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Isolation of Fungal Flora in Carpet and Floor Dust Samples As an Indicator of Indoor Air Quality (IAQ): A Case Study of a Nigerian Institution

Abu- Saeed Muhammad Buhari[1], Abu-Saeed Kamaldeen[2], Hassan Ummu K. M.[3]

[1]Microbiology Department, University of Ilorin Teaching Hospital, Ilorin. Nigeria.
[2]Research Unit, Peace Standard Pharmaceuticals, Ilorin. Nigeria.
[3]Microbiology Department, Usmanu Dan Fodio University, Sokoto. Nigeria.

Abstract- Porous surfaces such as carpets have been examined as a possible source for the introduction of indoor particle levels. Indoor airborne mold and/or mycotoxin exposure causes many multi-system adverse human health effects. This study is aimed at isolating and identifying fungal flora in the dust samples of various indoor environments in Usmanu Dan Fodio University, Sokoto, Nigeria. Samples were obtained from six (6) carpeted old buildings, two (2) carpeted new buildings, two (2) uncarpeted old building and two (2) uncarpeted new buildings. Samples were inoculated onto Potato Dextrose Agar (PDA) and identified. Ten (10) fungi species from 12 dust samples involving 12 indoor environments were isolated and identified. Total mean microbial concentration was $1.65x10^3$ cfu /g, average concentration $1.37x 10^2$ cfu/g and standard deviation of 6.73×10^1 cfu/g. The isolates include *Aspergillus niger, Aspergillus flavus, Apsergillus fumigatus, Aspergillus ustus, Mucor racemosus, Mucor hiemalis, Rhizopus stolonifer, Scopulariopsis spp., Trichoderma spp., Wallemia Spp. A flavus,* (100%) and A. Niger (91.7%) had the highest frequency of occurrence while A. ustus (8.3%) had the lowest frequency of occurrence. Conclusively, fungi are present in dust samples including infectious and toxigenic species. Therefore, attention should be given to our indoor air quality.

Index Terms- Indoor air quality, fungal flora, indoor environment, adverse health effects.

I. INTRODUCTION

Indoor air is made up of numerous airborne particles including bacteria, fungi, allergens and dusts. In a typical indoor environment, such as a home, school, workplace, particle levels are influenced by occupant activity, internal maintenance practices, the quality of interior maintenance and everyone of outdoor air that is brought into the facility by the ventilation system. Study of indoor air quality is a fairly new scientific discipline and the impact of interior particulate levels is only now been researched. Some researchers believe interior furnishings may play a role in these airborne particles levels. Porous surfaces such as Carpets have been examined as a possible source for the introduction of indoor particle levels [1]. Studies have observed that floor coverings could be pollutant sources as well as sinks and safes. (A ***source*** is something that emits contaminants, a ***sink*** is a reservoir for pollutant and a ***safe*** traps contaminants)[2].

Over the past decade, there has been growing concern regarding the role of toxigenic fungi in damp indoor environment, however, there is still a lack of field investigation on exposure to mycotoxins [3]. Fungus is an eukaryotic, spore bearing organism that has absorptive nutrition and lack chlorophyll; that reproduces asexually, sexually or both methods and that normally has filamentous hyphae surrounded by cell walls, which usually contain chitin [4]. Molds are forms of fungi found all year round both indoors and outdoors. Its growth is encouraged by warm and humid conditions although it can grow during cold weather. Most molds found indoor comes from outdoors sources. It needs moisture to grow and becomes a problem only where there is water damage, high humidity or dampness. Common sources of indoor moisture that can cause mold problems include flooding, roof and plumbing leaks, damp basements or crawl spaces, or any moisture condensation on cold surfaces. Bathroom showers and steam from cooking may also create problems if not well ventilated [5]. Many microbial organisms including molds are found in dust deposited on surfaces and are suspended into air by activities that stir up dust such as walking or vacuuming. Molds are also found on indoor surfaces and can release spores into the indoor air [6].

Indoor airborne mold and/or mycotoxin exposure causes many multi-system adverse human health effects. Health care professionals, building managers, homeowners and the general public need to be much more aware of their potential adverse health effects, the need for proper building remediation and the need for appropriate patient diagnosis and treatment. There is sufficient data from the medical literature and large number of clinical reports to substantiate the reported adverse health effects of indoor airborne mold. Indoor mold and mycotoxin exposure absorbed through the respiratory route can be a major pathway of injury by all 3 mechanisms: Infection, Allergy and Toxicity [7]. Therefore, this study is aimed at isolating and identifying fungal flora in the dust samples of carpets and floors used in selected offices, laboratories and common rooms in Usmanu Dan Fodio University, Sokoto, Nigeria.

