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Assessment of Airborne Microflora in the Indoor Micro-Environments of Residential Houses of Lahore, Pakistan

Safdar Sidra[1,6], Zulfiqar Ali[1*], Sikander Sultan[2], Shakil Ahmed[3], Ian Colbeck[4], Zaheer Ahmad Nasir[4,5]

[1] *Environmental Health and Wildlife, Department of Zoology, University of the Punjab, 54590, Lahore, Pakistan*
[2] *Department of Microbiology and Molecular Genetics, University of the Punjab, 54590, Lahore, Pakistan*
[3] *Department of Botany, University of the Punjab, 54590, Lahore, Pakistan*
[4] *School of Biological Sciences, University of Essex, Colchester, Essex, CO4 3SQ, United Kingdom*
[5] *School of Energy, Environment and Agrifood, Cranfield University, Cranfield, Bedfordshire MK43 0AL, United Kingdom*
[6] *Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore, Pakistan*

ABSTRACT

The presence of micro-organisms in air is taken for granted, but understanding the identities, distribution and abundance of airborne micro-organisms remains in its infancy. Indoor exposure to micro-organisms has been related to range of adverse health outcomes. The indoor levels of particulate matter and bioaerosols were monitored in thirty houses across Lahore, Pakistan. Two DustTrak aerosol monitors (model 8520, TSI Inc.) were run simultaneously in the kitchens and living rooms of the selected sites to measure fine particulate matter. At the same time, agar coated petri plates were exposed face upwards for twenty minutes to sample the micro-organisms present in surrounding air of both micro-environments. A total of 7 bacterial species and 11 fungal species were identified including *Staphylococcus spp., Bacillus spp., Micrococcus spp.* and *Serratia spp.* while the predominant fungal species were *Alternaria alternata* and *Aspergillus spp.* The concentrations (cfu m^{-3}) for bacteria ranged from 472 to 9829 in the kitchens and from 275 to 14469 in the living rooms. Likewise, the fungal cfu m^{-3} ranged between 236 and 1887 in the kitchen and from 315 to 1887 in the living room. A seasonal variation in bioaerosols was evident in the kitchens while being not so pronounced in the living rooms. A linear regression model showed a direct association of temperature with bacteria and fine particulate matter but not with fungi. Ventilation was also observed to have a significant impact upon PM levels. Out of 30 households sixteen had the presence of at least one individual with allergenic reactions. These findings highlight the enhanced risk of exposure to fine particulate matter as well as bioaerosols in the urban residential built environment in Pakistan.

Keywords: Bioaerosols; Fine particulate matter; Residential area; Indoor air quality.

INTRODUCTION

Air is an important route of disease transmission as it is laden with a variety of pollutants which can enter the human body simply through breathing. Many studies have shown that concentrations of pollutants in the indoor environment are often higher than those typically encountered outdoors, while the estimated risk to human health from indoor sources is about 1000 times higher than the one resulting from exposure to outdoor pollutants (Colbeck *et al.*, 2010a).

Particulate matter is known to bind with compounds of biological origin to create bioaerosols (Cox and Wathes, 1995). It acts as a carrier matrix for bacteria, fungi, pollens and/or their derivatives/parts affecting their shapes, sizes, chemical and biological composition and resultant aerodynamic behavior, dispersion and deposition (Behrendt *et al.*, 1992; Glikson *et al.*, 1995; Knox *et al.*, 1997; Ormstad *et al.*, 1998; Risse *et al.*, 2000; Monn, 2001). As evident from previous studies (e.g., Lierl and Hornung, 2003), although each pollutant has its own adverse effect, the synergistic effects of particulate matter and bioaerosols can increase the chances of allergies and pulmonary problems (Adhikari *et al.*, 2006).

