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Assessment of Airborne Culturable Fungal Load in an Indoor Environment of Dormitory Rooms: The Case of University of Gondar Student's Dormitory Rooms, Northwest Ethiopia

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ABSTRACT: Pollution caused by fungal contamination on building materials contributes to poor indoor air quality. Fungi are known to cause several health-related problems, such as acute toxicity, hypersensitivity, invasive mycoses, and respiratory problems. Thus, this study aimed to determine the load and diversity of airborne culturable fungi in the dormitory rooms. An institution-based cross-sectional study was conducted from March to June 2019 at the University of Gondar students' dormitory rooms. Statistical analyses were carried out using Stata/SE 14.0. Spearman ranks correlation was used to assess the correlation of fungal load with indoor physical parameters. The median fungal loads were 250 CFU/m^3 and 157 CFU/m^3 in the morning and afternoon, respectively. Most commonly identified fungal genera/species were *Aspergillus, Penicillium, Alternaria, Fusarium, Candida, Trichophyton, Piedraia, Microsporum, Geotrichum, Saccharomyces, Rhodotorula, Rhizopus, Exophiala, Arthroderma, Cladosporium, Gliocladium*, and *Botrytis*. Formaldehyde ($r = -0.2859$, $P = .0031$), temperature ($r = -0.2153$, $P = .0274$), and CO_2 ($r = -0.3785$, $P = .0001$) were negatively correlated with airborne indoor fungal load in the morning and CO_2 ($r = 0.3183$, $P = .0009$) and temperature ($r = 0.2046$, $P = .0363$) positively correlated with airborne indoor fungal load in the afternoon. As a conclusion, the airborne fungal load in the dormitory room was intermediate according to the European standard of nonindustrial premises. Substantial action should be taken to reduce the fungal contamination of indoor environments.

KEYWORDS: Airborne fungal load, dormitory room, indoor air

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Introduction

Fungal contamination on building materials has been recognized as a cause of pollution that contributes to poor indoor air quality.[1,2] Besides being airborne, microbial spores are microscopic. The existence of these microscopic spores in the air affects the inhaled air quality and hurts human health. As human beings spend 85%–90% of their time indoors, indoor air quality continues one of the most unique parameters critically examined when considering occupants' health and well-being in a house.[3-5]

Over a wide range of environmental conditions, fungi are ubiquitous in nature. Approximately 2.2 to 3.8 million fungal species are found in almost all the earth's terras. *Aspergillus spp., Fusarium spp., Scedosporium spp.*, and *Mucorales spp*. are pathogenic fungal species. These fungi are known to cause several problems associated with health, such as acute toxicity, hypersensitivity (mostly asthma), invasive mycoses, and respiratory problems.[6] By circulating through doors, windows, ventilation systems, and air conditioning systems, molds easily enter indoor environments. Spores in the air also deposit prevalent mold carriers into indoor settings on humans and livestock, bags, and pets. *Cladosporium, Penicillium, Aspergillus*, and *Alternaria* are the most prevalent indoor molds.[7] Nearly all known species of fungi can propagate through sporulation (in air or water as a particle). In a broad range of physicochemical circumstances, fungal spores can survive to make them a persistent bioaerosol.[6]

People may inhale mycotoxins through the air, ingest them through food, and may also come into contact with the skin, causing several diseases. Different molds produce various mycotoxins, which also depends on the type of material on which the toxic mold grows. Gastrointestinal, respiratory, and reproductive disorders are among the diseases induced by mycotoxins.[8]

Indoor fungal contaminants depend on many factors such as meteorological parameters (ie, temperature and humidity), ventilation, organic matter current in construction materials, and human operations (ie, sneezing/coughing, washing floors/toilet cleaning, walking/talking, etc) that can have a significant impact on the extent of their formation and dispersion owing to their controlling impact on indoor surroundings.[3,6] Cigarette smoke, insulation materials, particulate board, furniture in the formaldehyde-based resin, nontoxic paint, manufacturing, and various other consumer products are indoor sources of fungal load.[9]

