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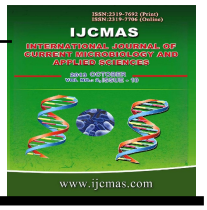

Original Research Article

Microorganisms associated with deteriorated desurface painted concrete buildings within Sokoto, Nigeria

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Keywords

Desurface painted; bacteria and fungi; deterioration; bacterial count.

A B S T R A C T

Samples from the buildings showing signs of deterioration like discolouration, cracking and peeling where collected and examined for the presence of both bacteria and fungi. Bacteria of varying gram reactions, shapes, and arrangements were isolated. They include gram positive cocci and positive rods. The isolates include *Bacillus polymyxa, Bacillus alvei, Bacillus cereus, Bacillus coagulans, Bacillus firmus, Bacillus laterosporus, Staphylococcus epidermidis and Staphylococcus kloosii.* Seven (7) species of fungi belonging to five (5) genera were isolated. They include *Aspergillus niger, Aspergillus fumigatus, Mucor racemosus, Rhizopus stolinifer, Cephalosporium* specie, *and Penicillium* specie. No, 32 Sultan Abubakar road has the highest bacterial count of 1.20 x 10^5 cfu and No. 7 Wurno road has the least bacterial count of 1.1 x 10^4 cfu. All the deteriorations were associated with two or more different species of fungi. Also most of the deteriorations caused by bacteria were found to be by more than one type of bacteria.

Introduction

Concrete is one of the strongest construction materials applied in centuries all over the world. However, they can get destroyed for a variety of reasons including the material limitations, poor quality design and construction practices, as well as the hard exposure conditions (Wazny, 1980). Desurface painted concrete is the removal of paint from the surface of concrete due to the environmental factors or activities of microorganisms (Perego, 2001).

Many architectural and other buildings structures undergo biodeterioration when exposed to contact with soil, water, sewage, as well as food, agricultural products and waste materials. Biodeterioration refers to undesirable changes in a material, caused by living organisms (Anonymous, 2002). Living organisms form specific communities that interact in many different ways with mineral materials and their external environment. This complex phenomenon

occurs in conjunction with many physical and chemical destructive processes. Thus, it is difficult to distinguish an extent of the damage caused by biotic factors from that resulting from abiotic ones. However, according to US estimation, the contribution of microorganisms to the deterioration of materials as a whole may be within the range of 30% of deterioration (Vindela and Harrerii, 2005). Biologically influenced corrosion of concrete has most often been detected in building foundations and walls, and also in constructions such as dams, harbour and maritime structures, bridges, tanks, pipelines, cooling towers, silos and many others (Roberts, 2002). This type of concrete deterioration occurs often in the food processing and storage works and in the abattoirs and buildings of holdings, in which the different microorganisms including bacteria, microscope fungi, and algae are usually present at increased concentration (Sand, 2001). They colonize the material surface, and its pores, capillaries and micro-cracks, and cause the concrete damage resulting in aesthetic, functional or structural problems (Sand *et al*., 2006).

Concrete buildings though having a hard texture are still subject to the very slow but inevitable process of corrosion and microbial deterioration (Braums, 2002). These buildings can not resist deterioration for a long time because some of their components are utilizable by microorganisms. The concrete building is made up of stone, cement and pain (Berthelin, 2003). The paint on its own is compose of pigment, binder or medium, thinner and drier some of which are attacked by microorganisms (Pelczar *et al*., 2002).

Stone is subjected to both physical and chemical deterioration but yet how microorganisms contribute to these deterioration is not very clear (Bock and Sand, 2001). Agents of concrete building deterioration include both microbial and non-microbial. The non microbial include temperature, moisture and acid rain. The microbial agents on the other hand are the fungi and bacteria. It has being known for many years that mass of microorganisms are present in the eroded masonry monuments (Bock and Sand, 2001).

Marble and calcerous blocks are sensitive to environmental impacts. Fungi has an upper hand than bacteria in concrete building spoilage and lichens are responsible for local marble disintegration (B). (2005) isolated bacteria from ancient monuments in South Britain showing that some unidentified strains were able to solubilize calcium from stone (Lewis, 2005). The types of microorganisms associated with concrete building deterioration include fungi like *Aspergillus, Penicillium, Alternaria, Cladosporium* and others (Vindela). The bacteria are the member of the *Cyanobacteria,* nitrifying bacteria and *Thiobacilli* (Bock and Sand, 2001).

