

**Biodeterioration of classroom wall surfaces in the University of Port Harcourt, Nigeria**

**ABSTRACT**

This study investigated the biodeterioration of classroom wall surfaces in the University of Port Harcourt, Nigeria. The microbiological and physicochemical parameters of the classroom wall surfaces were determined. The population of culturable bacterial and fungal biodeteriogens was determined by plating. Antibiotic susceptibility pattern of the bacterial isolates was determined using the disc diffusion method. The total culturable heterotrophic bacterial counts ranged from 6.48 to 8.23 log CFU/g while the total fungal counts ranged from 5.00 to 7.28 log CFU/g. The bacterial isolates identified by biochemical characterization and their frequency of occurrence are *Micrococcus* spp. (7.3%), *Citrobacter* spp. (3.2%), *Bacillus* spp. (39.1%), *Serratia* spp. (3.2%), *Corynebacterium* spp. (10.9%), *Staphylococcus aureus* (20.1%), *Proteus* spp. (9.2%) and *Shigella* spp (7.0%). The fungal isolates and their frequency of occurrence are *Aspergillus flavus* (39.1%), *Penicillium* spp. (20.1%), *Microsporium canis* (14.3%), *Coccidioides* spp. (10.9%), *Aspergillus fumigates* (3.2%) and *Tricophyton* spp (3.2%). All antibiotics used showed activity against all bacterial isolates except *Proteus* spp. From the results of the physicochemical parameters, pH values ranged from 6.15 to 9.01, nitrate ranged from 5.30 to 14.83 mg/kg, phosphate ranged 2.19 to 5.94 mg/kg, sulphate ranged from 12.97 to 19.07 mg/kg and Total Organic Carbon ranged from 74.89 to 119.43 mg/kg. This study has shown the potential public health risk associated with classroom building deterioration owing to the presence of pathogenic microorganisms. Therefore, measures towards prevention and mitigation of classroom building biodeterioration should be in place.

**Keywords:** Biodeterioration, buildings, public health risk, antibiotics, resistance

**1.0 Introduction**

Biodeterioration can be defined as undesirable changes to a product or substance or material, influenced by living organisms. Organisms are able to interact with nutrients and material

30 environment to form specific communities. This interaction and association could bring about  
31 many physical and chemical destructive processes. Both biotic and abiotic activities contribute  
32 simultaneously during the deterioration of building materials. Hence, the level of  
33 biodeterioration is difficult to quantify due to the involvement of uncontrollable external  
34 (abiotic) factors. However, the involvement of microorganisms in biodeterioration of materials in  
35 the environment has been estimated to be up to 30% in the United States [1].

36 Buildings, just like every other material are subject to microbial colonization, deterioration and  
37 degradation or "weathering". Architectural structures including buildings and bridges in contact  
38 with water, soil, waste, sewage, plant materials or any organic matter, can undergo deterioration.  
39 The hard and firm nature of these structures only limits the biodeterioration process to a slow,  
40 eventual and inevitable process of corrosion after microbial colonization, under conducive  
41 conditions [2]. The presence of utilizable substrates as part of the building components makes  
42 some building more prone to microbial deterioration. For examples, pigment, thinner, binder and  
43 drier are the main components of paints used to coat walls, and the most prone to attack by  
44 microorganisms [3].

45 Microorganisms use parts of building components for energy generation [4]. Painted surfaces  
46 provide the nutrients and micro-environment for microbial colonization before access to the  
47 building proper is later gained. During this attack and colonization, microorganisms produce  
48 different forms of corrosive acids which can solubilize the lattice structure [5]. *Bacillus* spp. for  
49 example produce sulphuric acid from the oxidation of reduced sulphur compounds [6].

50 Common building biodeteriogens include nitrifying bacteria, *Cyanobacteria*, and *Thiobacilli* and  
51 fungi of genus *Aspergillus*, *Fusarium* *Penicillium*, *Alternaria*, *Tricophyton* and *Cladosporium*

52 [1,5]. The major environmental parameters affecting biodeterioration are water availability,  
53 humidity, temperature, UV light and inadequate ventilation [7].

54 Despite the widespread knowledge of building deterioration, research on biodeterioration is  
55 lagging. It is curious though, as studies have pointed to the severe impact of paint components  
56 and their degradation products on human health [8,9]. Spoilage of building components come  
57 with proliferation of undesirable microorganisms and their degradation products. Consequently,  
58 human health and the environment are threatened. This study aimed to assess the microbiological  
59 and physicochemical properties of deteriorating painted building surfaces of University of Port  
60 Harcourt Faculties and the health implication on students.

