	0.82±0.05	0.61±0.05
	8 (coated)	14.30±5.50	1.09±1.28	0.29±0.36	0.27±0.28
<i>Fungi</i>	5 (uncoated)	7.00±0.00	1.90±0.37	0.65±0.15	0.68±0.13
	6 (coated)	7.33±2.08	1.26±0.04	0.49±0.01	0.44±0.07
	7 (uncoated)	9.67±2.52	2.09±0.44	0.68±0.12	0.65±0.14
	8 (coated)	7.67±0.58	1.24±0.72	0.42±0.27	0.42±0.24

785 Mean values and standard deviation of triplicates of each condition. ASVs, Amplicon
 786 Sequence Variants; H', Shanon-Weaver's index; D, Simpson's index; Equitability,
 787 Pielou's evenness.

Figure 1. Glass reactor (A) and top view of the arrangement of the coupons (B).

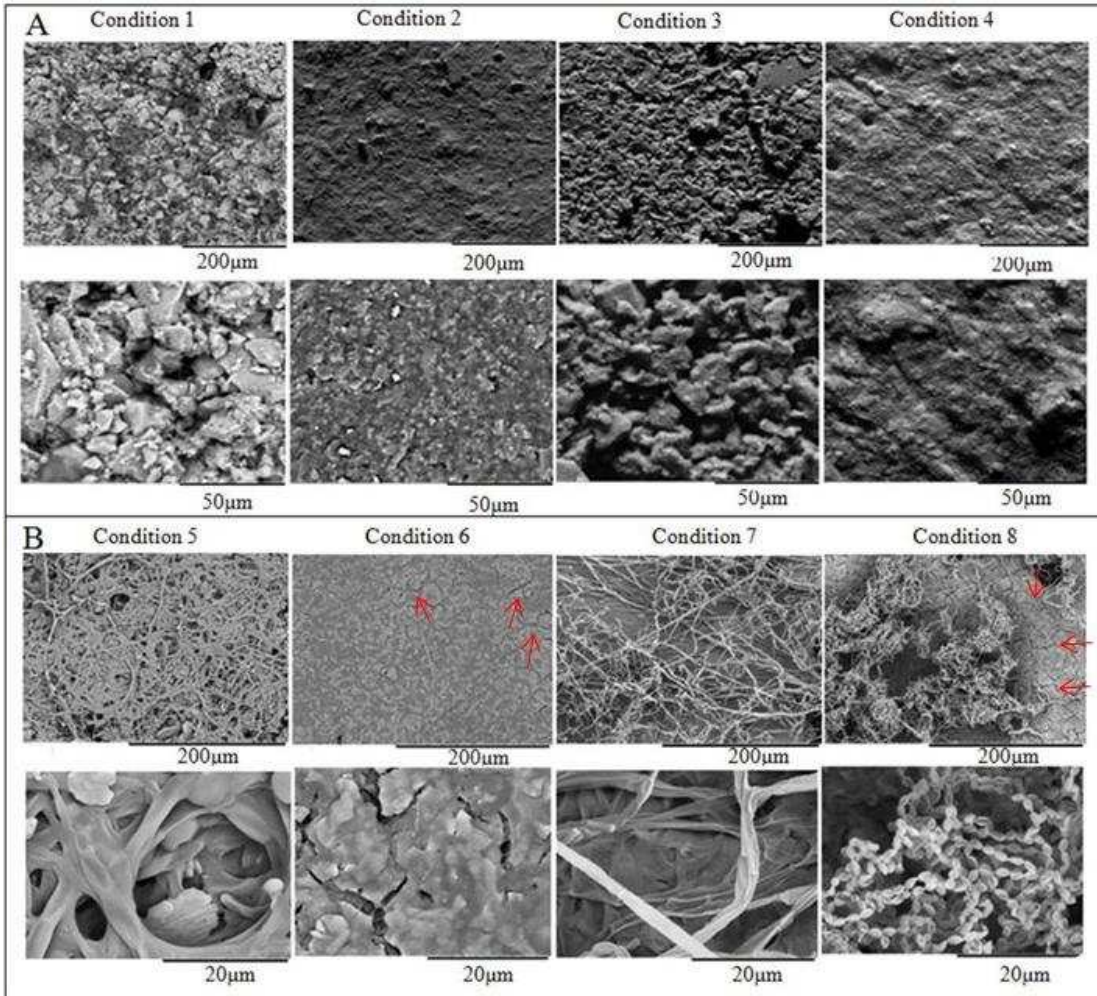
Figure 2. SEM image of the uninoculated (A) and inoculated (B) mortar coupons after 100 days of incubation. Conditions 2, 4, 6 and 8 represented coated mortar and 1, 3, 5 and 7 represented uncoated. Treatment with acid mist were used in conditions 3, 4, 7 and 8.

Figure 3. Image of mortar coupons at the beginning of the experiment (left, uncoated; right, coated), after 100 days of incubation and after scrapping off the biofilms. Conditions 2, 4, 6 and 8 represented coated mortar and 1, 3, 5 and 7 represented uncoated. Treatment with acid mist were used in conditions 3, 4, 7 and 8.

Figure 4. Bacterial (A) and Eukayotic (B) communities structures. Non-metric multidimensional scaling analysis (Bray-Curtis) based on T-RFLP profiles from 16S and 18S rRNA genes from biofilm developed in inoculated coupons under different conditions: uncoated coupons (C5 in green, condition 5-MUW), coated coupons (C6 in blue, condition 6-MCW), uncoated coupons under acid mist (C7 in cyan, condition 7-MUA) and coated coupons under acid mist (C8 in red, condition 8-MCA). The vectors connect the triplicates. The percentages indicate the average similarity (SIMPER) of samples in each group (condition).

Figure 5. Relative abundance of bacterial community at the genus level (A), fungal community at the class level (B) and ASVs distribution of the two dominants classes of Fungi (Sordariomycetes and Tremellomycetes) (C) in coated (conditions 5 and 7) and uncoated (conditions 6 and 8) mortar treated with water mist (conditions 5 and 6) and acid mist (conditions 7 and 8).





Initial Condition				
Condition 1	Condition 2	Condition 3	Condition 4	
				
Condition 5	Condition 6	Condition 7	Condition 8	
				
		After scrapping		
				

