Diversity of Algae and Cyanobacteria Associated with Bathroom Wall Biofilms within Diobu Port Harcourt, Nigeria

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Abstract: Algal and Cyanobacterial growths on surfaces are responsible for their discolorations and degradations. Ten concrete, ten wooden and five tiled bathroom wall surfaces were sampled by scrapping and the growths of these two organisms on them were compared using the morphological method. Cyanobacteria were found to predominate the wall surfaces with the genera *Chroococcus* ranking the top in the tile and concrete surfaces and *Osillatoria,* in the wooden surface. The wooden surface showed a predominance of the Chlorophytes with *Chlorella* occurring most times in the three surface types. The concrete and wooden surfaces showed equal number of Bacillariophyta taxa. The tiled wall surfaces were found to have the least number of taxa of all the genera, showing a reduced potential for support of microbial growth due to low porosity of its material type. This study had confirmed the diversity of cyanobacteria and algae on different bathroom wall surfaces and the influence of material surface types on their growth.

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1. Introduction

Biofilm formation on the wall surfaces of buildings lead to aesthetic deterioration, acid/alkaline production, moisture retention and temperature altering due to the differential heat absorption by coloured surface deposits and these result in most cases, in the biodegradation of the structural materials (Crispim *et al*, 2004). Microorganisms that could be detected in biofilms include algae, cyanobacteria, heterotrophic bacteria, fungi and protozoa (Crispim *et al*, 2003). In all climatic zones, cyanobacteria and algae occur as biofilms on the exposed surfaces of solid substrata (Samad and Adhikary, 2008; Adhikary *et al*, 2015). These kinds of growths are common in humid places on uneven surfaces such as holes, crevices and also on damp building walls due to leaking, roof guttering, inadequate drainage of flat areas or from adjacent water courses. The growth is rarely uniform, frequently forming streaks that follow areas of dampness (Samad and Adhikary, 2008). Algal growth results in the formation of bright green or grey-green patches and streaks on construction materials (Rajkowska *et al*, 2014) and also the retention of water thereby supporting the growth of other more dangerous organisms (Gaylarde and Morton, 1999). However, they can actively degrade structural materials by the production of acid metabolites, siderophores or other chelating materials and osmolytes which can degrade siliceous materials as well as by penetration into the substrate by unknown mechanisms (Crispim *et al*, 2003).

A bathroom is any room where people care for their personal hygiene. It is any building or room made for people to have their bath, usually with soap and water. Most bathrooms comprise of integrated toilets facilities and sinks for other related washings (Ajayi and Ekozien, 2014). It is a place known to be constantly moist due to its frequent usage by a large or numerous numbers of people. Algae and cyanobacteria therefore, can thrive best in this environment. The sanitary conditions of this area is a health challenge as a lot of disease are easily spread or contacted in this area which serves as a perfect breeding ground for these organisms due to its constant moisture and humidity. This study therefore, was geared towards the assessment of the diversity of algae and cyanobacteria on the surface bathroom wall biofilms and also knowing the best structural material type that favours the growth of these organisms in Diobu. This will help in the development and planning of guidelines and strategies on the cleaning and maintenance of these bathroom areas in order to reduce the prolific growth of these organisms which are threats to health of its numerous users.

2. Methods

2.1 Sampling sites: Diobu is a densely populated neighborhood of Port Harcourt, Rivers State, located within the Port Harcourt metropolis with coordinate of 4^0 47' 24"N, 6^0 59' 36"E (Latitude 4.772152; Longitude 6.994514). Although the neighborhood ranks among most commercial vibrant places in the city, about a third of its residents live below the poverty level (Nwisi, 2013). Sanitation and health have also deteriorated in these areas because of overcrowding and the absence of public sanitation facilities (Obinna *et al*, 2010), and so is suspected to have a high concentration of microorganisms and also a wide variety of them. The concrete bathrooms used for this study have all stood for more than fifteen years, the wooden bathrooms were all above five years and the tiled bathrooms have all been in use for more than four years. The areas (streets) chosen for sample collections were almost the longest streets and have a greater number of the people residing in them.