## 61 **2.0 MATERIALS AND METHOD**

### 62 **2.1 Collection of Samples**

63 Samples from visibly deteriorating classroom painted building surfaces were collected under  
64 aseptic conditions from selected Faculties of University of Port Harcourt. Samples were gotten  
65 by scraping off superficial material to a depth of 2-5 mm. Samples were moved to the laboratory  
66 for immediate analyses.

### 67 **2.2 Isolation and Enumeration of Bacterial and Fungal Isolates**

68 One (1) g sample of superficial scrapings was transferred into 9 ml sterile normal saline to make  
69 a stock solution. One (1) ml was pipette aseptically into a test tube containing 9ml of normal  
70 saline to make  $10^{-1}$  -  $10^{-5}$  dilutions. Nutrient agar and potato dextrose agar were prepared used  
71 for plating out the diluted samples. Triplicate plates were inoculated with 0.1ml aliquot of each  
72 dilution and spread using a flame sterilized hockey stick. Bacterial plates were incubator at  $37^{\circ}\text{C}$   
73 for 24 hours while fungal plates were incubated at  $27^{\circ}\text{C}$  for 48-72 hours. The number of colonies

74 that developed from each plate ranging between 30 and 300 after incubation was counted and  
75 recorded.

76 The bacterial isolates were identified based on their cultural and biochemical characteristics  
77 with reference to Holt *et al.* [10]. Morphological characteristics such as shape, colour,  
78 arrangement of spores, structure of the mycelium, and structure of hyphae and arrangement of  
79 sporangiophores were used in identifying the fungal isolates as described in Ellis *et al.* [11].

### 80 **2.3 Physicochemical Analyses**

81 The pH of building surface was measured in situ using a pH meter JENWAY 3071, model pH 82  
82 (degree of accuracy 0.01) equipped with a temperature probe. Determination nitrate, sulphate  
83 phosphate and Total Organic Carbon were carried out according to Anyanwu *et al.* [12].

### 84 **2.4 Bacterial antibiotic susceptibility test**

85 Isolated bacteria were subjected to antibiogram test. Susceptibility test was done using Muller  
86 Hinton agar with antibiotics discs effective against gram positive and gram negative bacteria.  
87 Following overnight incubation at 37°C, zones of inhibition (ZI) were determined and  
88 interpreted as sensitive, intermediate, or resistant for each of the assayed antimicrobial agent.  
89 Components of the antibacterial discs used include Erythromycin, Septrin, Ofloxacin,  
90 Gentamycin, Ampiclox, Pefloxacin, Amoxacillin, Rocephin, Cirpoflaxacin, Streptomycin and  
91 Zinnacef.

92

93

94 **2.5 Statistical Analysis**

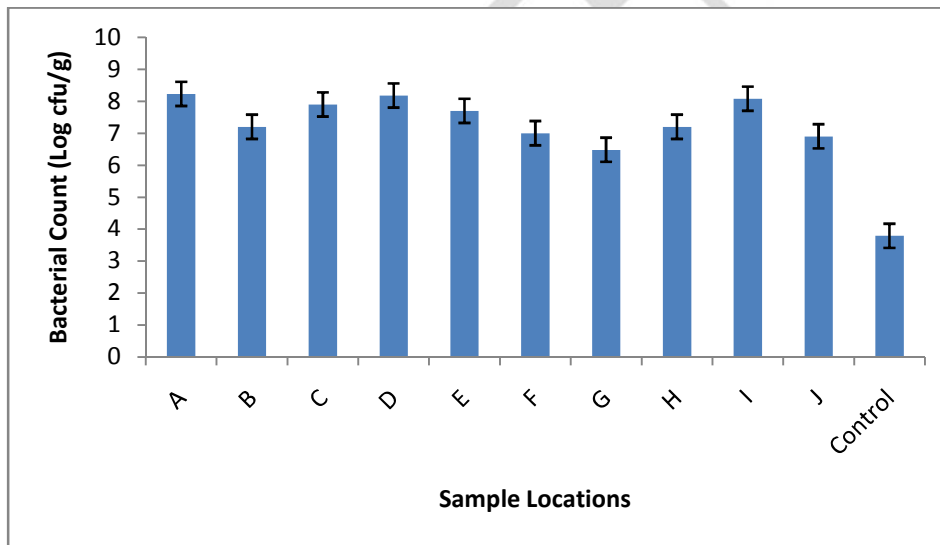
95 The physicochemical parameters for the different samples were analyzed using one-way  
96 Analysis of Variance (ANOVA) with the SPSS vs 20 software.

