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Characterization of Bioaerosols and Particulate Matter (PM) in Residential Settings of Asthmatic Patients of Lahore, Pakistan

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ABSTRACT

Airborne bioaerosols and particulate matter (PM) have been associated with asthma occurrence. Due to the adverse indoor environment and the absence of any baseline data for asthmatic patients of Pakistan, this **s**tudy was aimed to establish a correlation between microflora and PM of residential microenvironments of asthmatic patients.

This pilot study was conducted in different residential settings of asthmatic patients registered in the Jinnah hospital, Lahore. The characterization of PM (PM_{01}, $PM_{2.5}$, PM_{10}) and bioaerosols were carried out in the houses of fifty patients that were categorized into four groups; A-large (418.06 m^2), B-medium (211 m^2), C-medium (104 m^2), and D-small (62.71 m^2) houses. The PM concentrations were monitored; using the DustTrack8533 aerosol monitor and the bioaerosols were characterized up to the Genus; using the culture-based method and biochemical testing. The bioaerosols were sampled; using the expose plate method and were analyzed using morphological features and biochemical tests.

Eleven types of fungi and seven bacterial types were found in the air samples. The tendency of asthma occurrence is linked with higher *Alternaria spp* and *Aspergillus spp*. The mean indoor readings of PM_{01}, $PM_{2.5}$, PM_{10} were highest in D-category (331.75, 342.5, and 502.33 respectively). Moreover, the highest bacterial 9618 CFU/m^3) and fungal levels (3092 CFU/m^3) were also seen in D-category. According to two-way ANOVA, bacterial concentration was significantly different among the four groups while fungi concentration was non-significant (p<0.05). Pearson correlation showed a significant positive correlation among bioaerosol counts, relative humidity, and temperature. Moreover, a positive significant correlation was also observed among PM, bioaerosols, and temperature (p<0.01).

The multiple regression analysis confirms temperature as a significant predictor of bioaerosols and bacterial and fungal concentrations were observed to be a significant predictor for PM. Hence monitoring the PM levels could help in maintaining the indoor microenvironment for sensitive asthmatic patients.

Keywords: Asthma; Bacteria; Fungi; Particulate matter

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INTRODUCTION

The population of Lahore, Pakistan is about 12,188,000 till June, 2019.[1] The air of Lahore has

turned exceedingly polluted and unhealthy. This terrible condition has enhanced the commonness of airborne sickness.[2] Community spends about ninety percent of their time in a concealed environment that significantly influences the quality of life.[3] All over the world, switching from open-air to concealed indoor environments in the life trend has a severe consequence.[4] Occupants of concealed indoor environments especially patients experience adverse health effects.[5] Air is a big course of disease communication being laden with numerous pollutants by a single breath. Many researchers have revealed higher indoor bacterial and fungal concentrations than outdoors. The hazard to human healthiness from the indoor origin is almost 1000 folds greater.[6] Interest in indoor air quality is booming because it comprises a blend of biological and non-biological aerosols.[7] A big data of research have associated exposure to airborne particles, especially biological aerosols (bioaerosols), with detrimental health outcomes.[8] Exposure to indoor bacterial and fungal spores has been tightly linked with asthma aggravation.[9] Indoor pollutants may cause negative health effects especially pulmonary problems.[10] Infectious microbes like bacteria and fungi commonly found endogenous flora can originate from environmental sources.[11] Indoor bioaerosols have been strongly linked with asthma.[12] Currently many studies have highlighted bioaerosols concentration in various indoor environment.[13]

Asthmatics are a high-risk community for fungal contamination being most prevalent in Pakistan.[14] Fungal contaminations have been now named 'hidden killers'.[15] *Aspergillus* in the indoor environment can be a competent candidate for asthma development. *Aspergillus spp.* is the admitted cause of allergic asthma.[16] Previous research of outdoor fungi in Lahore has depicted a large-scale prevalence of *Aspergillus spp.* particularly during moist summer.[2,17] Information from the countryside highlights the actuality of infections in the population. *Alternaria*, *Aspergillus*, *Penicillium, Cladosporium,* and *Saccharomyces cerevisiae* from the ambient air of Lahore and Karachi has already been reported in all seasons.[2]