The mechanism of deterioration by microorganisms is through utilization of the organic and inorganic building components for energy generation (Parker, 2007). In painted surfaces, the paint which also contain nutrients such as latex, cellulose and the organic solvent used are attacked first before the microorganisms get access to the concrete, various forms of acid organic and inorganic are produced by the microorganisms which cause the solubilization of the concrete block (Bock and Sand, 2001). The ammonia oxidizing bacteria oxidizes the ammonia to nitrate. The bacilli generate energy from the oxidation of reduced sulphur compounds

producing sulphuric acid as the end product (Kelly, 2002).

In Nigeria, buildings are subjected to various forms of deterioration, but little or no effort has been made to study the microbial biodeterioration of the buildings. This study is therefore, aimed at isolation of some of the possible microbial biodeteriorating agents of concrete buildings in Sokoto metropolis.

Materials and Methods

Sample Collection

A total number of fifteen (15) samples were collected within Sokoto metropolis, namely: University Guest Inn, House No. 32 Sultan Abubakar road, School of Matriculation Studies Sokoto, House No. 10 Diplomat Area Sokoto, No. 1 Marina Kofar Atiku, 42 Gangaren Dadin Kowa, No. 1 Shuni Road, No. 8 Gandu Area, Corpers Lodge Kasuwa Daji Sokoto, No. 16 Bazza road Bazza area, No. 15 behind National Library, No. 5 Emir Yahya road beside Sultan Maccido Mosque, No. 7 Wurno Road, No. 1 Junaidu Road, and No. 10 Modibbo road Gidan Yari area.

The samples were obtained from the walls of buildings showing signs of microbial spoilage such as mouldy growth, decolouration and peeling of paint, cracking and loss of texture of the building. The samples were collected in a sterile small polythene bags. The samples were collected by scraping the surface of the buildings and inner part (1cm depth) with the use of sterile chisels. The polythene bags were labelled and took to the laboratory where further investigation was carried out. The samples were labelled according to their location.

Preparation of Dilution Factor

One gram (1g) of each sample was weighed and placed in 9ml of distilled water and serial dilution ($1:10^{-1}$ to $1:10^{-5}$) was carried out and they were allowed to solidify for proper culturing.

Isolation and Identification of Bacteria

0.1 aliquots of the dilutions were pipetted onto plates of nutrient agar (NA). Sterile bent glass rod was used to spread the inoculum on the plates. Nutrient agar plates were incubated at 37^0C for 24 hours. At the end of the incubation period, colonies were counted and different colony types were sub cultured on Nutrient Agar slants for subsequent identification.

Identification of Bacterial Isolates

Standard method of identification of bacteria was used. Microscopic morphology of the bacterial isolates was examined. They were differentiated into two: gram positive and gram negative, based on their gram reaction. Biochemical tests were carried out on the bacterial isolates and were identified up to specie level. The biochemical tests were as follows:

Indole Test

The test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan, in which one of the end products is indole which would be accumulated in the medium. This was detected using Kovac's reagent. The test organisms were incubated at 37^0C for 48 hours, after which 0.5ml of Kovac's reagent was added to each tube and allowed to stand for two minutes The red

colour at the top layer of the medium indicates a positive test (Cheesbrough, 1991).

Methyl Red (MR)

A heavy innoculum of the test organism was inoculated into MR medium contained in each tube. The tubes were then incubated at 37^0C for 48 hours. After that, 5 drops of methyl red indicator was added to the incubated test tube. An instant red colour signifies a positive test (Cheesbrough, 1997).

Voges Proskauer (VP)

A heavy innoculum of the test organism was inoculated into VP medium contained in different test tubes. They were then incubated at 37^0C for 48 hours. After which 0.5ml of alpha nephtol was added, then followed by 0.5ml of 40% KOH. It is then agitated and allowed to stand for 30 minutes; a red to pink colour signifies a positive test (Cheesbrough, 1997).

Hydrogen Sulphide Test (H_2S)

The prepared test medium was used to detect the production of H_2S from different test organisms. Each test organism was inoculated into test tube by stabbing the medium. The test tubes were then incubated for 24 hours at 37^0C. A black colour along the line of stabbing indicates a positive reaction (Cheesbrough, 1997).

Citrate Test

A heavy inoculum of the test organism was inoculated into a sterile citrate medium with the aid of a sterile wire loop. The inoculated test tubes were incubated at 37^0C for 72 hours. A positive test was indicated by a turbid and change of colour of the medium from light green to blue (Cheesbrough, 1997).