97 **3.0 RESULTS**

98 **3.1 Total Culturable Heterotrophic Bacterial Counts and Fungal Counts**

99 The total culturable heterotrophic bacterial counts and total fungal counts are shown in Figure 1  
100 and 2 respectively. Total culturable heterotrophic bacterial counts from the deteriorating  
101 buildings ranged from 6.48 to 8.23 log CFU/g while the control sample (non-deteriorated  
102 building) had 3.79 log CFU/g. Total spore counts from deteriorating buildings ranged from 5.00  
103 to 7.28 log cfu/g. Control sample had the least count with 2.92 log CFU/g.

104

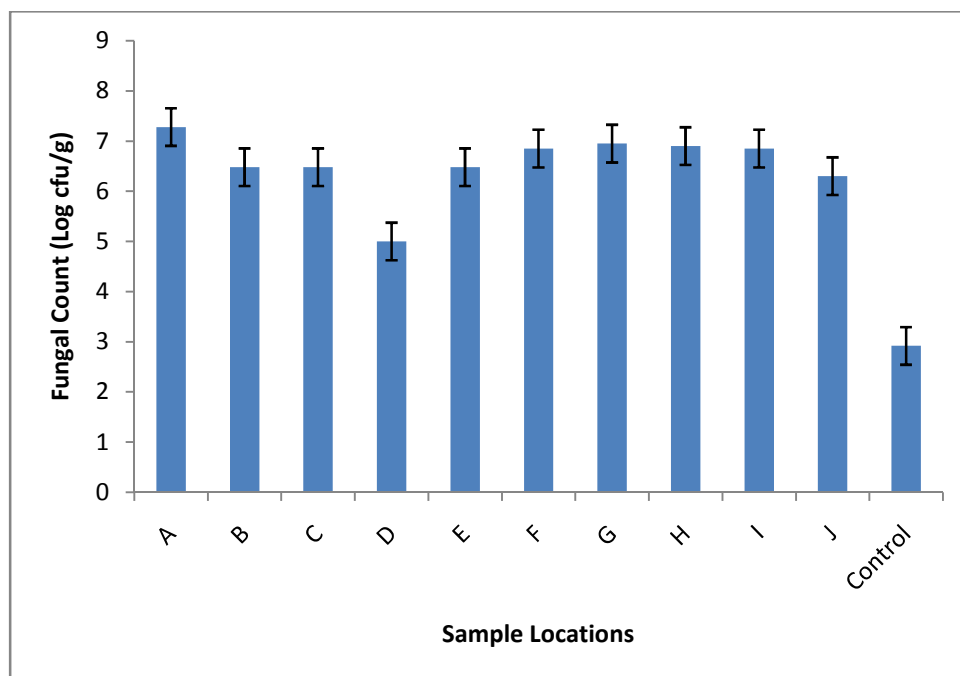


105

106 **Figure 1: Bacterial counts obtained from classroom wall scrapings**

107 **Keys:**

108 A= Dept of Marketing fin lecture Hall 1, B=Dept of crops &soil science, C=Faculty of Social  
109 Science, D=Dept of Human Physiology, E=Dept of Economics, F=Dept of Petroleum  
110 Engineering, G=Science MBS5, H=Dept of Educational Foundational, I=Dept of Fine Art  
111 &Design, J=Dept of Pharmaceutical



112

113 **Figure 2: Fungal counts obtained from classroom wall scrapings**

114 **Keys:**

115 Idem

116 **3.2 Bacterial and Fungal Biodeteriogens from wall scrapings**

117 The bacterial and fungal biodeteriogens isolated from wall scrapings and their percentage  
 118 frequencies of occurrence are presented in Tables 1 and 2 respectively. The bacterial  
 119 biodeteriogens include *Micrococcus* spp. (7.3%), *Citrobacter* spp. (3.2%), *Bacillus* spp. (39.1%),  
 120 *Serratia* spp. (3.2%), *Corynebacterium* spp. (10.9%), *Staphylococcus aureus*. (20.1%), *Proteus* spp.  
 121 (9.2%) and *Shigella* spp (7.0%). *Bacillus* spp were the highest occurring while *Serratia* spp. and  
 122 *Citrobacter* spp were jointly the least predominant. The fungal biodeteriogens include *Aspergillus*  
 123 *flavus* (39.1%), *Penicillium* spp. (20.1%), *Microsporium canis* (14.3%), *Aspergillus fumigates*  
 124 (3.2%) *Coccidioides* spp. (10.9%) and *Tricophyton* spp. (3.2%). *Aspergillus flavus* was the  
 125 predominant fungi in the study while *Coccidioides* spp. and *Tricophyton* spp. were the least  
 126 occurring isolates.