One of the major contributors to asthma is particulate matter (PM).[18] Ambient PM elevates due to their outdoor sources.[19] and 28% of illnesses are induced by indoor PM in developing countries.[20] Chronic and acute pulmonary diseases are because of PM_{10} and $PM_{2.5}$ inhalation,[21] which destroy respiratory system.[22] PM Exposure can lead to declined lung function,[14] lower respiratory inflammation, upper respiratory irritation, and asthma.[11] A big data demonstrated that aeroallergens and aero chemicals can join and enhance the aggravation of asthma expression.[23] Due to minute size, particulate matter enables it to enter deep into the lung.[24] People exposed to outdoor PM are linked with allergic rhinitis and asthma respectively.[25] Ultrafine particles (PM<0.1 mm) have more vigorous involvements in comparison to other breathable parts of the aerosol of urban areas, both at the molecular and respiratory level.[26] In the Italian population elevated indoor $PM_{2.5}$ has been linked with asthma.[27] Several studies mentioned the concentration of bioaerosols in various indoor environments.[13] Temperature and relative humidity have been known to affect concentrations of bioaerosols of ambient environment.[13] Outdoor motor vehicle exhausts increase levels of indoor air pollution, then airtight modern buildings result in decreased ventilation by increasing the danger of bioaerosols exposure.[28,29]

Estimation of the microbiological load of indoor air in different areas across the globe is necessary from a public health viewpoint, especially in the protection of vulnerable groups such as patients. But no trials have been produced to index their distribution in Lahore to probe the potential of these microbes. To address these challenges, the study was performed to analyze the bioaerosols (bacteria & fungi) concentration of various living environments of asthmatics and its relation with PM_{01}, $PM_{2.5,}$ and PM_{10}.

MATERIALS AND METHODS

Study Area

Lahore (31°15'-31°45' N & 74°01'-74°39' E) is the historical city and capital of the Province and 2nd large and thickly populated metropolitan city of Pakistan. Ravi (River) drifts alongside the north west. Lahore was designated administrative situation in 2001 and split into 9 managerial townships with a military cantonment area (Figure 1).[30]

Lahore undergoes summer, arid weather of 24.3°C midpoint temperature. Summers is of typically tropical type with an utmost mean temperature between 33-39°C whereas, winters event mean of 17-22°C.[30]

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Study Design and Settings

Fifty patients presented to the Jinnah hospital Lahore from May 2018 to June 2019 with acute asthma spasmodic episodes of breath shortness, cough, wheezing (Annex-III) as specified by the Global Initiative for National Asthma Guidelines,[17]for enrollment in the study (Annex-I). Written consent was taken from patients (Annex-II). The study was authorized by the ethical review committee to vide number D/1315/UZ.

Home Survey

A home visit was conducted within one week after enrollment. The home survey included inspection of the house, data collection related environment, and indoor air samples. Fifty houses were randomly selected as sampling sites for air quality monitoring of bioaerosols. To ensure the random mix of homes, a diverse floor area was chosen from each administrative town. The randomly selected houses were then categorized based on area and occupancy. Three categories (Figure 2) were designated as per the size of the houses given below details.

House selection was random so the surroundings were diverse markedly; houses were located in a variety of localities i.e. urban, industrial, and commercial. Selected houses were placed within the orbit of 1 kilometer of the main road with a variety of traffic all over the day.

Data Collection; Using Questionnaire

A questionnaire was applied to collect data on health-related variables, demography, and socioeconomic status. Lists of variables are as follows:

1. Health-related variables including earlier asthma episodes, cough, and family history.
2. Environmental variables including indoor pets, leaks of plumbing, moisture or leaks, musty smell, and tobacco smoke in the room.
3. Demographic variables including gender and age.
4. Socioeconomic variables including house size, room size, occupation, and the number of family members (Annex-III).

Bioaerosols Sampling

Following the study protocol, each site per day was sampled using the passive sampling method by the method of Cappuccino and Sherman, 2005described in detail by Balyan et al, 2020;[30] plates in triplicate were exposed for one hour at each sampling site. Sampling in every site was completed while at rest (early morning). The growth medium of bacterial and fungal was tryptic soy agar (TSA) and malt extract agar (MEA); respectively. Temperature and relative humidity were noted at the time of sampling.

Bacterial Analysis

Media Preparation

For TSA medium preparation, 40 g was dissolved in 1 liter purified water, recurrent agitation with boiling for 1minute to dissolve. It was autoclaved for 15min at 121°C, then flow in Petri dishes already sterilized and left overnight at 25±1°C.[31] Poured 20mL in sterilized Petri plates under biosafety cabinet solidify and incubated at 37°C overnight.

Laboratory Analysis

Koch sedimentation technique for bacterial and fungal sampling was used. A passive way of sampling was executed to calculate the IMA (index of microbial air contamination).[31] This correlates the number of CFU/ plate (colony forming units) calculated on Petri plate (9 cm) as per 1:1:1 pattern (one hour, one meter above the floor, one meter distant from walls) and incubated for growth and species identification.