Urease Test

This test is applied for bacterial that can decompose urea by enzymatic reaction to produce ammonia. The test organisms were inoculated into urea agar base medium contained in each bottle, which are then incubated at 37^0C for 48 hours. A positive test is indicated by a change in colour from yellow to pink as a result of ammonia production (Cheesbrough, 1997).

Catalase Test

It is used to differentiate those bacteria that produce the enzyme catalase, such as *Staphylococcus* from the non catalase producing bacteria such as *Streptococcus*. A drop of hydrogen peroxide (H_2O_2) was placed on a slide and a 24 hours growth culture was then emulsified with the drop of H_2O_2 on the slide. Immediately, it was observed for the presence of bubbles as indication for positive reaction (Cheesbrough, 1997).

Coagulase Test

This test was carried out to differentiate between the pathogenic *Staphylococcus* from non pathogenic *Staphylococcus*.

A drop of water was placed on a slide and a pure culture was then emulsified with the drop of water on the slide to obtain a suspension. A drop of blood plasma was then mixed with the suspension on the slide and it was immediately observed for agglutination, a positive test indicates that the plasma has undergone clothing (Oyeleke and Manga, 2008).

Sugar Fermentation Test

A twenty four hours old culture was stabbed into a sterile triple sugar iron agar slant (TSI) in a test tube and incubated at 37^0C for 24 hours. It was then observed for glucose, lactose, sucrose, gas production, and mortality, in a positive test for glucose was indicated by redness of the bottom of the test tube, while in lactose the media appeared yellow, for motility in the line of stabbation of the medium would not be sharply define and the rest of the medium would be cloudy (Cheesbrough, 1991).

Spore Detection Test

A smear of each test organism was made on a slide and solution of malachite green was used to flood the smear on a boiling water over a period of 10 minutes. They were then washed off with distilled water and a safranine solution was also flood on the same smear for two minutes after which all staining were washed off and allowed to air dry. The slides were then mounted under the microscope and examine for cell which is indicated by the presence of spore (Cheesbrough, 1991).

Starch Test

From pure culture of gram positive rod colonies, an inoculum was picked and subcultured in a petri dish plate containing starch medium and incubated at 37^0C for 24 hours. Colonies were formed and 1ml of Lugols iodine was poured inside the plate. Colour change was observed. Presence of dark purple colour is starch negative test, while absence of dark purple colour is positive test.

Isolation and Identification of Fungal Isolates

0.5ml aliquots of the dilutions were poured onto the potatoes dextrose agar (PDA) plates using a sterile syringe, shaken and the dilution spread over the surface of the media. PDA plates were incubated at room temperature for 72 hours. At the end of the incubation period growth of the fungi were observed and different growth were subcultured on Potato dextrose agar to obtain the pure culture. Pure culture were obtained and used for standard identifiThe colonial morphology of the fungal isolates on potato dextrose agar were observed for colour and type of growth that is wooly or cottony. Microscopic identification was carried out by preparing wet mount using lactophenol cotton blue to observe for the characteristics of fungi such as type of hyphae (whether septate or non septate). The fungi were identified using mycological atlas.

Result and Discussion

From the results obtained in this work, a total number of eight (8) bacterial isolates and seven (7) fungal isolates were identified. Tables 1 and 2 indicated the results of biochemical tests and morphological characteristics of the isolated organisms.Table 3 indicated the species of bacteria isolated which include gram positive rods and gram positive cocci. The isolates include *Bacillus polymyxa, Bacillus alvei, Bacillus cereus, Bacillus coagulans, Bacillus firmus, Bacillus laterosporus, Staphylococcus epidermidis,* and *Staphylococcus kloosii*.
Table 2 showed the fungi isolated which belong to five (5) genera: *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Mucor racemosus*. Others are