127

128

129 **Table 1: Bacterial Biodeteriogens from wall scrapings**

<b>Organism</b>	<b>% Frequency</b>
<i>Micrococcus</i> spp.	7.3
<i>Citrobacter</i> spp.	3.2
<i>Bacillus</i> spp.	39.1
<i>Serratia</i> spp.	3.2
<i>Corynebacterium</i> spp.	10.9
<i>Staphylococcus aureus</i>	20.1
<i>Proteus</i> spp.	9.2
<i>Shigella</i> spp.	7.0

130

131 **Table 2: Fungal Biodeteriogens from classroom wall scrapings**

<b>Organism</b>	<b>% Frequency</b>
<i>Aspergillus flavus</i>	39.1
<i>Penicillium</i> spp.	20.1
<i>Microsporium canis</i>	14.3
<i>Aspergillus fumigates</i>	3.2
<i>Coccidioides</i> spp.	10.9
<i>Tricophyton</i> spp.	3.2

132

133 **3.3 Antibiotic Susceptibility Pattern of Bacterial Isolates**

134 Results of the antibiotic susceptibility pattern of bacterial isolates are shown in Table 3. The  
 135 antibiotics used in the study include Erythromycin, Septrin, Ofloxacin, Gentamycin, Ampiclox,  
 136 Pefloxacin, Amoxacillin, Rocephin, Cirpoflaxacin, Streptomycin and Zinnacef. Results of the  
 137 antibiotic susceptibility pattern revealed susceptibility to the antibiotics by all the test organisms  
 138 except *Proteus* spp. The antibiotics showed more activity against *Bacillus* spp. and *Citrobacter*  
 139 spp.

140 **Table 3: Antibiotic Sensitivity Pattern of Bacterial Biodeteriogens of classroom wall**  
 141 **scrapings**

Organism	Antibiotic / Zone of inhibition (mm)									
	E	SXT	PEF	CN	APX	AM	R	CPX	S	Z
<i>Staphylococcus aureus</i>	0	10	0	0	0	0	0	15	10	0
<i>Micrococcus</i> spp.	10	9	4	12	5	0	0	20	15	8
<i>Citrobacter</i> spp.	20	20	20	20	0	0	20	20	20	0
<i>Proteus</i> spp	0	0	0	0	0	0	0	0	0	0
<i>Shigella</i> spp	20	15	24	20	0	0	10	21	20	0
<i>Bacillus</i> spp.	20	20	20	20	20	24	20	20	22	19
<i>Serratia</i> spp	17	17	21	20	0	0	0	20	20	0
<i>Corynebacterium</i> spp.	0	16	0	0	0	0	0	15	18	0

142  
 143 **Resistance range 0-13mm, Sensitive range 15mm and above**

144  
 145 Keys:  
 146 E= Erythromycin, SXT= Septrin, PEF=pefloxacin, CN=Gentamycin, APX=Ampiclox, AM=Amoxacillin,  
 147 R=Rocephin, CPX=Cirpoflaxacin, S=Streptomycin, Z= Zinnacef.

149 **3.4 Physicochemical Parameters of Deteriorating Buildings**



150 Physicochemical parameters of deteriorating buildings are shown in Table 4. The pH ranged  
 151 from 6.15 to 9.01, nitrate ranged from 5.30 to 14.83 mg/kg, phosphate ranged 2.19 to 5.94  
 152 mg/kg, sulphate ranged from 12.97 to 19.07 mg/kg and Total Organic Carbon ranged from 74.89  
 153 to 119.43 mg/kg. Results for Control sample (non-deteriorating building) were revealed to be pH  
 154 6.69; Nitrate 14.62 mg/kg; Phosphate 6.31 mg/kg; Sulphate 18.05 mg/kg; TOC 125.08 mg/kg.  
 155 Control sample had higher values for Nitrate, Phosphate, Sulphate and TOC.