Colony Forming Units (CFU)

Colonies found between 30 and 300 were selected. CFU for each sample was calculated by formula Omelyansky formula[30,32] expressed as CFU/m^3.

$N=5a.10^4/(b.t)$

Where:

N=colony forming units per m^3 (CFU/m^3) a=no. of colonies per Petri dish

b=surface area of a dish (cm^2)

t=exposure time (minutes).[30]

Bacterial Identifications

Morphology of the colony was noted for **s**hape, size, texture, margin, color pigmentation, and elevation of the colony.

Biochemical Test

Bacterial identification by many tests like Gram Staining, MacConkey agar plates, spore staining for rods, catalase test, oxidase test for rods, and test for Gram-positive cocci was carried out.[31]

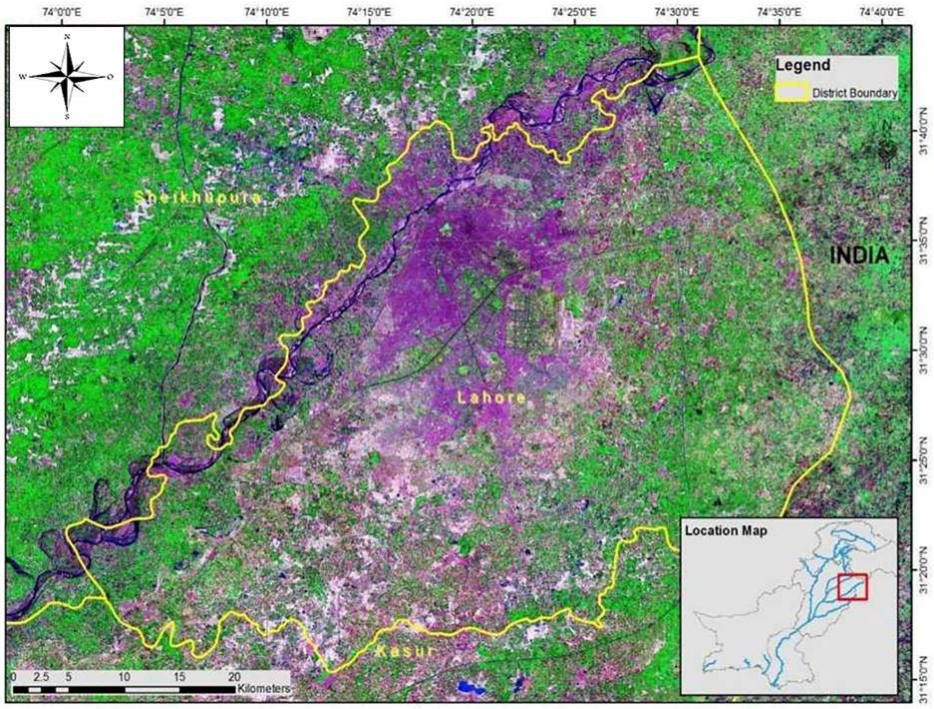

Figure 1. Map of Lahore city manifesting zone of sampling

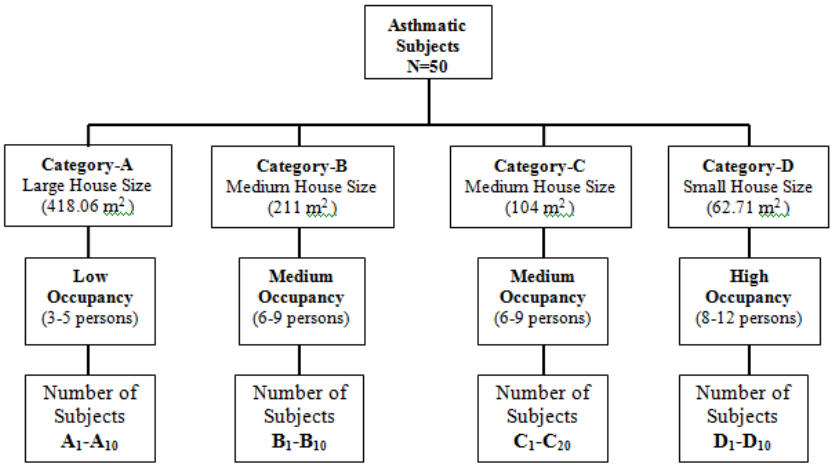

Figure 2. Categorization of the houses for sampling

Fungal Analysis

Media Preparation

Twenty grams of (MEA) Malt extract agar with 6 pH and twenty grams agar were mixed in 1000 mL H_2O (dist) autoclaved 121±1°C for 15 minutes media and Petri plates. After cooling antibacterial was put into for bacterial contamination and poured in sterilized dishes and left these plates at 25±1°C for one day. Petri dishes left for one day then incubated after taking air samples for 14-21 days at 30ºC.