Being ubiquitous in the indoor environment, bioaerosols are an important component accounting for up to 50% of all aerosol particles (Mandal and Brandl, 2011). An average person spends around 80% of their time indoors so exposure to these pollutants is inevitable. This can result in adverse health effects particularly respiratory problems (Hsu *et al.*, 2012). Airborne micro-organisms and/or their components

* Corresponding author.
Tel.: +92 300 4401535
E-mail address: zali.zool@pu.edu.pk

have been documented to be responsible for a variety of health problems such as asthma, rhinitis, sick-building syndrome, infections and many more (Fabian *et al.*, 2005; Roy and Reed, 2012). Horner *et al.* (1995) reported more than 80 genera of fungi to be responsible for respiratory problems. Around 10% people around the world are affected by fungal allergy (Burge, 2001). However it is also important to note that not all the bioaerosols we inhale are harmful. In fact most of them are harmless with only a small proportion being unhealthy (Heikkinen *et al.*, 2005).

Air is a dynamic system with various sources of particulate matter and bioaerosols. The levels of particulate matter, including bioaerosols, in the indoor environment depends on the number of the occupants, their activities, building structure, material, furnishings, and the outside air entering the building (Jaakkola and Miettinen, 1995; Douwes *et al.*, 2003; Gioulekas *et al.*, 2004; Bowers *et al.*, 2011, 2012). In fact microbial loads are significantly affected by the presence of human beings in the built environment (Loftness *et al.*, 2007; Hospodsky *et al.*, 2012).

Humidity, temperature and wind velocity are some of the many atmospheric factors that influence the concentration and spread of particulate matter and bioaerosols. Temperature has been known to affect the concentrations of air-borne pollen and particulate matter while relative humidity can decrease the particulate levels in the ambient air (Mirme and Ruuskanen, 1996; Bauer *et al.*, 2002; Beggs, 2004; Martuzevicius *et al.*, 2004). It is also noteworthy that since modern buildings tend to be more air tight, resulting in reduced ventilation, the risk of exposure to bioaerosols increases (Walinder *et al.*, 2001; Kembel *et al.*, 2012). It is therefore necessary to maintain an adequate ventilation rate so as to continuously circulate and replace the indoor air with outdoor air and thus resulting in diluting the indoor concentrations of the airborne microbes and particulate matter.

As a developing country, the environmental health status of Pakistan is poorly defined. There is very limited data about the state of indoor air quality. The Government has not yet considered the risks posed by exposure to pollutants and there are no guidelines available for assessing indoor air quality. While there are some studies on particulate matter indoors in Pakistan, bioaerosols have not been extensively investigated. Colbeck *et al.* (2008) and Nasir *et al.* (2012) have documented bioaerosol levels in rural and urban residential settings but apart from these, no other study was found. The current paper is an attempt to provide an insight into the various levels of bioaerosols and $PM_{2.5}$, in the indoor micro-environments in urban residential settings. Despite some limitations such as use of passive sampling for bioaerosols in contrast to the real time monitoring for $PM_{2.5}$, the study holds its significance as it provides preliminary knowledge about the levels of airborne bacteria and fungi and the common species present in the indoor environment in urban residential built environments in Pakistan.

Keeping these facts in view, the following study aims to investigate the concentrations and types of bioaerosols found in the air of various households of Lahore, Pakistan along with the fine particulate matter.

METHODS

Lahore (31.55°N and 74.34°E) is the second largest of Pakistan with a population of over 9,086,000. It is the provincial capital of Punjab. The city is situated along the bank of the River Ravi and covers an area of over 1772 km^2 (BOS, 2013). It is divided into nine administrative zones and a cantonment area. In order to select the sampling sites, three housing categories were defined on the basis of floor area as follows:

Category A: Small: ≤ 126.5 m^2
Category B: Medium: > 126.5 m^2 to 253 m^2
Category C: Large: > 253 m^2

Based on this categorization, three houses were selected from each administrative town i.e. one from each category making a total sample size of thirty houses (Fig. 1).