156 **Table 4: Physicochemical Parameters of Classroom Wall Scrapings**

PARAMETER	A	B	C	D	E	F	G	H	I	J	control
pH	8.47	8.59	8.61	7.94	8.43	7.52	9.01	6.15	8.30	7.55	6.69
Nitrate (mg/kg)	5.94	14.83	10.21	9.86	6.47	11.04	9.08	5.64	5.30	7.01	14.62
Phosphate (mg/kg)	5.89	3.88	2.19	4.62	5.85	5.07	5.94	3.41	3.74	3.88	6.31
Sulphate (mg/kg)	17.32	13.37	15.21	17.82	16.93	13.55	19.07	15.61	12.97	16.40	18.05
TOC (mg/kg)	119.43	74.89	93.60	92.71	103.53	87.65	91.70	109.06	89.51	95.75	125.08

157

## 158 **Discussion**

159 This study investigated the biodeterioration of classroom wall surfaces in the University of Port  
 160 Harcourt, Nigeria. The total culturable heterotrophic bacterial counts obtained from deteriorating  
 161 painted walls ranged from 6.48 to 8.23 log CFU/g while the total fungal counts ranged from 5.00  
 162 to 7.28 log CFU/g. The bacterial counts in this study exceeded those reported in a similar study  
 163 carried out by Shinkafi and Haruna [13], with bacterial counts range of  $1.1 \times 10^4$  CFU/g and  $1.20$   
 164  $\times 10^5$  CFU/g were recorded from buildings showing visibly signs of deterioration. The presence

165 bacteria on sampled walls might have been influenced by moisture, as seen in areas with visible  
166 discoloration and peelings. The moisture was traced to walls outside which were exposed to  
167 rainfalls.

168 Antimicrobial additives in paint formulation are intended to prevent biodeterioration. However,  
169 microorganisms have been reported to breakdown preservatives such the biocides used in paints  
170 and other paint components such as binders and resin [9]. The quality of biocides used in paints  
171 could be affected by harsh environmental conditions. These environmental conditions could  
172 diminish the quality of the paint thereby allowing microorganisms to thrive and colonize these  
173 surfaces [14].

174 From the results of the physicochemical parameter, pH ranged from 6.15 to 9.01, nitrate ranged  
175 from 5.30 to 14.83 mg/kg, phosphate ranged 2.19 to 5.94 mg/kg, sulphate ranged from 12.97 to  
176 19.07 mg/kg and TOC ranged from 74.89 to 119.43 mg/kg. The presence of phosphate, sulphate,  
177 nitrate and carbon, with pH within the neutral range suggests an appropriate environment for  
178 growth. Results of Control sample (non-deteriorating building) were revealed to be pH 6.69;  
179 Nitrate 14.62 mg/kg; Phosphate 6.31 mg/kg; Sulphate 18.05 mg/kg; TOC 125.08 mg/kg. While  
180 the pH was within the pH of the deteriorating surfaces, nitrate phosphate, sulphate and TOC were  
181 found to be generally higher but not statistically significant. This further suggests that these  
182 nutrients were present in higher concentrations until colonization and biodegradation began  
183 where the nutrients were utilized. These physicochemical parameters have effect on microbial  
184 growth. Warscheid and Braams [15] reported that pH, climatic factors, nutrient sources among  
185 others influence microbial colonization of building. The pH range in this study (6.15 to 9.01) was  
186 higher than the 3-6 range reported by Ogu *et al.* [14] from deteriorating painted buildings.

187 The bacterial biodeteriogens were *Micrococcus* spp., *Citrobacter* spp. (3.2%), *Bacillus* spp.  
188 (39.1%), *Serratia* spp. (3.2%), *Corynebacterium* spp., *Staphylococcus aureus*, *Proteus* spp., and  
189 *Shigella* spp. Similar bacteria were also isolated from painted surfaces in the study of  
190 Okpokwasili and Iteun, [16]. In a similar study by Ogu *et al.* [14] *Micrococcus*, *Bacillus* were  
191 isolated from deteriorating walls. Shinkafi and Haruna [13] isolated species of *Bacillus* and  
192 *Staphylococcus* from deteriorating wall surfaces.

193 In the present study, *Bacillus* was the highest occurring bacteria with 39.1%. *Bacillus* spp. are  
194 among the most abundant bacteria in the atmosphere [17] as they are spore formers and therefore  
195 can withstand adverse environmental conditions. These organisms might have gained their  
196 entrances onto painted surfaces through dust, dirt, soot and contaminants accumulating on the  
197 painted surfaces, which may also represent another significant source of nutrients to the  
198 microorganisms as alluded to by Ogu *et al.* [14].