Biochemical Test

Fungi were identified by microscopy by the use of lactophenol cotton blue staining.

Colony Morphology

Petri plates were analyzed under a microscope at 100X to know the morphology. Identification according to Dugan (2005) was accomplished.33

Sampling for Particulate Matter

The light scattering technique is considered better for initial aerosols measurements,[30] so the light scattering method was used in the current study. Amongst numerous accessible photometers, DustTrak™ DRX (model 8533, TSI Inc.) aerosol monitor of USA is acknowledged to display accurate and precise PM numerical readings,[30] which have been used for both indoor and outdoor PM concentrations. Its sensitivity lays 0.001-100 mg/m^3 with range of 0.1-10 PM particle size. Data logging interval (1 min) and the sampling duration (8 h) were set. Before the instrument running inlet, vents were cleaned and lubricated every time as per protocol given with the instruction manual. The monitor is electrically energized and the Cameleon C-size rechargeable power bank was used as a substitute source.

DustTrak monitor was positioned at 1 meter elevated above floor level. The Particulars of the data was latterly shifted to a computer by DustTrak™ DRX (model 8533, TSI Inc.) aerosol monitor USA using TrakPro™ data analysis software for more examination and analysis.[29] Monitoring of outdoor and indoor asthmatic patients began in May 2018 and continued till June 2019. Monitoring was conducted only once at each site because the devices were a bit loud and make disturbance for inhabitants was a remarkable constrain. Various family circle activities can be regarded to be a source of fluctuations in the mass of PM inside a house.[30]

Statistical Analysis

Statistics were used for mean, standard deviation, minimum and maximum observations for various bacterial and fungal spores. Two-way ANOVA was used to remark the association of PM and CFU/m^3 (bacterial & fungal). Pearson correlation was used to perceive the connection in-between temperature, relative humidity, and PM. The multiple regression was applied to check the impact of various environmental variables) on the concentration of PM, bacteria, and fungi using SPSS Inc., ver. 21.0. A *p*-value below 0.05 was taken as significant.

RESULTS

Temperature and Relative Humidity

The average temperature observed throughout the track was 29.60±3.66 to 38.7±3.27°C. The mean relative humidity varies from 44.60±9.89 to 57.20±8.85 (Table 1).

Bacteria and Fungi

The air-laden microflora of monitoring sites was expressed by totally seven bacterial and twelve fungal species. The mean and maximum bacterial CFU/m^3 ranged in category A-866 (1221), B-3716 (5801), C-6579 (9294), and D-9618 (12469). Similarly, fungal CFU/m^3 ranged in categories A-563 (905), B-980 (1326), C-1586 (1946) and D-3092 (3881) (Table 2). Based on two-way ANOVA, it was found that there was a significant difference between our groups at $p<0.05$ in bacterial concentration but the results were non-significant for fungi ($p<0.05$) (Table 2).

Particulate Matter (PM)

The mean levels of PM_{01} µg/m^3 (indoor & outdoor) observed in A-category has the lowest levels (158.75 & 277.16) whereas, C-category has the highest levels (374.40 & 507.86) of PM_{01}. A-category has the lowest level of outdoor $PM_{2.5}$ (254.16) and C-category has the highest levels of indoor PM (489.31). A-category has the lowest level of PM_{10} in the indoor (200.41) and D-category has the highest level of PM_{10} outdoor (636) (Table 3).

The prevalent bacterial species were *Staphylococcus spp*. (30%), *Micrococcus sp*. (24%), and *Bacillus sp*. (26 %) along with unidentified Gram-negative (8%), *Serratia spp* (1%), and Gram-positive rods (1%). The macroscopic identification of fungal species showed that *Alternaria alternate* (14%) and *Aspergillus fumigatus* (12%) were the much plentiful fungal species along with *Aspergillus nidulans* (12%), *Aspergillus flavus* (9%), *Aspergillus niger* (9%), *Aspergillus terreus* (8%), *Trichoderma spp* (8%), *Mucor spp* (7%), *Rhizopus spp* (7%), *Cladosporium spp* (5%) and *Penicillium spp.* (4%) (Figure 3-5). *Aspergillus fumigatus, Aspergillus niger,* and *Aspergillus terrus* were identified based on their morphological features (Annex-IV).