Even though in Ethiopia a few studies were carried out, that did not give adequate information about the different factors that contribute for fungal contamination such as compound

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sanitation, ventilation, number of windows, cleaning frequency, and other physical indoor parameters (such as temperature, humidity, particulate matter concentration, formaldehyde, and carbon dioxide). Determining the load and diversity of airborne fungi in the dormitory room and assessing environmental factors can be a good indicator of the cleanliness of these indoor environments.[6] Therefore, this study aimed to determine the load and diversity of airborne culturable fungi in the dormitory room. The findings of this study will enable policymakers and program managers, administrators, and other related stakeholders who work on this issue to understand the burden of the problem and its possible negative consequences which in turn directing them a way to take prompt actions and design appropriate preventive measure.

Methods and Materials

Study design, period, and study area

An institution-based cross-sectional study was conducted from March to June 2019. The study was conducted in College of Medicine and Health Sciences at University of Gondar which is located in the Northern part of Ethiopia in Amhara national regional state, Central Gondar zone at a distance of 727 km from Addis Ababa and 173.09 km from Bahir Dar at the 12°45′ North latitude and 37 ° 45′ East longitudes.

Sample size and sampling technique

The sample size was determined based on environmental sampling and sample size determination techniques.[10] Manly formula was used to determine sample size.[11] Using the following formula

$$n = \frac{4\sigma^2}{\delta^2}$$

where n = number of samples, σ = standard deviation, δ = acceptable error (δ is half of the width of a 95% the confidence interval on the mean [$X \pm \delta$]). To calculate sample size the following assumptions were considered; mean and standard deviation (σ) of dormitory building rooms were 78.13 and 7.4, respectively, and 5% acceptable error.

$$n = \frac{4(7.4)^2}{(2.5)^2} = 35$$

A total of 35 rooms were selected from 644 dormitories via a simple random sampling technique from 8 dormitory buildings proportionally.

Procedures for air sampling and laboratory analysis

Air samples were collected from 35 selected dormitory rooms from 8 dormitory buildings. The fungal load was determined by passive air sampling method (settle plate technique) using standard 9 cm diameter Petri dishes (63.585 cm^2) comprising malt extract agar as a culture media prepared with 50 g of powder mixed with 1000 mL of distilled water, by adding 500 mg of chloramphenicol to inhibit bacterial growth.

The determination of the fungal load was based on counting the microbial settlement on the Petri plates left open to the air for 30, 60, and 90 minutes at 1 to 1.5 m above the floor in the center of the dormitory room and 1 meter away from the buildings. After exposure for 30, 60, and 90 minutes, the Petri dishes were closed to determine the variation in fungal load during exposure. Airveda was used to measure PM2.5, PM10, CO_2, temperature, and relative humidity (RH).[12] Air quality meter was used to measure formaldehyde concentration.[13]

Sampling was performed at 6:00 AM and at 5:00 PM to determine the fungal load with regard to environmental variations. After exposure, the sample was taken to the Environmental and Occupational Health and Safety Department Laboratory at University of Gondar and finally incubated at 25°C for 3 to 5 days. Colony-forming units (CFU) were enumerated, CFU / m^3 fungal load was determined using the following equation.[14,15]

$$N = \frac{a \times 10000}{bt \times 0.2}$$

where N = microbial load CFU/m^3 of indoor air, a = number of colonies per petri dish, b = dish surface area (cm^2), t = exposure time in a minute. Microscopic (using lactophenol cotton blue staining) and macroscopic characteristics (with the aid of an Atlas of Mycology) were used to isolate fungal species.[16,17]

Data quality control. Standard procedures of collection, storage, and transport of samples were implemented. Culture media were tested for sterility and performance according to standard procedures. To monitor contamination during sample collection and transportation, one control media was used for each batch. Further quality assurance measures were taken at different levels of the laboratory process. At the analytical stage, the daily maintenance of the instruments was checked. Appropriate reporting and recording were done at the post-analytical stage. Besides, standard operating procedures for all laboratory tests were employed uniformly throughout the study.