Table.1 Biochemical and Morphological Characteristics

Morphology	Gram Reaction	Indole	Methyl red	VP	H_2S	Gas	Motility	Citrate	Urease	Sucrose	Lactose	Catalase	Coagulase	Spore	Starch
Bacilli (single)	+	–	–	+	–	–	+	–	–	–	–			+	+
Bacilli (pairs)	+	–	+	+	–	–	+	+	+	–	–			+	-
Bacilli (pairs)	+	–	–	+	–	+	+	–	+	+	–			+	–
Bacilli (pairs)	+	–	–	+	–	+	+	–	–	+	–			+	+
Bacilli (pairs)	+	–	+	–	–	–	+	–	+	+	–			+	+
Cocci (clusters)	+	–	–	+	–	–	+	+	+	+	–	+	-		
Bacilli (pairs)	+	–	–	+	–	–	+	+	–	–	–			+	-
Bacilli (pairs)	+	–	+	–	–	–	+	–	–	–	–			+	+
Cocci (clusters)	+	–	+	–	–	–	+	–	–	–	–	+	–		
Bacilli (pairs)	+	–	–	+	–	–	+	–	–	+	–			+	–
Bacilli (single)	+	–	–	+	–	–	+	–	–	–	–			+	+
Bacilli (pairs)	+	–	+	–	–	–	+	–	–	–	–			+	+
Bacilli (pairs)	+	–	–	+	–	–	+	–	–	–	–			+	+
Bacilli (pairs)	+	–	+	+	–	–	+	+	+	–	–			+	-
Bacilli (pairs)	+	–	+	–	–	–	+	–	–	–	–			+	+
Bacilli (single)	+	–	+	+	–	–	+	+	+	–	–			+	-
Bacilli (pairs)	+	–	–	+	–	+	+	–	–	+	–			+	+
Bacilli (pairs)	+	–	+	–	–	–	+	–	+	+	–			+	+
Cocci (clusters)	+	–	–	+	–	–	+	+	+	+	–	+	-		
Bacilli (single)	+	–	–	+	–	–	+	–	–	+	–			+	–
Cocci (clusters)	+	–	+	–	–	–	+	–	–	–	–	+	–		
Bacilli (single)	+	–	+	–	–	–	+	–	–	–	–			+	+
Cocci (single)	+	–	+	–	–	–	+	–	–	–	–	+	–		
Cocci (cluster)	+	–	+	–	–	–	+	–	–	–	–	+	–		

Table.2 Characteristics of Fungi Isolated from the Samples

Fungus	Colonial Morphology	Microscopic Characteristics
Aspergillus niger	Cottony growth which is brownish black in colour underside yellow/orange	Roundish conidiophores brownish vesicles globose and produce primary and secondary stenigmata and has branch hyphae
Aspergillus flavus	Cottony white irregular shape greenish-yellow with purple edges	Conidiophores septate and colourless. Terminates at the base with septate rhizoids sterigmata and the conidia is globose.
Aspergillus fumigatus	Velveting and spread over the plate. Light brownish colour at the centre and whitish edges.	Conidiophore coloured in shades of green and short. Conidia in chain on sterigmata, vesicle brownish coloured and hyphae septate
Penicillium spp	Surface of colony bluish-black spreading with yellow underside	Hyphae septate and branched conidiophores septate with branchelets. Conidia in chain giving broom shape appearance.
Mucor racemoses	White wooly colony mycelium arising from PDA	Non-septate sporangiosphore. Simple bend branched. Round cylindrical or pear shaped columelar.
Cephalosporium spp.	White colony with orange underside	Septate mycelium, non-septate conidiophores arise at random from hyphae has elongated conidia and colour at the tip of the conidiophores.
Rhizopus stolonifer	White colony with brownish underside	Brown-black sporangia, globose to subglobose, sporangiosphore are irregular in shape, slightly rough walled stolons opposite the branched rhizoid.

Table 3: Bacteria isolated from the samples

Bacterial isolates	**Sample Sites**
Bacillus polymyxa	University Guest Inn, School of Matriculation Studies No. 1 Shuni road Mabera No. 7 Wurno road No. 8 Gandu Area
Bacillus alvei	No. 4 Marina Kofar Atiku No. 16 Bazza road, Bazza Area University Guest Inn
Bacillus cereus	House No. 10 Diplomat Area, Sokoto University Guest Inn, No. 1 Junaidu road Corpers Lodge Kasuwar Daji
Bacillus coagulans	House No. 32 Sultan Abubakar road No. 42 Gangaren Dadin Kowa No. 5 Behind National Library
Bacillus firmus	No. 3 Emir Yahya road beside Sultan Maccido Mosque House No. 32 Sultan Abubakar road School of Matriculation Studies
Staphylococcus epidermidis	No. 10 Modibbo road Gidan Yani Area School of Matriculation Studies
Bacillus laterosporus	House No. 10 Diplomat area Sokoto No. 8 Gandu Area
Staphylococcus kleosii	No. Junaidu road No. 10 Diplomat Area Sokoto No. 32 Sultan Abubakar road

Rhizopus stolonifer, Cephalosporium specie and *Penicillium* specie. Table 3 indicated the total bacterial count obtained from the samples. No, 32 Sultan Abubakar road has the highest bacterial count of 1.20 x 10^5 cfu and No. 7 Wurno road has the least bacterial count of 1.1 x 10^4 cfu.