199 The fungal biodeteriogens include *Aspergillus flavus*, *Penicillium* spp., *Microsporium canis*,  
200 *Aspergillus fumigates*, *Coccidioides* spp. and *Tricophyton* spp. [13,14,18-20] also reported  
201 similar fungal genera in their respective studies. Previous studies have largely attributed the  
202 colonization of buildings by fungi and subsequent deterioration to moisture [13,20]. Hence, it  
203 can be said that fungal development on painted surfaces could imply that moisture is absorbed  
204 within the room walls and there is sufficient organic material on the walls to support fungal  
205 growth and by extension poses health risk to humans through possible inhalation of those spores.

206 Fungi just like every other living organism require some sets of conditions to thrive. Some of  
207 these conditions are optimal temperature, nutrient availability, oxygen and relative humidity. For  
208 fungi to conveniently colonize a painted surface, these conditions would have either been  
209 provided by the paint or the environment. Their ability to form spores makes them highly

210 resistant to high environmental temperature. According to Milica and Jelena[21] fungi are ideally  
211 suited as biodeteriogens of buildings due to their morphology and physiology. This further  
212 explains their presence on the sampled walls. Elumalai *et al.* [22] attributed visible discoloration  
213 of painted surfaces as signs to possible fungal effect.

214 Results of the antibiotic susceptibility pattern revealed susceptibility to the antibiotics by all the  
215 test organisms except *Proteus* spp. The antibiotics showed more activity against *Bacillus* spp.  
216 and *Citrobacter* spp. It is imperative to add antimicrobial additives to paints to mitigate  
217 biodeterioration. It is worrisome however that some of the bacterial isolates exhibited resistance  
218 to the antibiotics used. Microorganisms are known to cause sick building illnesses [5] and  
219 antibiotic resistant genes can be transferred within this environment to further worsen the problem  
220 of antibiotics resistance.

## 221 **Conclusion**

222 This study has shown that bacteria are prevalent in deteriorating buildings suggesting they play a  
223 critical role as deteriorating agents. The study also showed the diversity and abundance of  
224 microorganisms in the affected buildings. Furthermore, the study revealed the influence of some  
225 physicochemical parameters (pH, nitrate, sulphate, phosphate and organic carbon) on the  
226 microbial bioburden of painted surfaces. The need to control the colonization and proliferation of  
227 microorganisms on building surfaces is emphasized.

## 228 REFERENCES

- 229 1. Videla HA, Herrero LK. Microbiologically influenced corrosion: looking to the future.  
230 *International Journal of Microbiology*. 2005; 8(3):169-180.
- 231 2. Brauns JM. Optimizing concrete mixtures. *Concrete Inter*. 2002; 33-38.

- 232 3. Pelcza JR, Michael J, Chan ECS, Noel, RK. Microbiology. Tata McGraw Hill publication  
233 company limited 7 west pated naga. Nw Delhi Edn 5<sup>th</sup>. 2002; pp 851-852.
- 234 4. Parker K. Detection, assessment and evaluation of mould in buildings in relation to indoor  
235 environment and effects on human health. Report from the R and D-programme climate 2000.  
236 Norwegian Building Research Institute.
- 237 5. Bock SS, Sand W. Microorganisms, Sick and Building related illness. 2000; Pp. 1107-20.
- 238 6. Kelly CJ, Robentson CW, Kuenen HJ. Comparison of Non-destructive testers of hardened  
239 concrete. 2002; 84(5):374-386.
- 240 7. Singh A. Biodegradation of building material. Biodeterioration of stone surfaces. St. Clair LL  
241 and Seaward MRD Ed., Kluwer Academic Publisher, 2004.
- 242 8. Mendell MJ. Indoor residential chemical dmissions as risk factors for respiratory and allergic  
243 effects in children: a review. *Indoor Air*. 2007 17(4): 259-77.
- 244 9. Ravikumar HR, Rao SS, Karigar CS. Biodegradation of paints: A current status. *Indian*  
245 *Journal of Science and Technology*. 20012; 5(1): 1977-1987.
- 246 10. Holt JG, Krieg NR, Sneath PHA. (Ed.). Bergey's Manual of Determinative Bacteriology (9<sup>th</sup>  
247 Ed.). Lippincott Williams & Wilkins. 1994.
- 248 11. Ellis D, Davis S, Alexiou H, Handke R, Bartley R. Descriptions of Medical Fungi. Mycology  
249 Unit Women's and Children's Hospital School of Molecular and Biomedical Science University  
250 of Adelaide. 2007; pp 1-204.
- 251 12. Anyanwu CU, Nwankwo SC, Moneke AN. Soil Bacterial Response to Introduced Metal  
252 Stress. *International Journal of Basic and Applied Sciences*. 2011;11(1): 73-76.