Data processing and analysis

Statistical analyses were carried out using Stata/SE 14.0. Spearman ranks correlation was employed to assess the correlation of fungal load with indoor physical parameters like formaldehyde, carbon dioxide concentration, particulate matter concentration (PM2.5 and PM10), temperature, and RH.

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Table 1. Statistical summary of fungal load in the dormitory rooms at the University of Gondar, northwest Ethiopia, 2019.

BUILDING CODE	N	MEAN	SD	CV	MIN.	MAX.	MEDIAN
Morning fungal load							
B17	15	197.06	107.40	.54	52	367	170
B18	12	357.25	193.95	.54	131	773	318.5
B19	15	236.06	10.09	.04	220.26	251.87	236.06
B20	15	269.94	10.09	.04	254.13	285.74	269.93
B21	12	222.20	46.92	.21	145.9	288	227.85
B22	12	132.08	84.52	.64	26	349	137.5
B8	12	368.33	151.84	.41	207	708	336.5
B9	12	441.17	215.32	.49	262	996	358
Total	105	274.27	150.46	.55	26	996	250.2
Afternoon fungal load							
B17	15	315	118.45	.37	175	594	314
B18	12	111.58	28.48	.25	70	157	113
B19	15	179	37.45	.21	122	262	170
B20	15	161.49	63.75	.39	87	315	157
B21	12	226.33	77.79	.34	105	393	223
B22	12	262.92	152.28	.58	140	655	218.5
B8	12	107.25	42.00	.39	52	183	104.5
B9	12	114.17	28.55	.25	78	175	113.5
Total	105	187.61	106.71	.57	52	655	157

Abbreviations: CV, coefficient variation; Max., Maximum; Min., Minimum; N, number of samples; SD, standard deviation.

Results

Fungal load in dormitory rooms

A total of 210 samples in the morning and afternoon were collected at 30, 60, and 90 minutes exposure time from 35 selected dormitory rooms. The results of this study showed that the largest fungal load of 996 CFU / m^3 was found in building B9 at 6 AM at 30 minutes exposure time and the smallest fungal load of 26 CFU / m^3 at 5 PM at 30 minutes exposure time in building B22 with the median fungal load 250 and 157 CFU / m^3 in the morning and afternoon, respectively (Table 1).

In this study, the degree of fungal pollution by fungal populations throughout the dormitory rooms was largely intermediate. The overall median fungal load in the morning (250 CFU / m^3) was higher than in the afternoon (157 CFU / m^3). The maximum of the median fungal load was recorded at 30 minutes time exposure 259 CFU/m^3 in the morning (Table 2).

The Kruskal-Wallis H test showed that there was a significant airborne fungal load difference in dormitory buildings ($\chi^2 = 47.306$, $df = 7$ and $P = .0001$) in the morning and ($\chi^2 = 64.63$, $df = 7$ and $P = .0001$) in the afternoon.

Physical parameters of indoor air environments

No heating, ventilation, and air conditioning systems were found during physical parameter measurement. The ranges of indoor physical environment parameters were CO_2 (464-8835 ppm), temperature (13°C-56°C), RH (19%-70%), formaldehyde (0.5-1.99 μg/m^3), particulate matter concentration (PM2.5 [12-68 μg/m^3] and PM10 [25-210 μg/m^3]) (Table 3).

Isolated fungal genera/species

Quality characteristics of fungi isolated from the air in targeted rooms showed fungi genus/ species like *Aspergillus, Penicillium, Alternaria, Fusarium, Candida, Trichophyton, Piedraia, Microsporum, Geotrichum, Saccharomyces, Rhodotorula, Rhizopus, Exophiala, Arthroderma, Cladosporium, Gliocladium*, and *Botrytis* (Figure 1).

