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Levels of Fungi Aerosols in Residential Houses in Benin City, Southern Nigeria

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Abstract
Lack of monitoring residential homes for fungi contamination, has reportedly resulted in various health outcomes in vulnerable occupants. This study assessed the level of indoor and outdoor airborne fungi contamination and reveal the identity of air borne fungi species in the study area.
Forty-five households were randomly selected across the five local council areas in Benin city for airborne sampling. Airborne fungi were assessed bimonthly using the passive sampling technique. Discrete colonies of fungi were enumerated and mean values of triplicate concentrations were expressed in Colony forming unit / m^3 (CFU/m^3) Airborne fungi isolates were characterized and identified using standard procedures. The mean indoor and outdoor meteorological parameters ranged between 31.2 to 32.3°C and 31.1 to 32.5°C (Temperature); 71.6 to 74.1% and 63.0 to 74.1% (Relative humidity) respectively. The fungi concentrations varied from 301.0 to 747.1 CFU/m^3 and 337.67 to 554.6 CFU/m^3 (indoor); 223.8 to 450.4 CFU/m^3 and 378.64 to 532.7 CFU/m^3 (outdoor) in wet and dry seasons. The prevalent fungi genera isolated from the indoor and outdoor air across the sampling sites were Penicillium, Cladosporium, and Aspergillus. The outdoor fungi concentrations showed a significant association ($R = 0.360$ and 0.260; $R^2 = 0.130$ and 0.032; $p < 0.01$) with indoor fungi concentrations in both seasons. The study revealed high airborne fungi counts and fungi species of public health interest in the houses. Regular cleaning and maintenance practices to reduce the increasing effect of fungi spores is highly recommended.

Keywords: Airborne fungi, concentrations, homes, outdoor, indoor

INTRODUCTION
Human exposure to airborne microorganisms is inevitable due to their abundance in nature, and ability to grow in numerous habitats (Sharma *et al*., 2010). One of the major indicators of health risks in the indoor and outdoor environment is airborne fungi contamination (Samet and Spengler, 2003; Khan, 2009). Diverse fungi species have been reportedly isolated from different indoor and outdoor microenvironments (Portnoy *et al*., 2005; Khan *et al*., 2009). Notable among these species in indoor air samples include *Aspergillus* sp., *Penicillium* sp., *Alternaria* sp., and *Cladosporium* sp. (Zorman and Jersek, 2008; Bernasconi *et al*., 2003).

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Nearly all-natural and synthetic materials in the presence of moisture are substrates for fungi growth (Samet and Spengler, 2003). Studies have shown that fungi spores can occasionally be dispersed into the air through construction materials made of wood, food substances, house dust, pets and their beddings, clothing, carpets, and furniture (Portnoy *et al*., 2005; Kalogerakis *et al*., 2005; Sailer *et al*., 2010; Doherty *et al*., 2011).

Urbanisation and economic development have resulted in changes in lifestyles as it affects the pattern of buildings in most cities in developing countries. Insecurity has occasioned the reduction of the dimension of doors, and windows with little or no regard for indoor ventilation. The incessant power outage predominant in most cities has also contributed to the inactive air conditioning systems in homes which may lead to water condensation, increase in moisture content, and humid conditions that aid the growth of fungi (Hsu *et al*., 2011). Most buildings are reportedly having leakages in roofs and walls resulting in damp floors and surfaces which favours the growth of mould and fungi spores.

The prevailing atmospheric conditions, microclimatic factors, number of occupants, predominant anthropogenic activity, seasons, ventilation rates and quality of housing are major factors determining the load, types and distribution pattern of microbial population in the indoor environment (Adams *et al*., 2014). Overcrowding, various human activities, agitation and re-suspension of dust extensively also favour microbial growth (Udoh and Uyanga, 2013; Prussin and Matt, 2015; Tham, 2016). Exposure through inhalation, ingestion or skin contact with spores or secondary metabolites of airborne fungi has been linked with various health outcomes ranging from allergic diseases, respiratory symptoms, neuropsychiatric problems, hypersensitivity, and rheumatic diseases (Franks and Galvin, 2010; Bush, 2008; Breda *et al.,* 2010; Cann *et al.,* 2011; Yike, 2011; Ana and Fakunle, 2015).

Lack of monitoring residential homes for a range of biological hazards especially microbial contamination of indoor apartments has become a problem especially in developing countries.