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Table 1. Comparison of different parameters of sampling sites

Category	Sampling Sites	Number of houses	Temperature (Mean±SD)	*p*	Relative humidity % (Mean±SD)	*p*	House Size	Room Size
A	A1~A10	10	29.60±3.66	0.730	44.60±9.89	0.974	418.10±0.00	83.89±5.27
B	B1~B10	10	33.70±2.67		48.30±9.89		211.40±0.00	58.89±5.53
C	C1~C20	20	35.55±3.86		48.95±10.22		104.50±0.00	41.86±3.12
D	D1~D10	10	38.7±3.27		57.20±8.85		62.70±0.00	26.09±2.61

Table 2. The mean values of microbial counts (CFU/m^3) in the bioaerosols samples collected from sampling sites of different categories

Category	Sampling Sites*	Bacteria CFU/m^3	SD	*p*	Fungi CFU/m^3	SD	*p*
A	A1-A10	866	215.68	**0.021**	563	164.81	0.197
B	B1-B10	3716	1317.53		980	197.53	
C	C1-C20	6579	1331.03		1586	273.42	
D	D1-D10	9618	1760.56		3092	236.19	

* CFU/m^3 (colony forming units per cubic meter)

Category-A large sized house (418.06 m^2); Category-B medium sized house (211 m^2), Category-C medium sized house (104 m^2) and Category-D small sized house (62-71 m^2).

Table 3. The mean (Min-Max) values of three different sizes of indoor and outdoor particulate matters in various locations

Sampling Location*	PM_{01}	*p*	$PM_{2.5}$	*p*	PM_{10}	*p*
			Indoor PM (µg/m^3)			
			Mean (Min-Max)			
A1- A10	158.75 (106-211)		163.33 (109-217)		200.41 (120-296)	
B1-B10	278.18 (209-435)	**0.002**	285.90 (213-450)	**0.002**	474.63 (285-907)	**0.001**
C1-C20	374.40 (217-620)		386.31 (244-644)		601.86 (286-975)	
D1-D10	331.75 (219-471)		342.5 (224-487)		502.33 (287-751)	
			Outdoor PM (µg/m^3)			
			Mean (Min-Max)			
A1- A10	277.16 (138-407)		254.16 (199-318)		347 (281-421)	
B1-B10	381 (199-549)	0.562	380.36 (315-515)	**0.005**	529.72 (415-708)	**0.001**
C1-C20	507.86 (341-850)		489.31 (273-744)		699.27 (410-955)	
D1-D10	421.5 (305-651)		431.83 (321-547)		636 (405-905)	

* Category-A large size house (418.06 m^2); Category-B medium size house (211 m^2), Category-C medium size house (104 m^2) and Category-D small size house (62-71 m^2).

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Correlation

Pearson correlation test was applied with a significance level of 5% on $PM_{2.5}$, PM_{01}, PM_{10} to know the association between particulate levels and bioaerosols of each site. A significant positive correlation was observed PM_{01}, $PM_{2.5}$, PM_{10}, and bioaerosols (bacterial and fungal CFU/m^3) (Table 4).

Multiple regression was applied to check the impact of various environmental variables on the concentration of PM, bacteria, and fungi. The estimated regression models were developed for PM_{01}, $PM_{2.5}$, PM_{10}, bacteria, and fungi. The prediction power of models was analyzed; using the R^2 value. It was noticed that bacterial and fungal concentrations were the only significant contributing factors to PM_{01}, $PM_{2.5}$, PM_{10} levels. However, in the case of bioaerosols, the temperature was the only significant contributing environmental variable (Table 5 and 6).

Table 4. Parameters in an indoor environment

		Temp	Relative humidity	Bacterial-CFU/m^3	Fungal-CFU/m^3	Indoor-PM_{01} µg/m^3	Indoor-$PM_{2.5}$ µg/m^3	Indoor-PM_{10} µg/m^3
Temperature	Pearson Correlation	1						
Relative humidity	Pearson Correlation	0.267	1					
	p	0.061						
Bacterial-CFU/m^3	Pearson Correlation	0.657**	0.362**	1				
	p	**0<0.001**	**0.010**					
Fungal-CFU/m^3	Pearson Correlation	0.607**	0.375**	0.886**	1			
	p	**0<0.001**	**0.007**	**0<0.001**				
Indoor-PM_{01} µg/m^3	Pearson Correlation	0.433**	0.226	0.573**	0.348*	1		
	p	**0.002**	0.114	**0<0.001**	**0.013**			
Indoor-$PM_{2.5}$ µg/m^3	Pearson Correlation	0.439**	0.259	0.577**	0.360*	0.994**	1	
	p	**0.001**	0.069	**0<0.001**	**0.010**	**0<0.001**		
Indoor-PM_{10} µg/m^3	Pearson Correlation	0.428**	0.155	0.491**	0.288*	0.922**	0.928**	1
	p	**0.002**	0.283	**0<0.001**	**0.043**	**0<0.001**	**0<0.001**	

