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Isolation And Characterization Of Most Probable Molds Associated With Some Damaged Materials.

Ananso, G. N[1]., Okougha A. F[1]., Sanni, J. D[1]., Itoandon, E. E[2]., Shobowale, O. O[3]. And Oje, N. C[2].

[1]*Science Laboratory Technology And Research Department, Nigerian Building And Road Research Institute, Ogun State.*
[2]*Biotechnology Department, Federal Institute Of Industrial Research, Lagos State.*
[3]*Supreme Education Foundation, Magodo, Lagos, Nigeria.*
Corresponding Author: Ananso, G. N1.,

Abstract**:** *Damage of some building materials caused by microbial activity resulting to deterioration or degradation has been studied extensively. Many studies have examined the colonization of different building materials by various organisms while other research has determined different level of damage by measuring changes in the physical properties. Fungal growth in damp or water-damaged buildings worldwide is an increasing problem, which has adverse effects on both the occupants and the buildings. In this investigation, scrapings of the following: (i) burnt brick, (ii). concrete wall, (iii). aluminium sheets, (iv). wood, (v). laterite stabilizers and (vi). asbestors were used as samples for identification of most probable mold strains that cause damage due to influence of favourable factors such as pH, moisture and other nutrients. These samples were collected from Lagos and Ogun states separately within the southwestern region of Nigeria. The aim of this investigation was to identify filamentous fungi present on these damaged building materials. The results showed the following filamentous fungi: (i). Aspergillus niger, (ii). Aspergillus flavus, (iii). Trichederma harzanium and (iv). Rhizpous stolonifera, as most probable causative microbial agents acting on each sample. This investigation has demonstrated the level of microbial association with some building materials within different environment. Although several studies have shown similar results, it is very important to understudy mechanism of each microbial activity and also initiate a more a protective method in addition to improving longer shelf of building materials thus promoting both environmental and market value.*

Keywords*: Filamentous Fungi, Building Materials, Biodegradation, Biodeterioration.*

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I. Introduction:

Deterioration as well as degradation of building materials can be described as a loss of structural capacity with time due to action of external agents such as microorganisms, insects, man due to influence of some environmental conditions (20). It has many dimensions and depends on the type of structure, the constitutive material, the environmental conditions and the operation characteristics, as well as other factors. The actions are primarily dependent on the availability of water and nutrients (23). Thus, material specific parameters, like porosity and permeability, and architectural conditions, which determine exposure and environmental factors at the site, will determine the intensity and rate of damage (22). Progressive damage has perhaps the broadest impact on the long term performance of infrastructure systems and the largest potential economic consequences (4).

It has been observed that the loss of structural capacity with time is caused mainly by chloride ingress, which usually leads to loss of effective cross-section of steel, concrete cracking, aggregate-hydrated cement paste, wood degradation, burnt bricks and spalling (2,4). Among these causes of structural degradation, it has been noted that damage arising from biological sources is significant in harsh environments (20). Nevertheless, the role of biologically-induced degradation with respect to reinforced building materials remains uninvestigated. Also higher porosity values can also lead to increase surface wear, reducing the depth of the protective concrete cover over the reinforcement. More so higher diffusivity and lower concrete covers can facilitate other deterioration processes such as corrosion of the reinforcement. Microorganisms can penetrate inside any kind of material even if there are no observable cracks and the most common mechanism for their ability is through the capillaries in the material (20). Some research has been performed concerning the analysis of some structures to verify whether microorganisms could be responsible for some of the damage observed (23,24,25). Laboratory analysis of building samples has shown that many microorganisms such as fungi (Yeasts,

Cladosporium, mycelia, hypha etc.), bacteria (*Actinomycetes*, *Thiobacillus*, among others), algae (the most popular are diatom algae) and even protozoa, can be found within the concrete matrix (13,14,5).

The consequences of microorganisms within the microstructure are different. Although there is insufficient experimental evidence, it has been observed that the action of microorganisms increases porosity, which in turn can change the diffusivity of most materials (20). The action of microorganisms on structures of some building materials can be classified according to their effects on concrete surfaces, concrete matrices, and on cracking and crack growth (3). According to (20), the action of microorganisms affect the surface of materials mainly by contributing to the erosion of the exposed concrete surface, reducing the protective cover depth, increasing concrete porosity, increasing the transport of degrading materials into the concrete that can accelerate cracking, spalling, and reduce the service life of the structure (11). Filamentous fungi are the main microorganisms influencing the concrete bio deterioration or degradation. The productions of biogenic organic acids, carbon dioxide as well as hydrolytic enzymes have been attributed to damage of different building materials (25,9). The identification of the most important microorganisms in bio deterioration/degradation are in various stages processes and must be defined by carrying out careful experiments so that treatment procedures can be developed. The aim of this investigation is to enumerate and identify most probable filamentous fungi associated with some scrapings.