2.2 Sampling and culture techniques: A total of twenty eight (28) biofilm samples were scrapped from three bathroom wall surface types using sterile scapel knife. Ten (10) from concrete bathroom wall surfaces, ten (10) from wooden bathroom wall surfaces and five (5) from tiled bathroom wall surfaces. They were collected from ten different locations in Mile 2 Diobu namely, Abel Jumbo1 and 2 (AJ1 and AJ2), Obidianso 1 and 2 (OB1 and OB2), Echue 1and 2 (EC1 and EC2), Timber 1 and 2(TIM1 and TIM2) and Akokwa 1 and 2(AK1and AK2) for the concrete and wooden surfaces. The tiled samples were collected only from five locations; one from each street. The samples were preserved in 4% formaldehyde solution. Cyanobacteria and algae were identified using a binocular microscope connected to a video capture system (Chinde-200) using the lower power objectives of the optical microscope, by the morphological features of cells, colonies and thalli, based on published literatures of George (1976), Belcher and Swale (1978), Van Vuure (2006), Samad and Adhikary (2008) Bellinger and Sigee (2010) and Adhikary *et al*, 2015).

3. Results

The physicochemical conditions influencing the study bathroom environments were as shown in Table 1. The mean temperature was 23.98^{O}C for the tiles, 25.18 ^{O}C for woods and 26.44^{O}C for concretes. Mean pH was 8.55 for tiles, 4.70 for woods and 7.47 for concretes while the mean moisture content was 34.80% for tiles, 39.45% for woods and 39.02% for concretes (Table 1).

Table1: The physicochemical parameters of the bathroom wall surface biofilms from the different material types

Physicochemical parameter	Bathroom surface type		
	Tiles	Woods	Concretes
Mean temperature (^{0}C) (Range)	23.98 (24-27)	25.18 (24-27)	26.44 (24 -27)
Mean pH (Range)	8.55 (8.00 – 9.13)	4.70 (4.00-5.50)	7.47(8.00 – 9.13)
Mean Moisture content (%) (Range)	34.80 (33.20– 39.10)	39.45(31.40-48.40)	39.02(33.20 – 39.10)

On direct examination of the samples, the cyanobacteria (Table 2) and algae (Table 3 and 4) present in them were noted. Their genera are as contained in Table 2, 3 and 4. Table 2 shows Cyanobacteria abundance in the bathroom wall surface biofilms in Diobu, Port Harcourt. Chroococcus (19.05%) was most predominant genus on the tiles surfaces, followed by Oscillatoria (14.29%). Stigonema, Nostoc, Aphanizomenon, Microcoleus, Scytonema and Gloeothece were the least predominant having a percentage frequency of 4.76%. However, Lyngbya, Aphanothece, Tolypothrix, Anacystis and Calothrix were absent (Table 2). On the wooden surfaces, Oscillatoria (17.54%) was most predominant, followed by Spirulina (12.28%), Microcoleus (12.28%) and Chroococcus (12.26%). Lyngbya, Aphanizomenon, Tolypothrix, Anacystis and Calothrix were the least predominant (1.75%). Gloeocapsa was the only genus absent (Table 2). On the concrete surfaces, Chroococcus, and Microcoleus was most predominant (18.42%), followed by Oscillatoria (13.16%) while Spirulina, Nostoc and Gloeothece was the least predominant (2.63%). Stigonema, Aphanothece, Tolypothrix, Anacystis and Calothrix were absent (Table 2).

Table 3 shows Algae (Chlorophyta) abundance in the bathroom wall surface biofilms in Diobu, Port Harcourt. It showed that Chlorella was the most predominant algae (Chlorophyta) on bathroom wall surfaces having a percentage of 50.0, 35.29, and 46.15 for tiles, woods and concretes surfaces respectively. Genus Pandorina, Scenedesmus, Oedogonium, Eudorina, Cosmarium, Klebsormidium, Micractinum and Sphaerocystis were absent on the tiles surfaces (Table 3). Genus Scenedesmus, Eudorina and Sphaerocystis were absent on the wooden surfaces while Ullothrix, Cosmarium, Klebsormidium, Micractinum were absent on the concrete surfaces (Table 3).

Table 4 shows Algae (Bacillariophyta) abundance in the bathroom wall surface biofilms in Diobu, Port Harcourt. It showed that Navicula was the most predominant algae genus (Bacillariophyta) on the tile surfaces having a percentage of 33.33 while Gyrosigma, Melosira and Eunotia were not found on tiled wall surfaces. On the wooden surfaces, Nitzschia was most predominant (50.00%), followed by

Navicula and Synedra with 14.29 percentage frequency while Cracticula and Eunotia were not found on the wooden surfaces. (Table 4). On the concretes surfaces, Nitzschia was also the most predominant (28.57%), followed by Navicula (21.43%). Fragillaria was the only algae (Bacillariophyta) absent on concrete surfaces (Table 4).