Although there has been tremendous scientific progress in knowledge about air pollution due to bioaerosol contamination in developed countries, little or no contributions have been made in low- and medium-income countries including Nigeria. This is majorly due to the absence of established nationally approved standards for assessing exposures to indoor and outdoor bioaerosols, and few numbers of institutions committed to comprehensive environmental monitoring of bioaerosols Settle plates technique was used to estimate bacterial load in the indoor and outdoor air of buildings in the study area. Passive air sampling uses "settle plates", which are standard Petri dishes containing culture media, that are exposed to the air for a given time in order to collect biological particles which "sediment" out on the agar medium. The passive method of airborne microbial sampling provides a quantitative risk assessment as it quantifies the harmful component of the bioaerosols populations which is deposited on the surfaces of the substrate (Haig *et al*., 2016). Moreover, the method is applicable when the microbial load is perceived to be high.

In addition to the few existing studies on the research area in Nigeria (Durugbo *et al*., 2005; Ana and Fakunle., 2015; Odebode *et al*., 2020), this study seeks to provide comprehensive data on the composition and concentrations of airborne fungi species in residential buildings across Benin metropolis. This information will assist the relevant institutions with measures of control and interventions to mitigate the health hazards related to airborne fungi pollution in the area.

MATERIALS AND METHODS

Study area

Benin City is located within latitudes 6°20′N and 6°58′N and longitudes 5°35′E and 5°41′E, and situated in the humid tropical environment. Its rainfall element strongly determines the occurrence of the rainy and dry seasons (Agboola and Holder, 1979). The total annual rainfall amount recorded in the city ranged between 2,000 and 3,000 m. The rainy season is between April and October with a short dry period in August. The dry season begins in November and ends in April with a humid and dusty harmattan period between December and January. Also, high relative humidity between 75 and 85% is regularly experienced in the area (Okhakhu, 2014). The study area comprises of five area councils namely Oredo, Ikpoba okha, Egor, Ovia North East and Uhunmwonde.

Measurement of Meteorological Parameters

Indoor and outdoor temperature and relative humidity were measured with the aid of Lutron 4 in 1 environmental tester (LM-8000) at each sampling location. The measurements were taken bimonthly in triplicates throughout sampling.

Airborne Microbial Sampling

Microbial air sampling was carried out in both indoors (sitting room) and outdoor environments in the selected forty- five households across the five area councils. The passive method of airborne microbial sampling was utilized during the fungi measurements. Sampling was carried out between 9:00 am to 1:00pm, bimonthly in triplicates for one year covering wet and dry seasons between 2019 and 2020. Petri dishes of 9 cm in size containing Potato dextrose agar (PDA) were used for fungal sampling respectively. The Potato dextrose agar was impregnated with Chloramphenicol to inhibit bacteria growth (Napoli *et al*., 2012). The plates containing the sterile culture medium were placed for sampling at the height of 2 m above the ground level and with a distance of more than 1 m from the walls, doors, windows and barriers. The plates were exposed for a period of 30minutes to allow for appropriate surface density for counting with respect to time. The Petri dishes were immediately transferred to the laboratory and incubated at 28 ^{o}C for 7 days for fungi (Nasir *et al*., 2010).

Enumeration of airborne fungi flora

The quantitative analyses of the indoor and outdoor fungal isolates were carried out to determine the fungi concentrations or numbers in the sampled air. Mean values of triplicate airborne concentration were expressed in CFU/m^3 using the empirical conversion formula as stated by Gizaw *et al*., (2016).

$$N=5a*10^4(bt)^{-1}$$

a = number of colonies on the Petri dish, b = Surface area of the Petri dish (cm^2), t = Time of exposure (minutes).

Identification of airborne fungi

The qualitative analysis of fungi isolates was performed through observation of colonial microscopic features. Different characteristics which include surface and bottom colours, underside pigmentation colony elevation, presence of spores, shape and type of spores, presence of septa, hyphal mass and structure were important phenotypic characteristics used in the identification of fungi.

In addition, fungi colonial nature such as woolly, fluffy, powdery, cottony, fuzzy and slimy) were also used to compare each fungal morphological and cultural characteristics (Guan *et al.,* 2007; Obire and Anyanwu 2009; Sharma, 2009; Pincus, 2009).
Several physiological tests were also conducted to further identify the sexual characteristics, assimilation of carbon compounds, sugar fermentation, nitrate utilization, urea hydrolysis, and growth of yeast isolates (Barnett *et al.,*2000; Harley and Prescott, 2002).