II. METHOD

Sample Collection

Samples were obtained from six (6) carpeted old buildings, two (2) carpeted new buildings, two (2) uncarpeted old building and two (2) uncarpeted new buildings. A total of twelve (12)

samples sites all within the premises of Usmanu Dan Fodio University, Sokoto, Nigeria. Samples were collected using new carpet brush and new plastic collector which were surface sterilized before each collection by washing with 70% ethanol. Each dust sample was collected into a new sterile polythene bag and appropriately labeled. A square meter of the carpet was surface sampled by brushing in multiple directions such that the available dust could be practically collected.

Sample Inoculation

Samples were analyzed for fungi that can be cultured using a procedure described. One gram (1g) of individual samples was weighed and suspended in 10ml sterile distilled water. From the above suspension, 1ml was then diluted to 10^1. Then, 1m1 of the diluted sample (that is, 10^1) was then inoculated onto Potato Dextrose Agar (PDA) prepared according to manufacturer's instruction as a general screening medium and incubated at room temperature (about 32^0C) between 5-7 days. This procedure was carried out in duplicate.

Identification

Fungal growth observed were identified and reported in colony forming units per gram of dust (cfu/g of dust). Macroscopically, the colour and appearance were noted. Microscopic examination was carried out and identification was speciated where possible.

A portion of the obtained culture was placed and teased out into a clean glass slide upon a drop of lactophenol cotton blue using sterile inoculating needles and covered with clean coverslip. It was then viewed under the microscope using x4, x10 and x40 objectives. Identification was based on characteristic morphology, which was compared to the mycological atlas for confirmatory identification.

III. RESULT

At the end of this study, a total number of 10 fungal species were isolated. The following results were recorded and presented in four (4) separate tables as follows:

Table 1: This highlights the exact locations where the samples were obtained and the characteristics of such floors whether carpeted or uncarpeted and the relative age of such building simply put as new or old**.** Designations were also assigned to each location for easy reference in other table presentations and discussion.

Table 2: This highlights the specific fungal species isolated from each sample site. Level of contamination of each site was expressed in percentage of the total number of fungal species obtained from the study which was ten (10) species in all.

Table 3: Highlights the result of the study expressed in colony forming units per gram of dust sampled (cfu/g). Total colony count was 1.65×10^3 cfu /g, average count was 1.37×10^2 cfu/g while standard deviation was 6.73×10^1 cfu/g.

Table 4: This shows the frequency or occurrence of each fungal species isolated among the twelve sites expressed in percentage.

TABLE 1: SAMPLE SITES, CHARACTERISTIC OF LOCATIONS AND DESIGNATIONS.

LOCATIONS	CHARACTERISTICS	DESIGNATIONS
Head of Unit, Microbiology Office	Old building, Carpeted	Sample site 1 (S.S.1)
Head of Unit, Zoology Office	Old building, Carpeted	Sample site 2 (S.S.2)
Head of Unit, Botany Secretary's Office	Old building, Carpeted	Sample site 3 (S.S.3)
Dean of Sciences secretary's Office	Old building, Carpeted	Sample site 4 (S.S.4)
Computer room Bursary department	Old building, Carpeted	Sample site 5 (S.S.5)
'I.B.B' Centre Male common room	Old building, Carpeted	Sample site 6 (S.S.6)
Personal Assistant to Dean of Post Graduate School's Office	New building, Carpeted	Sample site 7 (S.S.7)
Deputy Dean Post Graduate School Office	New building, Carpeted	Sample site 8 (S.S.8)
Microbiology (Undergraduate III) Laboratory	Old building, Carpeted	Sample site 9 (S.S.9)
Microbiology (Undergraduate IV) Laboratory	Old building, Carpeted	Sample site 10 (S.S.10)
New male hostel common room 1	New building, Carpeted	Sample site 11 (S.S.11)
New male hostel common room 2	New building, Carpeted	Sample site 12 (S.S.12)

TABLE 2: TYPES OF FUNGI IDENTIFIED AT SPECIFIC SAMPLE SITES AND LEVEL OF CONTAMINATION

SAMPLES SITES	**FUNGI IDENTIFIED**	**LEVEL OF CONTAMINATION (%)**
Sample site 1	*Aspergillus niger, Aspergillus flavus*	20
Sample site 2	*Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus*	30
Sample site 3	*Aspergillus niger, Aspergillus flavus, Mucor racemosus, Scopulariopsis spp, Rhizopus stolonifer*	50
Sample site 4	*Aspergillus niger, Aspergillus fumigatus Mucor racemosus, Rhizopus stolonifer*	40
Sample site 5	*Aspergillus niger, Aspergillus flavus, Mucor racemosus, Mucor hiemalis, Wallemia Spp.*	50
Sample site 6	*Aspergillus niger, Aspergillus flavus, Aspergillus Ustus.*	30
Sample site 7	*Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Mucor racemosus, Mucor hiemalis, Rhizopus stolonifer*	60
Sample site 8	*Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Mucor racemosus, Rhizopus stolonifer.*	50
Sample site 9	*Aspergillus niger, Aspergillus flavus, Mucor racemosus, Rhizopus stolonifer, Scopulariopsiss spp, Trichoderma spp., Wallemia spp.*	70
Sample site 10	*Aspergillus niger, Aspergillus flavus, Mucor racemosus, Rhizopus stolonifer, Trichoderma spp.*	50
Sample site 11	*Aspergillus niger, Aspergillus flavus, Mucor racemosus, Trichoderma Spp.*	40
Sample site 12	*Aspergillus niger, Aspergillus flavus, Mucor racemosus, Wallemia spp.*	40