From the present study showed that the bacteria *Bacillus alvei, Bacillus cereus, Bacillus coagulans, Bacillus firmus, Bacillus laterosporus, Bacillus polymyxa, Staphylococcus epidermidis* and *Staphylococcus kleesii* were found to be present in the walls of investigated buildings in Sokoto metropolis. Fungi *Aspergillus fumigatus, Aspergillus niger, Cephalosporium* spp, *Mucor racemoses* and *Rhizopus stolonifer* were the common inhabitants of the walls of the investigated buildings.

The concentrations of bacteria were higher in University Guest Inn and School of Matriculation Studies buildings than in the other buildings. The results obtained in this study are comparable to the findings of a study conducted in Homes Finland (Pelczer *et al*., 2007)

Table.4 Fungi isolated from the samples

Fungal isolates	**Sample Sites**
Aspergillus niger	House No. 32 Sultan Abubakar road House No. 32 Diplomat Area, Sokoto No. 1 Junaidu road University Guest Inn. Corpers Lodge Kasuwar Daji
Aspergillus flavus	University Guest Inn School of Matriculation Studies No. 7 Wurno Road No. 1 Shuni road
Aspergillus fumigates	No. 4 Marina Kofar Atiku No. 3 Emir Yahya beside Sultan Maccido Mosque No. 8 Gandu Area
Rhizopus stolonifer	No. 42 Gangaren Dadin Kowa Corpers Lodge Kasuwar Daji No. 10 Modibbo road Gidan Yani Area No. 16 Bazza road, Bazza area
Penicillium specie	No. 5 Behind National Library
Mucor racemoses	No. 1 Junaidu road No. 7 Wurno road

which indicated the concentration of bacteria were higher in buildings that had contact with water. Similarly, the concentration of fungi were higher in No. 32 Sultan Abubakar road, Junaidu Road and Wurno Road than in the other buildings. Significant differences were observed only in Diplomat Area and University Guest Inn. This is indicative of possible water sources due to the existing moisture which is in accordance with previous studies reported by Parker (2007) whose results suggested that the concentration of viable bacteria seem to be very high which can be attributed to the prevailing moisture and environmental conditions in the buildings. Also, the occurrence of fungi in higher concentration in the buildings investigated may indicate that the substrates are made available for their growth. These fungi deteriorate the substrates in order to obtain energy. It can be inferred from the findings that moisture damage may change the fungal composition of the buildings which support the earlier conclusion that certain microbes indicate moisture damage (Goodman, 2006).

In conclusion, it can be surmised from the study that the distribution and abundance of microorganisms in the affected buildings is a consequence of moisture and other environmental conditions. Fungi and bacteria play an essential role in our global mycology/bacteriology and survival on

Table.5 Total bacterial count obtained from the Samples

Sample Site	Total bacterial Count (CFU)
University Guest Inn	7.8×10^4
House No. 32 Sultan Abubakar Road	1.20×10^5
School of Matriculation Studies	8.2×10^4
House No. 10 Diplomat Area Sokoto	9.4×10^4
No. 4 Marina Kofar Atiku	7.1×10^4
No. 42 Gangaren Dadin Kowa	T N C
No. 9 Shuni road Mabera	4.2×10^4
Corpers Lodge Kasuwar Daji	2.0×10^4
No. 16 Bazza road Bazza area	6.3×10^4
No. 5 Behind National Library	7.2×10^4
No. 3 Emir Yahya beside Sultan Maccido Mosque	7.9×10^4
No. 7 Wurno road	1.1×10^4
No. 1 Junaidu road	5.8×10^4
No. 10 Modibbo road Gidan Yani area	8.5×10^4
No. 8 Gandu area	1.02×10^5

plant and animal, but can also pose a significant health risk when they are permitted to grow in buildings. Fungal and bacterial colonization occurs when biodegradable materials are chronically damp or wet. On the basis of the results found, it can be concluded that moisture content of the habitat is the most important factor for the growth of microorganisms.

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