II. Materials and Methods

Sample collection:

Scrapings of the following: (i) aluminum roof, (ii) asbestos roof, (iii) burnt bricks, (iv) concrete wall, (v) wood and (vi). Laterite stabilizer were collected as samples from factories located in Lagos and Ogun states separately using sterile sample collection bottles, knives, spatula, and gloves. These samples were taken to the laboratory of the biotechnology department of the Federal Institute of Industrial Research, Oshodi, Lagos state, Nigeria for the evaluation of microbial agents causing damage on the finished products.

Preparation of media:

Potato dextrose agar (Merck Cat. No: 69775 containing: agar 15g/l, dextrose 20g/l, potato extract 4g/l, pH 5.6 + 0.2) was prepared according to manufacturer's direction and used for the isolation, characterization, sub-culturing and preservation of mold isolates. 200ml of the medium was prepared in a 500ml conical flask and further sterilized at 121°C for 15mins under 15 psi pressures. After sterilization, medium was cooled to 45^{o}C before use. 50µg|ml streptomycin was added to inhibit the growth of bacteria that could occur as contaminant.

Isolation of molds:

1g of each sample was dispensed into 9ml of distilled sterile water to form a solution in sterile test tubes. Using the micropipette (Name), serial dilution was carried out by taking 1ml of solution from test tube No. 1 and serially diluting in a ratio of 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8 and 1:9. From ratio 1:3, 1:6 and 1:9, 1ml was inoculated into an empty sterile Petri Dish and pouring the prepared using pour-plate method. The culture media were allowed to solidify and further incubated at 30^{o}C for 5 days using a static incubator (VMR International, 1585/1585R Shaking Incubator)(16).

Microbial enumeration and characterization

After incubation period, the microbial growth count was taken per dilution. The method was further used for identification. Clean sterile glass slide, lactophenol cotton blue, wire loop, sterile cover slips and fluorescence microscope were used for morphological identification of each specimen. Sterile wire loop was used to collect a loop full of the lactophenol cotton blue and placed on the slide. The wire loop was then passed over a flame, used to pick the mycelium of a microbial growth and added to the loop full making a smear. The cover slip was then placed over the smear and then examined under the microscope at X40 magnification for imaging (17,18).

Identification of mold isolates

The method of (8,9,11) was carried out for representative colonies on the basis of their morphological traits (shape, size, and color of the colony; shape of cells).

III. Results

Building materials are decayed by environmental conditions and often depend on type of material and present condition such as pH, moisture and other nutrienst. According to (19) these materials provide ecological niches in their ecosystem for settlement, growth proliferation of a variety of mold species. (20) reported that mould growth intensity and rate depends on nutrition and pH level of material surface. This results presented in Figure 1 below are scrapings of different damaged materials collected for analysis.

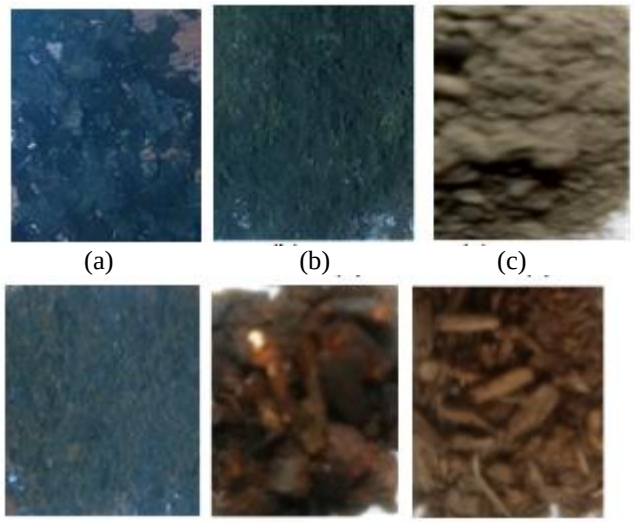

Figure 1: Scrapings of damaged building materials. The following samples are as follow: (a. Burnt Bricks, b. Asbestors roof , c. Laterite, d. Concrete wall, e. Aluminium roof, and f. Wood)

Microbial enumeration is to determine colony count per gram of sample. Since microbial load is usually high in sample, dilution allows dispersion and further enables assessment. The Table 1 below indicates difference in microbial load per sample from different location. Although similar microbe did appear from each sample, it shows microbial diversity. Similar report was made by (25).