TABLE 3: AVERAGE FUNGAL CONCENTRATIONS BY SPECIFIC SAMPLE SITES.

Sample sites	**Average number of colonies**	**Dilution factor (10^1)**	**cfu/g of dust**
Sample site 1	14.5	10	1.45×10^2
Sample site 2	8.0	10	8.0×10^1
Sample site 3	9.5	10	9.5×10^1
Sample site 4	5.5	10	5.5×10^1
Sample site 5	16.0	10	1.6×10^2
Sample site 6	18.0	10	1.8×10^2
Sample site 7	10.5	10	1.05×10^2
Sample site 8	10.0	10	1.0×10^2
Sample site 9	26.5	10	2.65×10^2
Sample site 10	12.0	10	1.2×10^2
Sample site 11	8.5	10	8.5×10^1
Sample site 12	25.5	10	2.55×10^2

Total Colony count - 1.65×10^3 cfu/g
Average count - 1.37×10^2 cfu/g
Standard Deviation - 6.73×10^1 cfu/g

TABLE 4: FREQUENCY DISTRIBUTION BY FUNGI SPECIES ISOLATED

Fungi Species Isolated	Total number of sample sites	Number of sites of observed presence	Frequency (%)
Aspergillus niger	12	11	91.7%
Aspergillus flavus	12	12	100.0%
Aspergillus fumigatus	12	4	33.3%
Aspergillus ustus	12	1	8.3%
Mucor racemosus	12	9	75.0%
Mucor hiemalis	12	2	16.7%
Rhizopus stolonifer	12	5	41.7%
Scopulariopsis spp.	12	2	16.7%
Trichoderma spp.	12	3	25.0%
Wallemia spp.	12	3	25.0%

IV. DISCUSSION

Based on the result presentation and analysis of this study, certain useful assumptions vis-à-vis the sample sites could be made after discussing the results based on their merits and facts. However, in order to appreciate the economic values of the isolated species in this study, it will be logical to appraise them based on the extent to which they can cause hazards. This hazards classification has been used by a number of researchers where they classified into:

Hazard Class A: This includes Fungi or their metabolites that are highly hazardous to health. These fungi or metabolites should not be present in occupied dwellings. Presence of these fungi in occupied building requires immediate attention.

Hazard Class B: This includes fungi, which may cause allergic reactions to occupants if present indoor over a long period.

Hazard Class C: Includes fungi not known to be a hazard to health. Growth of these fungi indoors, however, may cause economic damage and therefore should not be allowed [8].

Tables 2 and 3 reflect the level of contamination and concentration respectively. The most striking of the results is that from site 7 and 8, which are sites of a new building and new furnishing of less than 1 year old. The fungal concentration of 1.95 $x10^2$ cfu/g and 1.0 x 10^2 cfu/g respectively are high as well as their level of contamination – 60% and 50% respectively.

Comparatively, they are higher in concentration and level of contamination than some of the old carpeted buildings like sites 2, 3 and 4. The assumption is that the postgraduate school building, most mold found indoor originate from outdoor sources. Spores settle on virtually all building materials throughout the manufacturing process, storage and delivery to the construction site. Mold spores continue to infiltrate during the construction process. After the construction was completed, mold spores will continue to enter building through air current through open doors and windows, through heating, ventilation and air condition systems, through new materials and furnishings introduced into the building, and through the clothes and persons of people coming into the building [9]. All these could contribute to the high concentration and level of contamination recorded

since the building was not specially washed and disinfected after construction before putting it into use.

Another comparism worth of note is site 2 and site 11. Site 2 is an old carpeted office while site 11 is a new hostel common room with bare floor. Their concentrations are 8.0 x 10^1 cfu/g and 8.5 x 10^1 cfu/g respectively and their level of contamination are 30% and 40% respectively. Both sites show similar values despite their different features. Site 2 is an office with high foot traffic likewise site 11 but site 2 is more frequency swept and experience better sanitation while site 11 is used by students, less frequently swept and sanitized. Naturally, much higher concentration and contamination should be expected from site 11 than site 2. Therefore, one could assume that the presence of carpet in site 2 could be a factor, that is, the carpet may be serving as 'safe' and 'sink' for fungi spores by trapping and harboring which may not be completely removed by the routine sweeping [2].