The sampling was carried out from January 2012 to March, 2013. Passive monitoring of airborne microbes was conducted for twenty minutes each at each site. The Koch sedimentation method was applied to collect bioaerosol samples from the two micro-environments. Agar coated petri plates were exposed face upwards at a height of 1 meter from the ground. There are relatively few studies which have employed passive sampling for bioaerosol sampling and the exposure time varies from 15 minutes (Stryjakowska-Sekulska *et al.*, 2007) to 30 minutes (Bogomolova and Kirtsideli, 2009). The exposure time in this study was set to be twenty minutes during which the petri plates were kept open to allow settling of airborne bioaerosols present in the surrounding air. In order to minimize the impact of household activities upon the bioaerosol levels, petri plates were exposed one hour after the last activity was performed in the room.

Two real time aerosol monitors (DustTrak model 8520, TSI Inc.) were run simultaneously for 72 hours in the kitchens and living rooms of the sampling sites to record the mass concentration levels of $PM_{2.5}$. The $PM_{2.5}$ levels during twenty minutes of bio-aerosol monitoring were also separated from the data for correlation with microbial levels observed at that time.

Temperature, relative humidity and CO_2 were recorded using a Gas probe IAQ (BW technologies). Other relevant information regarding the number of occupants, their activities, and surroundings of the house, type of fuel and their health condition was obtained through direct questioning.

The medium used for bacterial sampling was Tryptic Soy Agar (TSA) while Malt Extrose Agar (MEA) was used for fungal sampling. The petri plates were incubated at 27°C for 24 to 48 hours. The number of colonies was then counted by direct count and the species were identified using light microscopy by studying the morphological features of the colonies. The number of colony forming units per cubic meter (cfu m^{-3}) was determined using the Omelyansky formula (1940) as followed by Bogomolova and Kirtsideli (2009).