Correlation of fungal load with indoor physical parameters

A small negative correlation between formaldehyde and temperature with airborne fungal load ($r = -0.2859$, $P = .0031$) and

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Table 2. Fungal load variation at 30, 60, and 90 minutes exposure in the morning and afternoon in a dormitory room at the University of Gondar, northwest Ethiopia, 2019.

EXPOSURE TIME	N	MEAN	SD	CV	MIN.	MAX.	MEDIAN
Morning airborne fungal load							
30 min	35	292.67	199.21	.68	26	996	247
60 min	35	268.5	141.65	.53	79	773	249
90 min	35	261.64	94.59	.36	70	524	259
Total	105	274.27	150.45	.55	26	996	250
Afternoon airborne fungal load							
30 min	35	204.23	104.27	.51	78	524	183
60 min	35	174.8	104.53	.59	66	655	157
90 min	35	183.8	112.10	.61	52	594	157
Total	105	187.61	106.71	.57	52	655	157

Abbreviations: CV, coefficient variation; Max., maximum; Min., minimum; N, number of samples; SD, standard deviation.

Table 3. Statistical summary of physical indoor air quality parameters in the dormitory rooms at the University of Gondar, northwest Ethiopia, 2019.

VARIABLE	MEAN	SD	CV	MIN.	MAX.	MEDIAN
PM2.5 (μg/m³) ~M	31.97	9.90	0.31	12	68	32
PM2.5 (μg/m³) ~A	26.14	10.10	0.39	12	63	26
PM10 (μg/m³) ~M	68.57	16.37	0.24	35	105	69
PM10 (μg/m³) ~A	48.31	29.70	0.61	25	210	42
Formaldehyde (μg/m³)~M	1.74	0.17	0.10	1.44	1.99	1.75
Formaldehyde (μg/m³)~A	1.70	0.142	0.08	1.49	1.92	1.69
Carbon dioxide (ppm) ~M	987.85	427.13	0.43	541	2371	850
Carbon dioxide (ppm) ~A	677.93	812.20	1.19	464	8835	553
Temperature ºC~M	17.28	1.60	0.09	13	19	17
Temperature ºC~A	19.37	6.39	0.330	15	56	19
Relative humidity % ~M	57.2	8.42	0.147	19	70	58
Relative humidity %~A	52.54	3.39	0.06	46	59	53

Abbreviations: A, afternoon; CV, coefficient variation; M, morning; Max., maximum; Min., minimum; PM, particulate matter concentration; SD, standard deviation.

($r = -0.2153$, $P = .0274$) was observed in the morning, respectively. Formaldehyde and temperature concentration explaining 8.2% and 4.6% of airborne fungal load variability, respectively. In the morning, there was a moderate negative correlation between carbon dioxide and fungal load ($r = -0.3785$, $P = .0001$) with carbon dioxide concentration explaining 14.3% of the fungal load variation. In the afternoon, there was a moderate positive correlation between carbon dioxide and fungal load ($r = 0.3183$, $P = .0009$) with carbon dioxide concentration explaining 10% of the variation in fungal load. Temperature ($r = 0.2046$, $P = .0363$) was positively correlated with an indoor airborne fungal load (Table 4).

Discussion

In this study, the median indoor fungal load was 216.5 CFU/m³; it ranges from 157 to 262 CFU/m³. This study was lower than a study conducted in Jimma university (Ethiopia),[18] Poland,[19] and Istanbul.[20] The variation in fungal load could be due to differences in environmental variables, compound sanitation, building conditions, and concentration of coarse airborne solid particles (dust) and some of the study samples have been collected where the rooms are active, this may contribute to the difference in fungal load.