Table 2: Cyanobacteria abundance in the bathroom wall surface biofilms in Diobu, Port Harcourt

S/N	Cyanobacteria Genus	Bathroom wall surface type: Tiles (% frequency)	Woods (% frequency)	Concretes (% frequency)
1.	Chroococcus	4 (19.05)	3 (5.26)	7 (18.42)
2.	Oscillatoria	3 (14.29)	10 (17.54)	5 (13.16)
3.	Anabaena	2 (9.52)	7 (12.26)	3 (7.90)
4.	Spirulina	2 (9.52)	7 (12.28)	1 (2.63)
5.	Phormidium	2(9.52)	5 (8.77)	3 (7.90)
6.	Gloeocapsa	2 (9.52)	0(0.0)	3 (7.90)
7.	Stigonema	1 (4.76)	2 (3.51)	0(0.0)
8.	Lyngbya	0(0.0)	1 (1.75)	7(5.26)
9.	Nostoc	1 (4.76)	2 (3.51)	1 (2.63)
10.	Aphanizomenon	1 (4.76)	1 (1.75)	2 (5.26)
11.	Aphanothece	0(0.0)	3 (5,26)	0(0.0)
12.	Microcoleus	1 (4.76)	7 (12.28)	7 (18.42)
13.	Scytonema	1 (4.76)	2 (3.51)	3 (7.90)
14.	Gloeothece	1 (4.76)	4 (7.02)	1 (2.63)
15.	Tolypothrix	0(0.0)	1 (1.75)	0(0.0)
16.	Anacystis	0(0.0)	1 (1.75)	0(0.0)
17.	Calothrix	0(0.0)	1 (1.75)	0(0.0)
	Total taxa	**21(100.0)**	**57(100.0)**	**38(100.0)**

Table 3: Algae (Chlorophyta) abundance in the bathroom wall surface biofilms in Diobu, Port Harcourt

S/N	Algae Chlorophyta Genus	Bathroom wall surface type: Tiles (% frequency)	Woods (% frequency)	Concretes (% frequency)
1.	Chlorella	3 (50)	6 (35.29)	6 (46.15)
2.	Ullothrix	1(16.66)	1 (5.88)	0(0.0)
3.	Mougetia	1 (16.66)	1 (5.88)	1 (7.69)
4.	Spirogyra	1(16.66)	1 (5.88)	1 (7.69)
5.	Pandorina	0(0.0)	2 (11.76)	1 (7.69)
6.	Scenedesmus	0(0.0)	0(0.0)	1 (7.69)
7.	Oedogonium	0(0.0)	1 (5.88)	1 (7.69)
8.	Eudorina	0(0.0)	0(0.0)	1 (7.69)
9.	Cosmarium	0(0.0)	1 (5.88)	0(0.0)
10.	Klebsormidium	0(0.0)	3 (17.65)	0(0.0)
11.	Micractinum	0(0.0)	1 (5.88)	0(0.0)
12.	Sphaerocystis	0(0.0)	0(0.0)	1 (7.69)
	Total taxa	**6(100.0)**	**17(100.0)**	**13(100.0)**

Table 4: Algae (Bacillariophyta) abundance in the bathroom wall surface biofilms in Diobu, Port Harcourt

S/N	Algae Bacillariophyta Genus	Bathroom wall surface type: Tiles (% frequency)	Woods (% frequency)	Concretes (% frequency)
1	Navicula	2 (33.33)	2 (14.29)	3 (21.43)
2.	Synedra	1 (16.67)	2 (14.29)	2(14.29)
3.	Nitzschia	1 (16.67)	7 (50.00)	4 (28.57)
4.	Cracticula	1(16.67)	0(0.0)	1 (7.14)
5.	Fragillaria	1 (16.67)	1 (7.14)	0(0.0)
6.	Gyrosigma	0(0.0)	1 (7.14)	2 (14.29)
7.	Melosira	0(0.0)	1 (7.14)	1 (7.14)
8.	Eunotia	0(0.0)	0(0.0)	1 (7.14)
	Total taxa	**6(100.0)**	**14(100.0)**	**14(100.0)**

4. Discussion

The data showed that both substrate material type and environment, determine the overall microbial (cyanobacteria and algae) colonization of a surface. The temperature of the studied biofilm samples fell within the mesophilic range of between 20°C – 45°C which supports the growth of most microorganisms including the pathogenic ones. Mesophiles are widespread in nature; in warm-blooded animals, terrestrial and aquatic environment in temperate and tropical latitudes and so are rightly found on these bathroom wall environments where they grew. This temperature conditions suggests why cyanobacteria is predominant in the biofilm samples more than the algae due to its ability to withstand high insolation by its production of protective pigments. This is also noted in the works of Crispim *et al*. (2003) where it is confirmed that *Scytonema* produced brown-sheathed coloured cells for protection from ultra-irradiation. This also supports the works of Genitsaris *et al*. (2011) and Adhikary *et al.* (2015) which confirms cyanobacteria to be dominant in the tropical regions while other eukaryotic algae (chlorophyta) dominates the temperate regions.