Data analysis
The measured concentrations of airborne fungi in CFU/m^3 and meteorological parameters were subjected to statistical analysis using mean, analysis of Variance (ANOVA) in SPSS version 21.0. Variations in the level of monitored indoor and outdoor air bone fungi during the wet and dry season was determined using the paired t-test. Correlation and Regression analysis were employed to determine the level of associations between temperature, relative humidity, outdoor fungi concentration and the indoor fungi concentrations

The relationship between the predictor (independent variables) that is temperature, relative humidity and outdoor fungi counts and the outcome (dependent) variable that is indoor fungi concentrations was determined using multiple regression test.

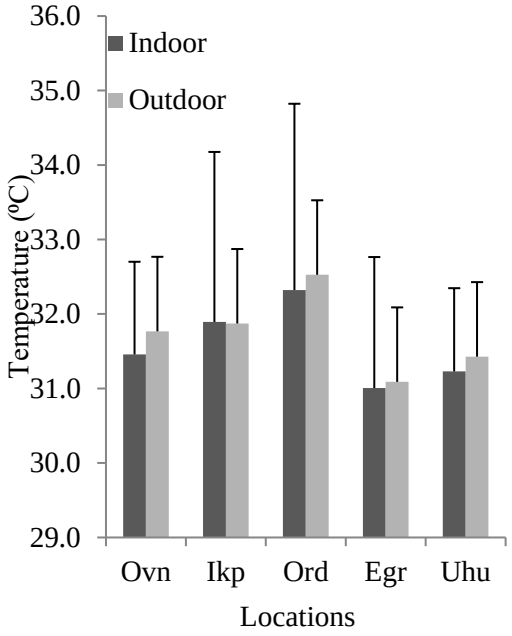
RESULTS AND DISCUSSION

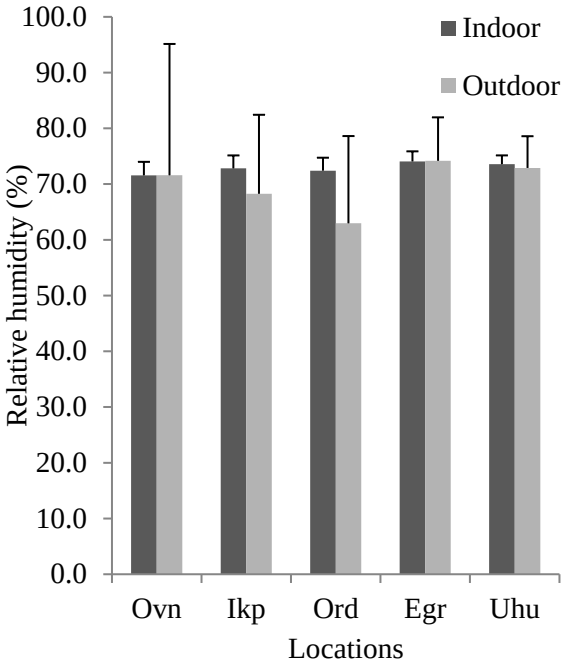
Variations of indoor and outdoor meteorological parameters
The mean indoor and outdoor temperatures ranged between 31.2 to 32.3°C and 31.1 to 32.5°C respectively, in the sampled locations (Figure 1). As expected, the ambient temperatures were slightly higher than the indoor temperatures across the locations. The highest temperature was recorded in sites located in Oredo area council while the least was measured at Egor. However, the relative humidity varied from 71.6 to 74.1% and 63.0 to 74.1% in the indoor and outdoor respectively (Figure 2). The humid conditions of the selected homes in Egor were highest while Oredo has the least relative humidity. Average indoor relative humidity varied from 71.9 to 75.4% in wet and 61.6 to 75.0% in dry seasons. These values were above the comfort level of (30% – 60%) (Ana and Umar, 2013). The high observed indoor RH in the homes could be attributed to high moisture levels due to leakages in the buildings across the study sites. Similar variations in meteorological conditions were also reported by (Akinbode *et al*., 2008) who examined the distribution of temperature and relative humidity in the western part of Nigeria.