- 253 13. Shinkafi SA, Haruna I. Microorganisms associated with deteriorated desurface painted  
254 concrete buildings within Sokoto, Nigeria. *International Journal of Current Microbiology and*  
255 *Applied Science*. 2013; 2(10): 314-324.
- 256 14. Ogu TC, Okaa AI, Ozokpo AC, Onochie CC. Biodeteriorated painted surfaces and In-can  
257 paints in Onitsha, Anambra State of Nigeria. *African Journal of Education, Science and*  
258 *Technology*. 2016; 3(1): 190-196.
- 259 15. Warscheid T, Braams J. *Biodeterioration of stone: a review. International Biodeterioration*.  
260 2000; 46: 343-63.
- 261 16. Okpokwasili GC, Iteun A. Fouling Microflora of painted surfaces. *Material und Organismen*.  
262 1996; 30: 155-159.
- 263 17. Hurst CJ. Disinfection of water: drinking water, recreational water and waste water. In:  
264 Disinfection, Sterilization and Preservation. (Block, S.S. Ed.) 5th ed. Lipincott Williams &  
265 Williams, Philadelphia, P.A, U.S.A. 2001; pP. 1023-1047.
- 266 18. Bashir U, Hafeez R. Deterioration of painted wall surface by fungal saprobes: isolation and  
267 identification. *Pakistan Journal Phytopathology*. 2016; 28(1):09-13.
- 268 19. Biswas J, Kavita S, Harris KK, Rajput Y. Biodeterioration agents: Bacterial and fungal  
269 diversity dwelling in or on the pre-historic rock-paints of Kabra-pahad, India. *Iranian Journal of*  
270 *Microbiology*. 2013; 5 (3): 309-314.
- 271 20. Mamta C, Padma, S. Building Deteriorating Fungi as Biocontaminant. *Asian Journal. Exp.*  
272 *Biological. Science*. 2012; 3(1): 209 – 213.
- 273 21. Milica VL, Jelena B. Role of Fungi in Biodeterioration Process of Stone in Historic  
274 Buildings. *Proc. Nat. Sci, Matica Srpska Novi Sad*. 2009; 116: 245—251.

275 22. Elumalai P, Elumalai EK, David E. Fungi associated with deteriorations of painted wall  
276 surfaces: isolation and identification. *European Journal of Academic Essays*. 2014; 1(3): 48-50.

277

278

279

280 Adeyemi, A.O, and Gadd, G.M. (2005). Fungal degradation of calcium, lead and silicon-bearing  
281 minerals. *Biometals*. **18**: 269-281.

282 Ahn, J.H., Kim, Y.J., Kim, T., Song, H.G., Kang, C. and Ka, J.O. (2009). Quantitative  
283 improvement of 16S rDNA DGGE analysis for soil bacterial community using real-time PCR.  
284 *Journal of Microbiological Methods*. **78**(2):216-222.

285 Albertano, P., and Bruno L. (2003). The importance of light in the conservation of hypogean  
286 monuments. In *Molecular Biology and Cultural Heritage*. Edited by Saiz-Jimenez C. Balkema,  
287 Lisse; 2003.

288 Allsopp, D., Seal, K. and Gaylarde, C. (2003). Built Environment, Structures, Systems, and  
289 Transportation. *Introduction to Biodeterioration*. Pp 111 - 160

290

291 APHA (1985). Standard Methods for the Examination of Water and Wastewater. American  
292 Public Health Association, Washington, DC. 1268 pp.

293

294

295

296 Cifferi, O. (1991). Microbial Degradation of paintings. *Journal of Applied Environmental*  
297 *Microbiology*. 65:879-885.

298 Crispim, C.A, Gaylarde P.M, Gaylarde C.C. (2003). Algal and cyanobacterial biofilms on  
299 calcareous historic buildings. *Curr Microbiol*. **46**:79-82.

300 Crispim, C.A., and Gaylarde C.C. (2005). Cyanobacteria and biodeterioration of cultural  
301 heritage: A Review. *Microbiological Ecology*, **49**:1-9.