Most natural environments have pH values between 4 and 9 and organisms with optima in this range are more commonly encountered (Madigan *et al*, 2009). Building materials with pH levels between 6 and 8 are more sensitive to microbial colonization (Verdier *et al*., 2014). This explains why we had growths on the different material types. The wooden surfaces showed a pH range of 4.00 – 5.50 which are acid tolerant. This could be because most of the cyanobacteria and algae are acid producers on their own making the environment of growth acidic.

Moisture is another key factor that controls the growth of all microorganisms (Madigan *et al*, 2009). The results showed that the wooden bathroom wall surface biofilms had moisture contents that exceeded the fiber saturation point (FSP) of wood; the threshold of moisture in wood which is approximately 26%. This can lead to a quick deterioration of the wood material and the efficient growth of microorganisms in the presence of the moisture. Concrete walls depending on the coatings, manufacturer's and owner's specifications should not have moisture contents well above 5% (Cole, 2015). The results have moisture contents well above the threshold of the different material types and so can lead to water entering the walls and moldings thereby promoting the growth of cyanobacteria, algae and other microorganism types. The result also showed different microbial types on the different substrate types despite the fact that they were collected from the same environmental zones confirming our earlier suspected belief for the sampled area. This can be seen from the varying total number of cyanobacteria and algae taxa identified from each surface material type in Table 2 and 3.

Tomaselli *et al*. (2000), did similar work and found out that some algae and cyanobacteria types were associated with calcerous substrates while *Nostoc* were frequently associated with artificial substrates. Crispim *et al.* (2003) and Blanton (2007) found out that this is due to the porosity of the material involved. Porosity is generally lower in tiled materials. Wood is known to be a porous material and retains a lot of waters in its pores and this relate to its easy penetration which affects microbial colonization. Algae are more frequent on humid than on dry sites (Crispim, 2003). This accounts for the increased algal taxa identified from the wooden bathroom wall surface type in table 2. Wood retains the water from the constant usage of the bathroom by its numerous users, as a result of bathing and splashing on the wall surfaces. In general, attachment of microorganisms to surfaces will occur most readily in surfaces that are rougher, more hydrophilic and coated by surface conditioning films (Donlan, 2002).

Most of the identified genera, according to Grbic *et al.* (2010) are known to produce gelatinous products which are related to mineral fixation. Several researchers also have shown that most of the identified genera are toxin producers in their natural environments. *Microcystis, Anabaena, Lyngbya* produce hepatoxic microcystis; *Oscillatoria* and *Anabaena* produce neurotoxic anatoxins while *lyngbya* produces skin irritating lyngbyatoxins and saxitoxins (Genitsaris *et al*, 2011). These cyanobacteria and algae might have been transported into these bathroom environments by its numerous users who serve as carriers, from the poor water quality in the area and from aerosols from toilet flushing and microbial laden (poor quality) air. These algae and cyanobacteria comfortably stick to the walls of these bathrooms because most produce gelatinous product which encourages attachment on surfaces. These algae and cyanobacteria on the wall biofilms, despite washings of the bathrooms therefore serve as reservoirs for the transmission of infections and diseases which could be contacted through the skin contact or inhalation of their toxins. Their presence also speed up the deterioration of the structural material type as most of them penetrate deep into the material and bore several holes, supports cracks and crevices which increase the porosity of the materials and if the threshold of its moisture content is exceeded, decay and deterioration sets in. This was confirmed from the visual survey of the study bathrooms, which showed a lot of discolorations due to pigment productions, fissures, cracks and crevices produced by these organisms.

This study therefore had shown the diversity of algae and cyanobacteria that colonize bathroom walls and also the different material types. There had not been previous publications on the algae and cyanobacteria of this environment. These results suggest that the much water (moisture) constantly present on the surfaces of these materials supports the easy growth of these organisms. The study also showed that tile is a better material for the construction of bathrooms as it is less porous when compare to other materials in the environment and does not support the growth of most organisms and so should be considered when materials for construction of bathrooms is of choice. The use of low porosity materials in the construction of bathrooms therefore, need be encouraged to reduce the diversity and rate of cyanobacteria and algae growth on its wall surfaces.

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