Table 1: Table of microbial colony count (cfu/g).

Samples	Dilution factors	Lagos state(cfu/g)	Ogun state cfu/g)
Aluminum Roof	1:3	2.0×10^{-3}	1.0×10^{-3}
	1:6	1.0×10^{-6}	Nil
	1:9	Nil	Nil
Asbestos roof	1:3	3.0×10^{-3}	1.0×10^{-3}
	1:6	1.0×10^{-6}	1.0×10^{-6}
	1:9	Nil	Nil
Burnt bricks	1:3	2.0×10^{-3}	1.0×10^{-3}
	1:6	1.0×10^{-6}	1.0×10^{-6}
	1:9	Nil	Nil
Concrete wall	1:3	3.0×10^{-3}	2.0×10^{-3}
	1:6	2.0×10^{-6}	1.0×10^{-6}
	1:9	Nil	Nil
Wood	1:3	4.0×10^{-3}	2.0×10^{-6}
	1:6	2.0×10^{-6}	1.0×10^{-6}
	1:9	1.0×10^{-9}	Nil
Laterite cement (stabilized)	1:3	1.0×10^{-3}	1.0×10^{-3}
	1:6	1.0×10^{-6}	Nil
	1:9	Nil	Nil

Wet mount is a microbiological practice which allows identification of variable microbial shape, cell, and spore formation. Table 2, 3 and 4 showed the most probable isolates as well as morphological characteristics of each isolate. From the Table 2 below, each sample had similar isolate growing which confirms the report of (24) describing fungi growth mechanism involving utilization of available conditions for growth and development.

Table 2: Table of most probable mold isolates from different scraped samples.

Samples	Most probable isolate
Aluminum Roof scrapping	*Rhizopus* sp., *Aspergillus* sp., *Trichoderma* sp.
Asbestos Roof scrapping	*Aspergillus* sp., *Rhizopus* sp.
Burnt Bricks scrapping	*Rhizopus* sp., *Aspergillus* spp.
Concrete Wall scrapping	*Trichoderma* sp., *Aspergillus* sp.
Wood scrapping	*Aspergillus* sp., *Rhizopus* sp.
Laterite Cement (stabilized rock) scrapping	*Trichoderma* sp., *Rhizopus* sp., *Asepergillus* sp.

Further identification of mold is by conventional method. The growth of isolate is often different in appearance on media. In this investigation, morphological characteristics of each culture growth are described in Table 3 and Table 4 as they appear on Potato Dextrose Agar shown below.

Table 3: Table of macroscopic characterization of identified isolates on Potota Dextrose Agar

Macrscopic Characterization	Identified isolates
Growth identified on substrates produced colonies of whitish wool felt like hyphae, turning black brown with the formation of conidia. Reverse of growth formed a yellow river pattern.	*Aspergillus niger*
Conidia appeared light sparse grey green to parrot green, mycelium fluffy creamy white to dull white color and exudates were present on surface, reverse uncolored to yellowish and wrinkled mycelial growth; soluble pigments were absent; very few sclerotia were present in wheat brown color	*Aspergillus flavus*
The surface appears with texture deeply cottony; white becoming gray-brown on surface, reverse often pale white. Very rapid growth.	*Rhiozopus stolonifera*
Trichoderma harzianum formed minimal concentric rings with green conidial production. The conidia production was denser in center then towards the margins.	*Trichoderma harzianum*

Table 4: Table of microscopic characterization of identified isolates on Potota Dextrose Agar

Morphological Characterization	Identified isolates
Conidiosphores are smooth, hyaline or faintly brownish near the apex. Apices are spherical but often quite small. Two series of conidia-bearing cells (supporting cells and phialides) are produced. Growth usually from whitish colorless to blackish brown.	*Aspergillus niger*
Conidiophores are heavy walled, hyaline, coarsely roughned, and usually Apices are elongated when young, becoming subspherical to spherical. There can be one or two series of conidio-bearing. Philiades are present.	*Aspergillus flavus*
Stolons are hyaline becoming brown towards nodes, near which a spectrum may occur. Rhizoids are short, brown and sometimes absent. Sporangiosphores arise singly or in small groups from nodes on the stolons. They are brown, smooth or finely roughned, non-septate. Sporangia are spreical, initially white but later black. Columellae are light brown and umbrella-shaped when dehisced. Sporangio-sphores are yellow to dilute brown, spherical or oval, longitudinally striped.	*Rhiozopus stolonifera*
Colonies are transparent at first on media. Conidia typically form within one week in compact or loose tufts in shades of green or yellow or less frequently white. Conidiophores are highly branched, Phialides may be held in whorls. Conidia typically appear dry but in some species they may be held in drops of clear green or yellow liquid.	*Trichoderma harzianum*