$$N = 5a \times 10^4 (b.t)^{-1} \quad (1)$$

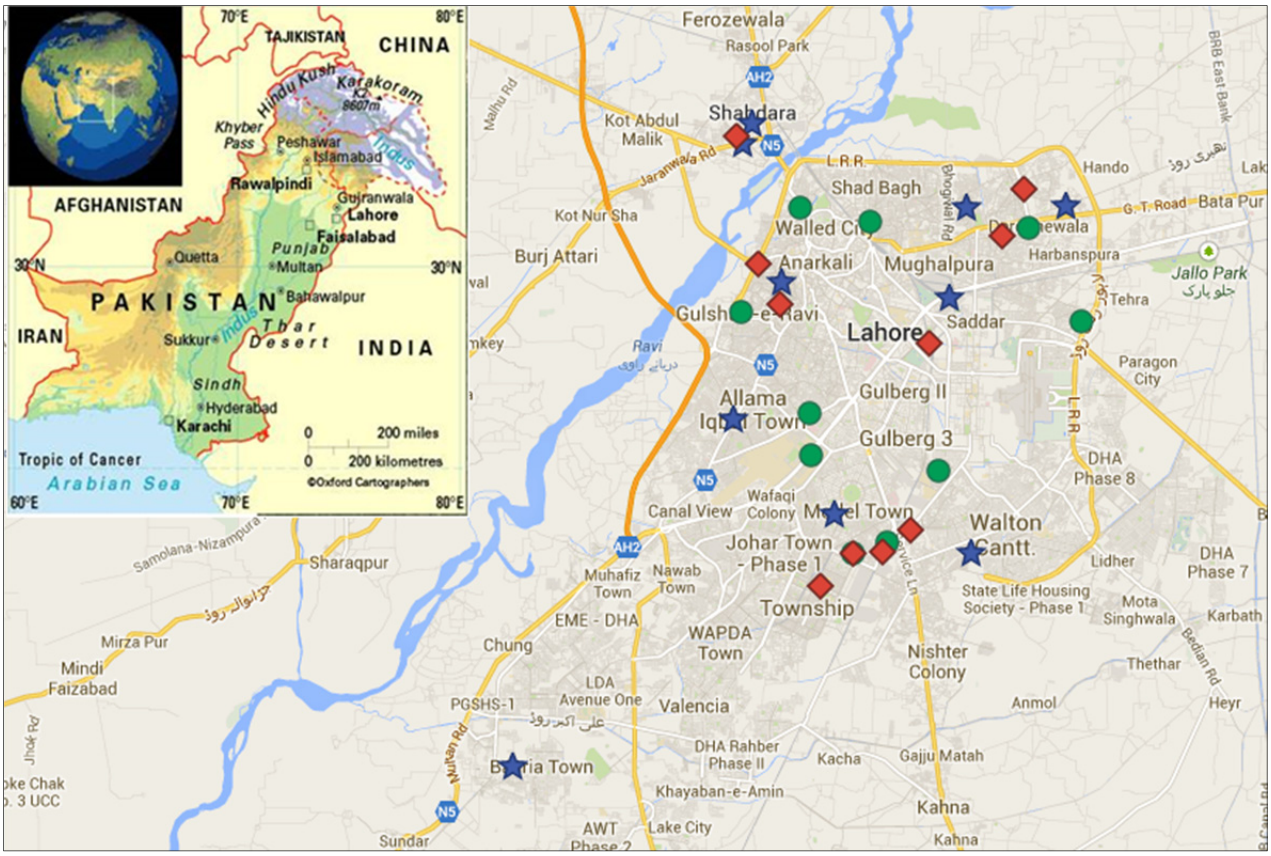

Fig. 1. Location of study sites within Lahore city marked according to size (◆ = Category 1; ● = Category 2; ★ = Category 3).

where N = microbial cfu m^{-3} of indoor air; a = number of colonies per Petri dish; b = dish surface (cm^2); and t = exposure time (minutes).

Ventilation rates were measured using a concentration decay method (Fischer-Mackey, 2010). CO_2 was used as the tracer gas and air exchange rate per hour was determined. Statistical analysis of the results was carried out using Microsoft Excel and SPSS (v. 16.0.0).

RESULTS AND DISCUSSION

The levels of pollutants in the indoor environment depend upon not only the sources but also other factors as well such as mode of ventilation or occupant's activities. However, since the sampling was conducted one hour after the performance of any last activity in the room, the levels of bioaerosols and particulate matter should not have any strong association with the activities of the surrounding environment at the time of sampling. The sampling was done during different times of the year so there was a considerable fluctuation of temperature and humidity levels in the selected sites.

Levels and Composition of Airborne Microflora

A total of 7 bacterial species and 11 fungal species were identified that dominated the indoor air of kitchens and living rooms. The bacterial concentrations (cfu m^{-3}) ranged from 472 to 9829 in the kitchens and from 275 to 14469 in the living rooms. Similarly, the fungal cfu m^{-3} ranged between 236 and 1887 in the kitchen and from 315 to 1887 in the living room (Tables 1(a) and 1(b)).

The predominant bacterial species from all thirty sampling sites were found to be *Staphylococcus spp*. (37% in kitchens and 35.4% in living rooms), *Micrococcus spp*. (28.3% in kitchens and 29.8% in living rooms), and *Bacillus spp*. (11.8% in kitchens and 14.2% in living rooms) along with *Serratia spp.* and some unidentified Gram negative and positive rods and Cocci in a few sites (Fig. 2). *Aspergillus fumigatus* (25.3% in kitchens and 22.9% in living rooms) and *Alternaria alternata* (18.9% in kitchens and 30.0% in living rooms) were the most abundant fungal species found at all sites along with other *Aspergillus* species, *Rhizopus*, *Fusarium spp. Trichoderma* and *Mucor* (Fig. 3).

Different studies have similarly reported a wide assortment of species of bacteria and fungi in indoor environments. Pastuszka *et al.* (2000) monitored air quality of various houses in Poland and observed *Micrococcus spp.* to be present in all houses with *Staphylococcus epidermidis* to be present in 76% of houses. The major fungal species were identified to be *Absidia glauca*, *Alternaria alternata*, *Cladosporium cladosporioides* and *Penicillium aurantiogriseum* in the majority of homes. In another study, Gorny and Dutkiewicz (2002) recorded the presence of numerous bacterial species including *Aeromonas*, *Bacillus*, *Kocuria*, *Micrococcus*,

Table 1(a). Summary of the parameters monitored in the kitchens of the sampling sites ($PM_{2.5}$ quoted in μg m^{-3}).