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed). Pearson correlation matrix of analyzed parameters.

Table 5. Bacterial and fungal colony-forming units per cubic meters in the residential houses of asthmatics

	Bacterial cfu/m^3			Fungal cfu/m^3		
Constant	**SE**	**B value**	***p***	**SE**	**B value**	***p***
Sampling site	108.9	-107	0.296	32.60	-2.48	**0.031**
Temrature (°C)	79.4	-4.75	**0.000**	23.70	0.521	**0.000**
Humidity (%)	32.3	0.141	0.108	9.68	0.198	0.088
Adjusted R^2		0.528			0.434	

Multiple regressions modeling for bacterial and fungal colony forming units per cubic meter

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Two-way ANOVA was used to analyze the difference between demographic variables like age, gender, and asthma allergy level between four groups. It was found that only the asthma allergy level was significantly different between the four groups ($p<0.05$) while there was no significant difference between groups based on gender and age (Table 7).

Bioaerosols Characterization

Staphylococcus spp. was found to be the predominant bacterial species in all four categories, *Micrococcus spp*. was also found dominant except in A-category. In all categories (A, B, C, and D), gram-positive rod and S*erretia spp.,* were minimum in numbers. The lowest (866 CFU/m^3**)** bacterial level was measured in A-category residences (Figure 3A), while the highest level (9618 CFU/m^3**)** was seen in D-category houses (Figure 3D).

Predominant Indoor Fungi

In all sampling sites, the most common fungi included *Alternaria alternate, Aspergillus fumigates, Aspergillus nidulans*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus.* The Highest level of fungi in the D-category (3092CFU/m^3) (Figure 3D), and the lowest level of the A-category (563CFU/m^3) (Figure 3A) was seen. The moderate level of both bacteria and fungi was seen in B-category (3716 & 980) and C-category (6579 & 1586) respectively (Figure 3B and C).

Table 6. Particulate matter (PM) concentration in the residential houses of asthmatics

	PM_{01} µg/m^3			$PM_{2.5}$ µg/m^3			PM_{10} µg/m^3		
	Standardized coefficients								
Constant	**SE**	**B value**	***p***	**SE**	**B value**	***p***	**SE**	**B value**	***p***
Sampling site	4.93	0.84	0.492	5.07	0.090	0.464	10.23	0.194	0.139
Temrature(°C)	4.01	0.112	0.454	4.12	0.114	0.447	8.32	0.177	0.263
Humidity (%)	1.40	0.065	0.590	1.44	0.099	0.411	2.91	0.007	0.958
Bacterialcfu/m^3	0.010	1.09	**0.000**	0.010	1.05	**0.000**	0.21	0.849	**0.004**
Fungalcfu/m^3	0.036	-0.703	**0.011**	0.037	-0.669	**0.015**	0.075	-0.54	0.058
Adjusted R^2	0.407				0.406			0.332	

Multiple regressions modeling for particulate matter (PM) concentration

Table 7. Comparison indoor temperatures in different sampling sites

Dependent Variable	Sampling-site	Sampling-site	SD	*p*	Lower Bound	Upper Bound
Indoor Temrature-^{0}C	A	D	1.565	**0.000**	-12.25	-5.95
		C	1.356	**0.000**	-8.68	-3.22
		B	1.565	**0.012**	-7.25	-0.95
	D	A	1.565	**0.000**	5.95	12.25
		C	1.356	**0.025**	0.42	5.88
		B	1.565	**0.003**	1.85	8.15

The error term is Mean Square (Error)=37772.202*. The mean difference is significant at the .05 level with 95% C. Mean will fall between these two lower and upper bound values at 95% confidence.* Category-A large size house (418.06 m^2); Category-B medium size house (211 m^2), Category-C medium size house (104 m^2) and Category-D small size house (62-71 m^2). Multiple comparisons two-way ANOVA.