The differences in the indoor and outdoor meteorological conditions could be as a result of the variations in the level of ventilation attributed to the variations in building characteristics such as density and activities of occupants, patterns of buildings.
Seasonal variations of airborne fungi (CFU/m^3) concentrations

The indoor fungi count recorded varied from 301.0 to 747.1 and 337.67 to 54.6 CFU/m^3 in wet and dry seasons respectively. The highest indoor fungi concentrations were observed in Ikpoba Okha while the least concentrations were determined at sites located in Uhunmwonde areas (Figure 3). The observed outdoor fungi concentrations in the wet and dry season across the sampling locations ranged between 223.8 to 450.4 CFU/m^3 and 378.64 to 532.7 CFU/m^3. Figure 4 revealed that the highest outdoor fungi concentrations (535 CFU/m^3) were recorded in Ikpoba Okha during the wet season. The least outdoor fungi count (223.8 and 245.9 CFU/m^3) were observed Uhunmwonde and Egor sites (Figure 4).

Although there are no uniform acceptable international standards for regulatory or acceptable limits for fungi loads in outdoor and outdoor air, The sanitary standards of the European Commission for non-industrial environment considered less than 50 CFU/m^3 as 'very low' bioaerosol load, 50–100 CFU /m^3 as 'low', 100–500 CFU/m^3 as 'intermediate', 500–2000 CFU/m^3 as 'high' and above 2000 CFU/m^3 as 'very high' load (CEC, 2003).

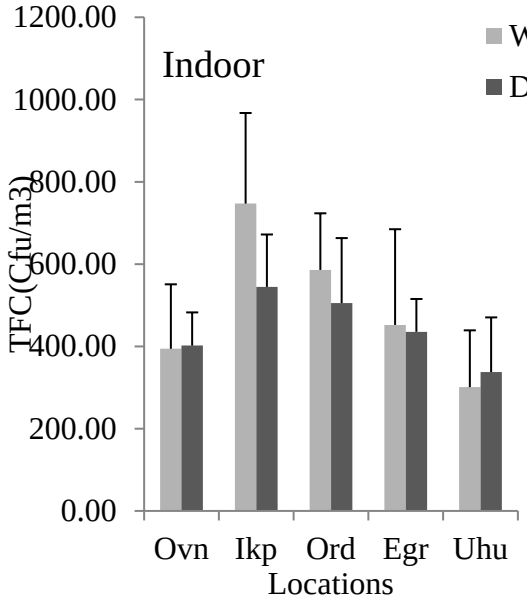


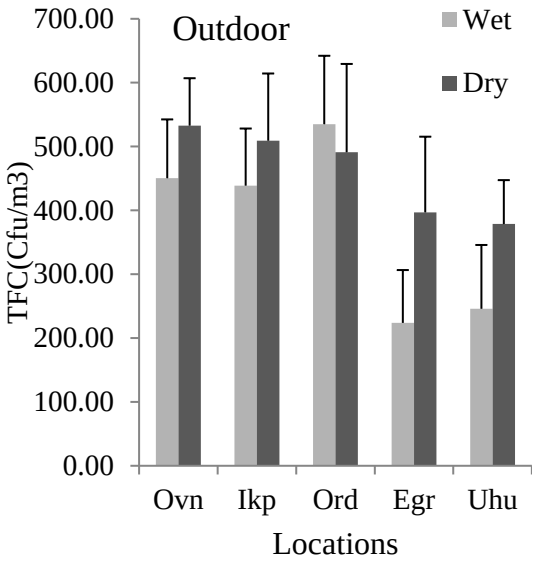


Ovn: Ovia North; Egr: Egor; Uhu: Uhumwonde; Ord: Oredo; Ikp: Ikpoba Okha

Figure 1: indoor and outdoor temperatures

Figure 2: indoor and outdoor Relative humidity





Ovn: Ovia North; Egr: Egor; Uhu: Uhumwonde; Ord: Oredo; Ikp: Ikpoba Okha

Figure 3: Total indoor fungi count in wet and dry seasons

Figure 4: Total outdoor fungi count in wet and dry seasons

In line with the above standard, the fungi load recorded in most of the sampling locations in this study is considered as "intermediate" and 'high'. This could be as a result of the increased relative humidity levels (63.0 – 74.1%) measured in the homes across the sampled locations.

This study is in line with the reports of several authors who reported high fungi load in air samples from homes, offices, hospital environments and classrooms (Chao *et al*., 2000; Oppliger *et al*., 2008; Pegas *et al*., 2010; Haas *et al*., 2006; Hargreaves *et al*., 2003). However, other studies reported that high mould concentrations in dwelling places were due to moisture, substrate availability and increased relative humidity, lack of ventilation in occupied homes (Hargreaves *et al*., 2003; WHO, 2009).