The degree of fungal pollution throughout the dormitory rooms by fungal populations was largely intermediate in the

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current studies following the European Commission's health standards for nonindustrial premises, the permissible fungal load limits were < 25 CFU/m^3 very low, 25 to 100 CFU/m^3 low, 100 to 500 CFU/m^3 intermediate, 500 to 2000 CFU/m^3 high and > 2000 very high CFU/m^3.[21] In healthy buildings, scholars considered that 750 CFU/m^3 was the limit for fungi where species were not infectious.[22] According to the WHO expert group on the evaluation of biological agents 'health risks in indoor environments, total microbial concentrations should not exceed 1000 CFU/m^3.[23]

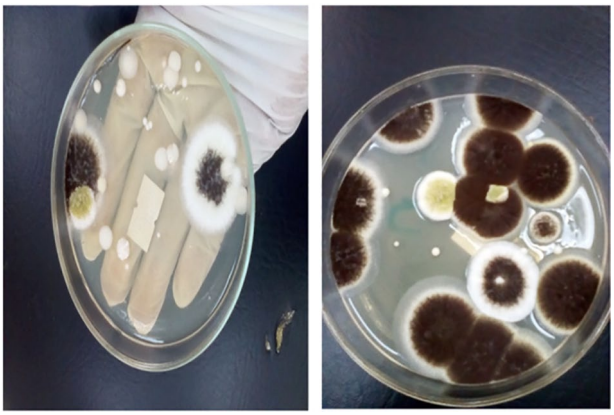

Figure 1. Sample fungal colony-forming units after incubation of 3 to 5 days.

In the present study, various kinds of fungal genera/species have been isolated in dormitory rooms such as *Aspergillus, Penicillium, Alternaria, Fusarium, Candida, Trichophyton, Piedraia, Microsporum, Geotrichum, Saccharomyces, Rhodotorula, Rhizopus, Exophiala, Arthroderma, Cladosporium, Gliocladium*, and *Botrytis*. The findings of this study partly agree with earlier studies.[6,18-20] The isolated fungal genera and species variation could be triggered due to variability in temperature, humidity, building condition, study settings, and other indoor physical parameters in dormitory rooms. The presence of fungi such as *Aspergillus* and *Penicillium* is an opportunistic human pathogen and is often associated with clinical manifestations such as respiratory allergies, asthma, and conjunctivitis.[6]

Regarding environmental factors, formaldehyde, temperature, and carbon dioxide were negatively correlated with indoor airborne fungal load in the morning. In the afternoon, carbon dioxide and temperature were positively correlated.

In the morning, formaldehyde was negatively correlated with the indoor airborne fungal load. It is a highly reactive compound widely used in medicine, agriculture, and industrial processes as a disinfectant for killing bacteria and fungi some of the fungal species such as *Paecilomyces sp., Aspergillus sp.*, and

Table 4. Spearman ranks correlation of fungal load and indoor physical parameters in dormitory rooms, at the University of Gondar northwest, Ethiopia 2019.

	FUNGAL LOAD	PM2.5	PM10	FORMALDEHYDE	CO_2	T (ºC)	RH (%)
Morning							
Fungal load	1.0000						
PM2.5	−0.0141	1.0000					
PM10	0.1280	−0.4032*	1.0000				
Formaldehyde	−0.2859*	−0.1979*	0.1047	1.0000			
CO_2	−0.3785*	0.1991*	0.0136	0.4565*	1.0000		
T (°C)	−0.2153*	−0.0480	0.3775*	0.4050*	0.4311*	1.0000	
RH (%)	−0.0516	0.2723*	−0.2124*	−0.1937*	0.2021*	−0.5604*	1.0000
Afternoon							
Fungal load	1.0000						
PM2.5	0.1304	1.0000					
PM10	−0.1759	−0.1477	1.0000				
Formaldehyde	0.1707	−0.0319	−0.0895	1.0000			
CO_2	0.3183*	0.3920*	0.0982	−0.0234	1.0000		
T (°C)	0.2046*	0.1600	−0.1992*	0.1775	−0.0297	1.0000	
RH (%)	0.1339	0.2289*	0.0913	−0.1430	0.4327*	−0.6228*	1.0000

Abbreviations: PM, particulate matter concentration; RH (%), relative humidity; T, temperature.
*Level of significance $P < .05$.