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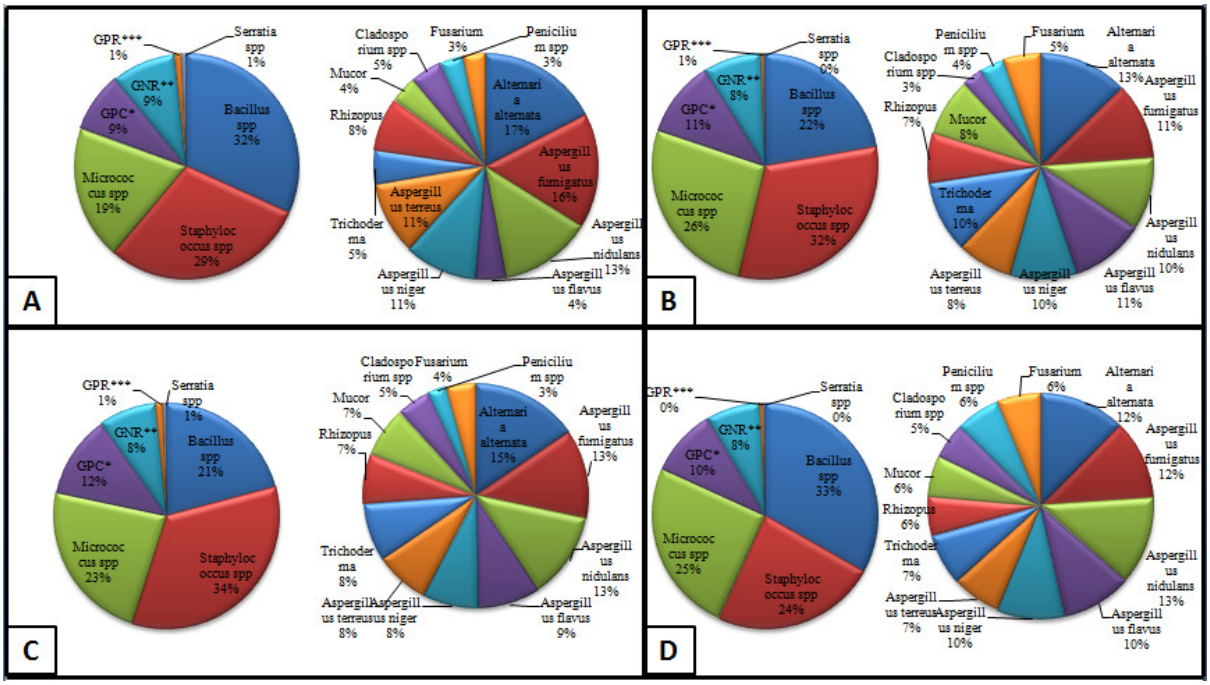

Figure 3. The proportion of bioaerosols from sampling sites A-category (3A), B-category (3B), C-category (3C) and D-category (3D).

DISCUSSION

PM and bio-aerosols are both very great air-borne components having a destructive effect on man's general health condition.[30] Allergenic enzymes are produced by upper respiratory tract mucosa upon exposure to bacterial endotoxins, fungal spores, and PM.[34,35] The average levels of PM_{01}, $PM_{2.5,}$ and PM_{10} µg/m^3 observed indoors of A-category were (158.75, 163.33 & 200.41) respectively which have been revealed earlier by a study.[36] The mean levels of PM_{01}, $PM_{2.5,}$ and PM_{10} µg/m^3 (278.18, 285.90 & 474.63) observed in the indoors of B-category were on the lower limit of the highest level. Chao and Cheng (2002) recognized the various origin of $PM_{2.5}$ in residential homes together with cuisines, smoking, and indoor human life activities.[37] The mean levels of PM observed in the C-category were similarly reported.[38] $PM_{2.5}$ produced were 1022 µg/m^3 with degree stretch so high as 1821 pg/m^3.[30] Smoking was noted in C and D categories, as it is notorious to increase PM levels considerably.[39] The mean high indoor levels of PM in the D-category may be due to personal activities as already reported personal activities and low ventilation rate can be linked with elevated indoor PM.[40] Though people movement cannot produce PM itself but inhabitant activity in a concealed room has been connected with the resuspension of earlier lodge PM that deposited on furniture surfaces.[41] Another examination concerned with levels of PM stated that in some homes, PM values peaked without any apparent source might be due to some metrological phenomenon. These observed PM levels in all the categories were 5 to 10 folds greater than the WHO-approved limits. Chunram reported outdoor sources of PM to be responsible for contributing factors to indoor PM.[42]