Indoor fungi levels were significantly ($p>0.001$) higher in Ikpoba Okha, Oredo and Uhunmwonde sites during the wet season than in the dry season, while outdoor fungi concentration was significantly greater in all the locations in the dry season compared to the wet season. This could be due to the resultant effect of rainfall associated with increased relative humidity which stimulates the growth of fungi population indoors while the washing effect of the rains removes airborne fungi from the outdoor environment. This study is similar to the findings of (Frankel *et al*., 2012). The high outdoor airborne fungi count recorded in the dry season could be attributed to decreased relative humidity, high temperature and wind speed which enhanced the release and dispersal of fungi spores in the atmosphere. This was similar to the report of (Agwu *et al*., 2004, Durugbo *et al*., 2005) who reported higher outdoor fungi counts during the dry season.

Distribution of airborne fungi isolates

The prevalent fungal genera isolated from the indoor and outdoor air across the sampling sites were *Penicillium, Cladosporium,* and *Aspergillus* (Table 1). Other fungal genera identified in this study include *Rhizopus, Mucor, Neurospora, Rhodotorula* and *Saccharomyces*. *Aspergillus niger* had the highest respective indoor and outdoor proportions of 23 and 24% across the sampling locations (Table 1).

Table 1: Distribution of airborne fungi from in and around residential buildings

	Indoor sites							Outdoor sites						
Fungal genera	Ovn	Egr	Uhu	Ord	Ikp	Total	%	Ovn	Egr	Uhu	Ord	Ikp	Total	%
Aspergillus niger	32	23	43	46	57	201	23	32	23	43	56	77	212	24
Aspergillus flavus	23	34	14	24	42	137	16	23	44	34	48	42	130	14
Penicillium sp.	14	12	15	38	42	121	14	15	16	13	41	45	157	17
Rhizopus stolonifer	23	18	22	43	24	130	15	21	17	12	41	24	97	11
Mucor sp.	28	16	13	21	45	123	14	30	16	18	27	43	113	13
Cladosporium sp	11	7	2	13	11	44	5	8	5	1	13	11	52	5
Neurospora crassa	18	5	8	23	32	86	10	18	14	7	22	30	74	8
Rhodotorula sp	2	3	0	11	7	21	2	5	4	2	11	17	43	5
Saccharomyces sp.	3	1	1	5	4	15	1	4	2	2	7	4	23	3
Total	154	119	118	224	264	879	100	156	141	132	266	293	901	100

Ovn: Ovia North; Egr: Egor; Uhu: Uhumwonde; Ord: Oredo; Ikp: Ikpoba Okha

This was followed by *Penicillium* sp, *Aspergillus flavus*, *Rhizopus stolonifer* and *Mucor* sp with percentage occurrence of 16 and 14%, 14 and 17%, 15 and 11%, 14 and 13% in indoor and outdoor samples respectively. *Saccharomyces and Rhodotorula* sp had the least percentage occurrence across sampling locations. in Ikpoba okha followed by *(*33.3 and 18.2%) and *Aspergillus flavus* (28.4 and 30.7%). The dominant indoor fungal genus across the locations were *Aspergillus, Penicillum, Mucor* and *Cladosporium* except in Ovia North where some of the fungi isolates have more fungi in the indoors than in the outdoor environment (Table 1). Most of the airborne fungi were isolated from Ikpoba okha and Oredo areas while the least occurrence of fungi was obtained in Egor and Uhumwonde areas. The variation in the amount

of occurrence of the fungi isolates across the sampling locations could be as a result of the availability of their selective substrates, human activities in such environments in addition to the prevailing environmental factors in the respective locations (Durugbo *et al*., 2005; Faridi *et al*., 2015).

This finding is similar to the report of (Abdel Hameed *et al*., 2012). The presence of *Penicillium, Cladosporium,* and *Aspergillus* in and around the homes in Benin City is a public health concern as a number of them are known to be allergens, while others could become opportunistic thereby causing different diseases in humans. Shelton *et al*. (2002) reported that fungi spores could be responsible for different adverse health outcomes in humans and it has been correlated with air pollution.

Some of the fungi species such as *Aspergillus, Mucor, Penicillium, Rhizopus* and *Cladosporium* reported in this study are pathogenic and could cause diverse health effects such as respiratory infections, runny nose and eyes (allergic rhinitis) and asthma related symptoms (Durugbo *et al*., 2005).