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Trichoderma sp. tolerant to formaldehyde.[24] Formaldehyde, a high-volume chemical, is omnipresent in the atmosphere because of natural sources and anthropogenic activities. Building materials (eg, wood products such as furniture, particleboard and plywood, and fiberboard of medium density), consumer goods, and combustion processes are significant indoor air sources.[25,26]

In this study, the temperature was negatively correlated with indoor fungal load in the morning while positively correlated in the afternoon. The results of this study are compliant with other study findings.[1,6,7,27,28] The possible explanation could be due to air movements favoring the maintenance of microorganisms in the aerial media while their deposition owing to their small size is hardly influenced by gravity. Determinants of microbial survival and abundance are factors such as moisture, light, and nutrient accessibility.[29]

Carbon dioxide was negatively correlated with indoor fungal load in the morning but positively correlated with indoor fungal load in the afternoon. This study is consistent with the study findings.[30,31] The variation could be due to carbon dioxide (CO_2 levels can be considered as an indicator of the ventilation rate). The CO_2 levels identified varied between dormitory rooms and depending on the type of ventilation, the operation of the window, and the number of occupants.[32] In the lack of adequate ventilation to decrease and eliminate the CO_2 that the occupants are constantly throwing out, CO_2 can collect and increase its strength.[33]

There was no correlation between humidity and indoor fungal load in this study. This study finding was similar to earlier studies.[18,28] But humidity has a positive correlation to indoor fungal load.[34] Temperature and RH are two significant variables in the generation, release, and dispersal of fungal spores, especially indoor environments[35] and the influence of meteorological parameters (ie, humidity) on bioaerosol formation and dispersion.[6] The possible explanation could be that the contributing variables for fungal load were carbon dioxide, temperature, and formaldehyde containing organic matter contribute to the fungal load and basic environmental conditions, such as temperature can have a significant impact on the extent of their formation and dispersion owing to their control impact on microorganism formation rather humidity was contributed to fungal load in rooms.[36]

Limitation of the study

Due to the nature of passive air sampling techniques; the efficiency collection of passive samples may be influenced by environmental parameters and reverse diffusion can underestimate or overestimate fungal load. Moreover, this study was conducted during the spring season and may not be generalized for the remaining seasons. For further studies in this area, we will recommend using both active and passive air sampling techniques to compare the findings.

Conclusions

The airborne fungal load dormitory room was intermediate. *Aspergillus, Penicillium, Alternaria, Fusarium, Candida, Trichophyton, Piedraia, Microsporum, Geotrichum, Saccharomyces, Rhodotorula, Rhizopus, Exophiala, Arthroderma, Cladosporium, Gliocladium*, and *Botrytis* were among isolated fungal genera/species. Formaldehyde, temperature, and carbon dioxide were correlated with the indoor airborne fungal load. Substantial action (such as wetting during cleaning, ventilation, habits of deodorant usage, etc) should be taken to reduce the fungal contamination of indoor environments of dormitory rooms.

Availability of Data and Materials

The datasets of this article are available from the corresponding author on a reasonable request.

Author Contributions

The authors actively participated during the conception of the research issue, development of a research proposal, data collection, analysis, and interpretation, and write up of various parts of the research report. Z.A. designed the protocol, analyzed the data, supervised the overall research process, and prepared the manuscript. Y.A., T.A., and B.H. participated in data collection and data entry and commented on the overall work. All the authors read and approved the final manuscript.

Ethical Approval and Consent to Participate

The study was approved by the Department of Environmental and occupational health and Safety University of Gondar College of Medicine and Health Sciences, Institute of Public Health Research Ethics Review Committee.

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