Sample site 9 and 12 are also within similar range of concentration but different levels of contamination. With 2.65 x 10^2 cfu/g (70%) and 2.55 x 10^2 cfu/g (40%) respectively. Both sites are high foot traffic and indoor daily activity with diverse people and that could account for high concentration of both sites. However, site 9 is a microbiology lab, which has obvious activities going on in it that could account for the diversity of species, isolated giving it a higher level of contamination as compared to site 12, which is a hostel room.

Sites 2, 3 and 4 carpeted and frequently swept offices of high foot traffic but their concentration and contamination are low and as explained earlier, the carpets may be trapping some of the fungal particles which cannot be removed by frequent sweeping.
Sample site 1, 5 and 6 are generally higher in concentration than sites 2, 3 and 4. All six are old carpeted sites. The assumption here could be the 'sink' and 'safe' function of carpet, which traps those fungal particles from being released.

Sites 9 and 10 are both microbiology labs but it is observed that site 9 exhibits a much higher concentration and contamination that site 10. The possible assumption here is that a much higher volume of sterilization and disinfection activities takes place in site 10 being that final year students' lab. Much of their project practical procedures take place in this lab which involve diverse forms of sterilization. It is known that microbial population will be reduced by the same fraction at constant intervals when exposed to lethal agents[4].

Based on table 4, showing the frequency distribution of isolates, *Aspergillus flavus* (100%) and *Aspergillus niger* (91.7%) are the most ubiquitous. Along with *Aspergillus fumigatus* (33.3%), the three species of *Aspergillus* are classified as Hazard class A [8]. They are known to cause invasive Aspergillosis and produce mycotoxins e.g. aflatoxins and ochratoxins which are known carcinogens [9]. Hence, there is the need to pay adequate attention to our general indoor environments since they are ubiquitous and possess infectious and toxigenic health hazards.

Mucor racemosus (75%), *M. hiemalis* (16.7%) and *Rhizopus stolonifer* (41.7%) are readily present, known to cause more of opportunistic disease (mycosis) e.g. mucormycosis causing severe paranasal pulmonary infections, they are usually disseminated infection in those with reduced host defenses e.g. patients with uncontrolled diabetes, leukaemia, burns and those being treated with cytotoxic drugs corticosteroids [10]. Although *Rhizopus spp*. has a lot of beneficial characteristics, it also sometimes acts as a contaminant. *Rhizopus* and *Mucor* species have been implicated in zygomycosis [11].

Scopulariopsis spp. (16.7%) and *Trichoderma spp.* (25%) are generally classified as Hazard class B as they are known to releases certain allergens, which could trigger allergies in occupants, especially the sensitive ones, over a long period of time [8]. They are not ubiquitous, hence, with regular sanitation; they could be kept to the barest minimum.

Wallemia spp. (25%) is generally classified as Hazard class C. They are generally known to biodeteriorate porous household materials. They reduce aesthetic values of carpets and walls but not known to cause any health Hazard to humans [8]. *Aspergillus ustus* (8.3%) is rare and uncommon species and demands little or no attention.

V. CONCLUSION

From this study, it may be concluded that dust samples from carpets and floors of indoor environment harbor fungi of diverse species. There are also indications that the indoor air qualities are influenced by the nature of fungi species present in the dust samples. Furthermore, one can say that a good percentage of the fungi isolated have adverse health effects on occupants or users of such indoor environment especially the Hazard class A fungi.

Therefore, carpet and floor dust samples could be an indicator of indoor air quality. Thus, government agencies and general public to give proper and adequate attention to factors that precipitate indoor fungi and possibly remediate such factors to maintain the healthy well-being of occupants living within such indoor environment.

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AUTHORS

First Author – Abu- Saeed, Muhammad Buhari, Bachelor of Sciences (Microbiology), Masters of Public Health, Microbiology department, University of Ilorin Teaching Hospital, Ilorin. Nigeria, Email: Niasse75@yahoo.com

Second Author – Abu-Saeed, Kamaldeen, Bachelor of Pharmacy, Masters of Public Health, Research Unit, Peace Standard Pharmaceuticals, Ilorin. Nigeria, Email: Kamal.abusaeed@yahoo.com

Third Author –Hassan Ummu K. M, Doctor of Philosophy (Microbiology), Microbiology Department, Usmanu Dan Fodio University, Sokoto. Nigeria.

Correspondence Author – Abu- Saeed Kamaldeen, kamal.abusaeed@yahoo.com, onlykas@yahoo.com, +2348033898351, +2348059529632