Influence of meteorological parameters and outdoor fungi counts on indoor fungi levels

In the wet season, indoor temperature significantly correlated positively but very weak with indoor fungal concentrations (r = 0.022) while the correlation between relative humidity and indoor fungi was 0.038 (Table 2).

Table 2: Influence of outdoor meteorological parameters and fungi levels on indoor fungi concentrations

	R	R^2
Wet season		
Indoor Temp	0.022	0.016
Indoor RH	0.038	0.017
Indoor TFC	0.360[a]	0.130
Dry season		
Indoor Temp	-0.079[a]	0.011
Indoor RH	-0.005	0.002
Indoor TFC	0.260[a]	0.032

Temp: Temperature; RH; Relative humidity; TFC; Total fungi counts
R represents correlation coefficient; R^2 stands for coefficient of Determination; [a] Correlation is significant at 0.01 level

The positive correlation between outdoor RH and indoor fungi populations is in tandem with (Jones and Harrison, 2004) who reported that relative humidity as an environmental factor, has more influence on the concentrations of fungi populations due to its role in the development of mycelia structures in different fungi species. The outdoor fungi concentrations showed a significant but weak positive correlation (r = 0.360; $p < 0.01$) with indoor fungi populations.

The significant positive correlation between the outdoor and indoor fungi populations indicated that the outdoor fungi concentrations in the sampling locations could influence the concentration of bacteria and fungi inside the homes. The spearman correlation result shows

that only outdoor temperature significantly correlated (r = - 0.079) with indoor fungi counts, while outdoor relative humidity did not have any significant association with indoor fungi concentrations. The negative correlation between outdoor temperature and indoor fungi concentrations could be a result of higher ambient air temperature than the indoor temperature in the dry season. This result is similar to the findings of (Zhan *et al*., 2018) who observed a negative association between meteorological parameters and microbial populations, stating that cold air could facilitate the release of fungi spores, while microbial release level increases with decreasing relative humidity.

The outdoor fungi concentrations showed a significant positive correlation (r = 0.260; p< 0.01) with indoor fungal populations in dry season. The positive correlation (r = 0.260; p< 0.01) between outdoor fungi concentrations and the indoor fungal populations dry season is in line with (Ponce-Caballero *et al*., 2013) who studied the seasonal variation of airborne microbial propagules in domestic environments and reported that the ambient bacterial and fungi are the sources of indoor microbial concentrations.

The regression analysis revealed that during the wet season, variations in temperature and relative humidity only explained about 1.6 and 1.7% of the recorded indoor fungi populations in the wet season Table 2. The outdoor concentration of fungi in wet season explained about 13.0% of the corresponding indoor concentration of fungi. The regression also showed that less than 2% of the fungi concentrations were explained by meteorological parameters (Table 2) in the dry season. In contrast with the wet season, outdoor fungi concentrations accounted for only 3.2% of the indoor concentration in the dry season. The relatively low explaination for indoor fungi concentrations by measured indoor temperature and relative humidity is in line with previous studies (Pasanen *et al*., 2000; Kalogerakis *et al*., 2005; Adams *et al*., 2015) that outdoor sources, human occupancy and activities, deposition and settling floor dust, moisture content of building materials largely influence the occurrence of \fungi in the indoor environments. Outdoor fungi concentration accounted for higher proportion of indoor fungi levels. This study is in tandem with the work of (Lee *et al*., 2006) who carried out a study on the relationship between indoor and outdoor bioaerosols in urban homes and reported that an increment in indoor bioaerosol level was due to high outdoor concentration after the rains.

CONCLUSION

This study assessed the level of indoor and outdoor fungi in air samples from homes. The indoor relative humidity of the homes was above comfort level. The concentrations of airborne fungi varied across the sampling locations and were high in most of the locations. The high concentrations of airborne fungi in some of the sites could be critical to the health of the occupants. The most frequently airborne fungi in the sites were *Penicillium, Cladosporium,* and *Aspergillus,* which are either allergy or opportunistic pathogens in exposed individuals especially the immunocompromised. The presence of medically important airborne fungi species and the relatively high indoor fungi concentrations in this study calls for urgent intervention in the area.

Personal hygiene and care must be taken to reduce the increasing effect of fungi spores in the indoor areas. Fixing of leaking roofs, broken walls to avoid damp and moist floors and wall that breed fungi spores is highly recommended.

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