Sampling site	KITCHEN						
	ACH	Temperature (°C)	Humidity (%)	Bacteria (cfu m^{-3})	Fungi (cfu m^{-3})	$PM_{2.5}$ (20 min mean value)	$PM_{2.5}$ (24-h mean value)
A 1	4.1	18.3	33	1927	354	106.5	224.5
A 2	3.2	21	40	511	315	119.5	188.8
A 3	11.1	27	51	6566	236	317.3	185.3
A 4	5.0	36	25	7942	747	68.2	69.9
A 5	5.4	31.8	59	7077	393	40.8	132.9
A 6	4.8	30.3	72	3460	550	100.1	202.3
A 7	11.6	32	65	6291	1101	109.5	342.7
A 8	2.7	33.1	47	6605	315	149.8	191.8
A 9	6.3	30.1	48	9436	1573	270.0	422.7
A 10	11.7	32.7	32	5937	1887	510.4	456.7
B 1	5.0	18.1	45	1848	315	201.2	983.0
B 2	4.7	20	55	1730	236	383.9	236.7
B 3	3.9	23.4	32	5308	354	207.5	445.6
B 4	4.2	29.9	33	4915	511	75.3	185.3
B 5	6.6	36.7	36	6723	590	91.3	250.5
B 6	7.7	32.5	74	6448	944	197.2	199.6
B 7	5.6	22.8	55	2359	1730	566.8	440.3
B 8	15.0	22.6	48	5151	786	700.4	736.2
B 9	4.9	32.5	20	3381	1101	456.5	383.5
B 10	6.7	20.9	60	786	393	102.5	743.5
C 1	3.6	24.7	46	472	275	303.9	342.6
C 2	2.9	33.2	31	9829	1100	88.2	79.6
C 3	2.5	30	72	7749	708	60.8	136.8
C 4	2.5	29.4	46	8257	708	283.0	321.4
C 5	10.2	19.7	55	6094	1494	297.5	851.8
C 6	3.9	19.7	56	4482	1337	615.8	504.8
C 7	2.8	26.9	36	3263	275	169.1	389.0
C 8	11.0	30.9	37	2556	1612	192.7	285.8
C 9	6.8	25.3	55	9436	432	155.9	255.4
C 10	3.7	30.9	32	5622	904	61.5	137.8

Table 1(b). Summary of the parameters monitored in the living rooms of the sampling sites ($PM_{2.5}$ quoted in μg m^{-3}).

Sampling site	LIVING ROOM						
	ACH	Temperature (°C)	Humidity (%)	Bacteria (cfu m^{-3})	Fungi (cfu m^{-3})	$PM_{2.5}$ (20 min mean value)	$PM_{2.5}$ (24-h mean value)
A 1	3.5	18.0	32	1455	472	89.1	139.7
A 2	3.0	20.5	42	1848	393	130.6	149.0
A 3	3.8	26.0	47	7785	315	329.0	168.4
A 4	3.5	35.5	20	6448	590	115.9	119.9
A 5	7.4	32.1	57	8650	432	39.9	177.2
A 6	6.1	30.3	72	2477	668	102.9	123.4
A 7	4.8	30.4	64	7785	983	117.8	336.3
A 8	5.5	32.0	45	7470	1101	184.0	179.7
A 9	8.2	30.1	49	10616	1612	312.3	509.3
A 10	2.8	30.4	33	8453	1573	210.6	383.2
B 1	2.7	18.5	51	432	432	671.3	462.7
B 2	3.4	18.0	55	1062	354	336.9	203.2
B 3	2.4	23.5	28	393	432	173.9	227.4
B 4	2.8	29.9	33	4522	432	69.6	166.5
B 5	3.8	37.9	30	6291	1258	80.0	114.6
B 6	5.4	32.8	74	7785	1533	179.9	213.6
B 7	5.0	22.7	57	275	1887	644.1	476.0
B 8	6.6	22.7	47	6684	1219	809.5	894.6
B 9	5.5	32.8	22	2988	629	579.5	657.2
B 10	2.4	30.3	33	747	1573	143.7	417.3
C 1	3.8	26.0	47	354	393	215.3	193.6
C 2	4.1	33.2	31	7274	826	93.0	68.4
C 3	2.4	30.0	70	6291	1062	67.8	137.6
C 4	3.2	29.6	45	2005	1180	386.1	388.5
C 5	5.1	19.7	56	1494	1140	273.3	974.1
C 6	8.1	21.5	52	5111	1101	732.7	729.7
C 7	5.9	28.0	35	9004	354	250.8	285.5
C 8	9.1	31.0	38	747	1691	303.1	386.9
C 9	5.0	34.9	29	14469	826	235.7	449.0
C 10	3.5	32.7	32	7116	944	117.3	149.0