The average range of indoor bacteria in the existing study was found to be 866-9618CFU/m^3. The microbial count was increasingly found from A to D categories likely due to a decrease in the size of the living room and increase in the number of occupants in the lower categories like C and D in comparison to A and B. This was in agreement with many other findings.[43] Moreover opening and closing of windows also affect the percolation of outdoor air.[44] The abundant bacterial species in this study has been reported the allergenic effects predominant indoor microbes based on 16S rRNA gene sequence analysis.[45] Moreover Mentese has reported the *Staphylococcus and Micrococcus* the much

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usual bacteria found in indoor environments of asthmatics.[46] As in this study 8% Gram-negative were recorded as already reported with the presence of endotoxin.[47] In the current study Gram-positive rods were only (1%) which was contrary to the previous findings that gram-positive are chiefly present airborne in indoor environments.[15] The relatively low presence of airborne gram positive bacteria may primarily reflect the short survival periods of such bacteria in the airborne state.

In A-category houses of asthmatics, the chiefly bacterial species were found to be 1221 CFU/m^3**.** In contrast, Lee reported in normal homes ranging between 10 and 10^3CFU/m^3.[48] In B-category dominant bacteria to be *Staphylococcus spp*., *Micrococcus spp*. and *Bacillus spp*. mean 3716 CFU/m^3.[48] Lee reported the bacteria 900 CFU/m^{-3}.[48] *Staphylococcus spp*., *Micrococcus spp*., and *Bacillus spp*. were the dominant species in C- and D-category predominant bacteria were 6579-9618CFU/m^3. In a study by Pegas, bacteria counts were 934-1634 CFU m^{-3} in all normal persons (934-1634 CFU/m^3).

Mucor spp., *Rhizopus spp.*, *Cladosporium spp.* and *Penicillium spp.* were less established in all the houses. Similar findings were reported by Sidra et al 2015 and Mushtaq et al, 2011.[29,48,51] Most commonly reported fungal species in congested indoor environments are *Alternaria*, *Aspergillus*, *Cladosporium,* and *Penicillium.*[50,51] Highest level of fungi in the D-category (3092 CFU/m^3) and the lowest level in the A-category (563 CFU/m^3)was seen. However, in previous studies the maximum concentration of *Aspergillus* between 50 and 160 CFU/m^3 and seen in offices, representing a potential health risk for asthmatic individuals.[52] Fungi found outdoors have been noted commonly from moist indoor environments. Similar to this study other workers,[53] reported the same genera predominant indoor. *Alternaria alternata* and *Aspergillus fumigatus* were the most abundant fungal species in the current study. Similarly, airborne fungal concentration in various indoor environments was already reported.[48] *Aspergillus spp.* were present 980-1326)CFU/m^3 whereas, other studies have shown low levels of fungal concentrations ranging 103-1116 CFU/m^3 in offices.[54] The C and D category sampling sites 1586, 3092 CFU/m^3 of *Aspergillus spp.* was noted. In comparison, almost similar fungal levels (463-3125 CFU/m^3) have been observed in residence places by other researchers.[55] High load of asthma and allergic bronchopulmonary aspergillosis with moldy sensitization in grownups asthmatic subjects in Pakistan is already reported by.[56]

The current study has a considerable positive correlation between temperature (38.9°C) and bacterial counts. Similar results were expressed in studies with a connection between the concentrations of bioaerosol and environmental factors like temperature.[57]The spectrum of fungi concerning the relative humidity and temperature has been already reported.[58]

Relative humidity levels in this study ranged from 44.60±9.89 to 57.20±8.85. Similarly found relative humidity positively correlated with airborne fungi levels in houses of northeast United States but, indoor temperature negatively correlated with levels of bacteria.[59] Temperature 20-25°C sponsors the culture of mesophilic fungi but temperature down to optimum value retard the growth.[60]

Readings of PM were taken only once. It was not convenient for respondents to visit their houses again and again weekly or monthly. Additionally, real-time observation of PM and passive sampling of bio-aerosols are considered as the limitations of the current study.

Pakistan is deficient in standard data of micro-flora of indoor environments of general public especially sick people like asthmatic. $PM_{2.5}$ recorded in the current study was 10 folds greater than WHO recommendable limits. The bacterial and fungal species identified in the monitoring sites were well-known common components of the indoor air and they are opportunistic pathogens. Improvement in air change rate per hour can improve air quality and heating, ventilation, and air conditioning (HVAC).

CONFLICT OF INTEREST

There is no conflict of interest.

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