302

303 Cruickshank, R., Duguid, J.P., Marmion, B.R. and swain, R.H. (1975): Medical Microbiology,  
304 12th Ed ., Living stone, London, New York, 812-825.

305 De Muynck, W., Maury, A., De Belie, N., and Verstraete, W. (2009). Evaluation of strategies to  
306 prevent algal fouling on white architectural and cellular concret. Elsevier. *International*  
307 *Biodeterioration & Biodegradation*. 63, 679–689.

308 Dupont, J., Jacquet C., Dennetière B. and Lacoste S. (2007). Invasion of the French Paleolithic  
309 painted cave of Lascaux by members of the *Fusarium solani* species complex. *Mycologia*.  
310 **99**:526-533.

311 Ellis, D., Davis, S., Alexiou, H., Handke, R. and Bartley, R. (2007). Descriptions of Medical  
312 Fungi. Mycology Unit Women’s and Children’s Hospital School of Molecular and Biomedical  
313 Science University of Adelaide. Pp 1 – 204.

314 Ezenna, E., Stanley, H.O., and Stanley, C.N.(2017). Spoilage Bacteria Associated with Selected  
315 Body lotions commonly used amongst Students of the University of Port Harcourt, Nigeria.  
316 *Journal of Pharmaceutical Research International*. **19**(5): 1-7.



317 Gadd, G.M., (2004). Mycotransformation of organic and inorganic substrates. *Mycologist*,  
318 **18**:60-70.

319 Harley, A.D and Gilkes, R.J. (2000). Factors influencing the release of plant nutrient elements  
320 from silicate rock powders: a geochemical overview. *Nutr Cycl Agroecosyst*. **56**:11-36.

321 Hudon, E., Mirza S., and Frigon D. (2011). “Biodeterioration of Concrete Sewer Pipes: State of  
322 the Art and Research Needs.” ASCE. *Journal of pipeline systems engineering and practice*. 2:42-  
323 52

324 tions and diversity. *International Biodeterioration and biodegradation*. 2002; 49: 27-37.

325 Ire, F.S., and Eruteya, O. C. (2017). Antimicrobial susceptibility of spoilage bacteria of Atama (  
326 *Heinsia critana*) soup. *International Journal of Current Microbiology Applied science*.  
327 **6**(4):2664-2672.

328 Jukes, T. H., and Cantor, C. R. (1969). Evolution of protein molecules. New York: Academic  
329 press. Pp. 21-132.

330 Kaur, G., Siddique R., and Rajor A. (2012). Influence of fungus on properties of concrete made  
331 with waste foundry sand. *Journal of Materials in Civil Engineering*.

332 Machell, J., and Boxall J. (2011). Field Studies and Modeling Exploring Mean and Maximum  
333 Water Age Association to Water Quality in a Drinking Water Distribution Network. ASCE.  
334 *Journal of Water Resources Planning and Management*.

335 Piñar G, Ripka K, Weber J, and Sterflinger K., (2009). The micro-biota of a sub-surface  
336 monument the medieval chapel of St. Virgil (Vienna, Austria). *International journal of*  
337 *Astrobiology*. **63**:851-859.

338 Prieto, B., Silva, B., Aira, N., and Alvarez, L. (2006). Toward a definition of a bioreceptivity  
339 index for granitic rocks: Perception of the cha nge in appearance of the rock. Elsevier.  
340 *International Biodeterioration & Biodegradation*. 58, 150–154.

341 Roldan, M., Clavero E., Castel S. and Hernandez-Marine M., (2004) Biofilms fluorescence and  
342 image analysis in hypogean monuments research. *Arch Hydrobiology Algologic Studies*.  
343 **111**:127-143.

344 Saitou, N. and Nei, M. (1987).’ The neighbor-joining method: a new method for reconstructing  
345 phylogenetic trees.’ *Molecular Biology and Evolution*. 4(4): 406-425.

346 Sanchez-Silva M. and Rosowsky D. (2008). Biodereriation of Construction Materials: State of  
347 the Art and Future Challenges. *Journal of materials in civil engineering*. 20 (5):352–365.

348 Suihko, L. M., Alakomi L.H., Gorbushina A.A., Fortune I., Marquardt, Saarela, M. (2007).  
349 Characterization of Aerobic Bacterial and Fungal Microbiota on Surfaces of Historic Scottish  
350 Monuments, *Syst. Applied Microbiology*. 30: 494-508.