IV. Discussion:

Filamentous fungi also regarded as mold are being described as artificial grouping of a number of species of microfungi that have common life strategies. They grow on the surface of materials, use easily assimilated compounds as nutrients and energy sources, and produce spores as dispersal and survival units (19). These spores are produced in high amounts and widely spread across different environments. When favourable conditions are present, the spore will germinate and a small germ tube will develop; if the favourable conditions prevail, a hypha will be produced. A hypha is a tubular cell structure which extends at the tip. By continuously branching during growth, the hyphae form a mycelium. Eventually, specialized structures (conidiophores) develop from the hyphae and from them the spores are produced and dispersed. It is in the hyphae that the activity of the fungi takes place (19).

The scrapings of some building materials used for analysis are shown in Figure 1. From the figure, the following samples; aluminum roof, asbestos roof, burnt bricks, concrete wall, wood and laterite stabilizer are amongst materials that usually attacked by microorganisms causing damage due to conditions that favour growth and reproduction. Research has shown that many building materials can support growth of microbes and mould problems are more common than decay damages. (19) have reported that typical mould found in damaged buildings are e.g. *Acremonium*, Aspergillus species (e.g. *fumigatus*), *Aureobasidium pullullans*, *Alternaria alternata*, *Cladosporiu*m species (e.g.*herbarum*, *sphaerospermum*), *Mucor* species, *Penicillium* species (e.g. *brevicompactum*) and *Stachybotru*s species (e.g. *atra*). A large number of species of microfungi are commonly found in damp buildings. Several studies have attempted to survey which are the most common species and on which building materials they exist. In similar reports by (1,2,3), growth found at least 45 species; (16) found fungi from at least 22 genera; (25) found at least 49 species. (13) lists 52 species isolated from building interiors. No matter how big the exact number, the conclusions are that there are many varied species, they all represent a broad range of demand for water, and they vary in their potential for growth. Some will only grow in very specific environments, while others may be able to colonize more diverse environments (9).

Growth count of probable mould isolated from different samples is shown in Table 1. From the table, most counts were recorded for dilution fact 1:3 for all samples. There was difference in enumeration mostly due to type of material composition. Scarping from wood sample recorded 4.0 x 10^{-3} as highest count followed by scrapings from concrete wall and asbestos respectively recording value of 3.0 x 10^{-3}. Several reports have shown species occur together on a building material ((1;16), while at other times only one species dominates, as reported for *Penicillium corylophilum* in crawl spaces in southern Sweden (6). This is confirmed in Table 2 recording isolates from different samples using potato dextrose agar. From the table the following most probable isolates were *Rhizopus* sp., *Aspergillus* sp., and *Trichoderma* sp. The taxonomical identification of mold was based on their morphological characteristic as observed under compound microscope after 5 days at 30°C.

Morphological characters were studied for identification of all these isolates for culture media. Tables 3 and Table 4 described the morphological characteristics of each isolate. From the tables, *Apsergillus niger*, *Aspergillus falvus*, *Rhizopus stolonifera* and *Trichoderma harzanium* were identified. Since the concentration of organic compounds, as well as other characteristics like pH, surface structure, etc., varies among materials, the expected critical moisture conditions also vary. One of the earliest studies of mould growth on different types of building materials was by (Ref). He concluded that the most sensitive materials will be subject to mould growth at 80% RH at room temperature. Numerous studies have since attempted to identify the climates in which different types of building materials begin to mould (19;). Some of the published studies were used to estimate the critical moisture limit for different groups of building materials (Johansson et al., 2005). However, differences in methodology and/or variations in evaluation of the data complicated the comparison between the different studies and the estimation of critical moisture levels. Factors that vary among the experiments include the fungi used, inoculation method, climate, duration, analytical method and frequency of analyses. Studies also vary in their criteria of when growth is considered to be critical.

V. Conclusion:

The conclusion from these observations is that mould growth on different materials in different area may be very diverse; thus, it is be very hard, if not impossible, to predict the composition of a fungal population, whether the growth will be visible or whether it will affect indoor air negatively.

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