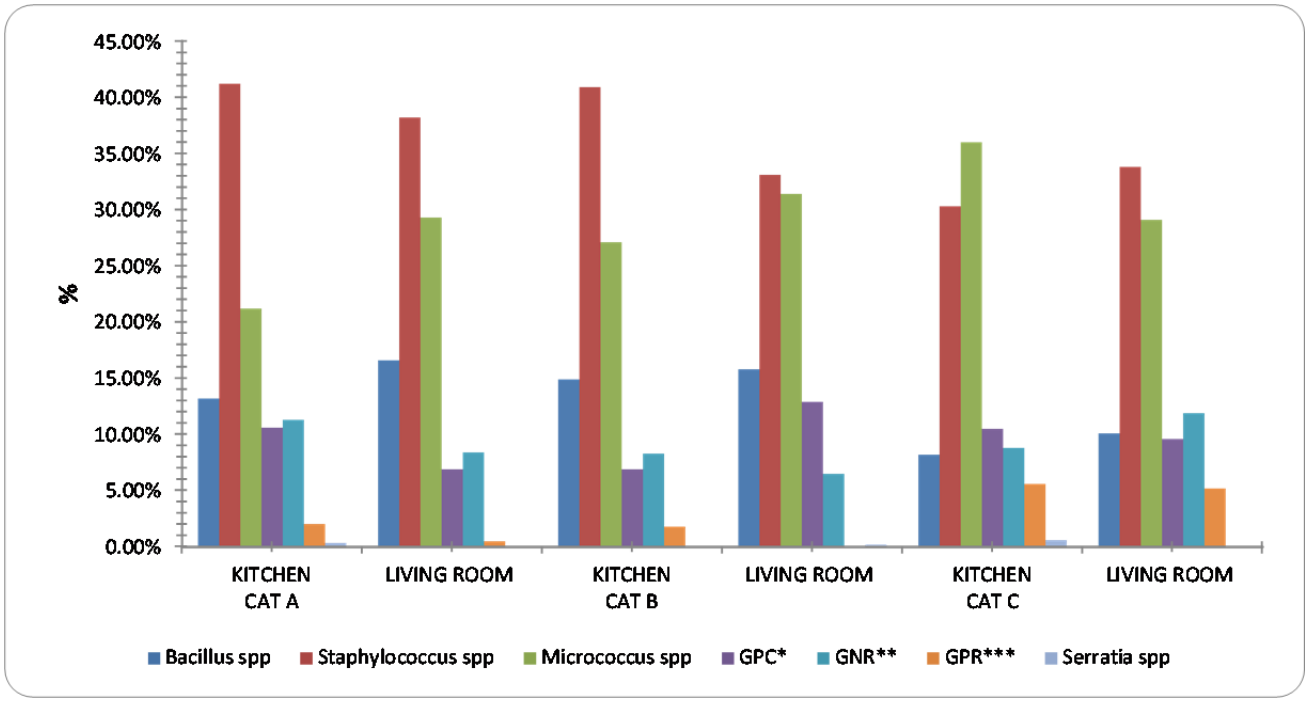

Fig. 2. Percentage of bacterial species observed in the kitchens and living rooms of different categories.

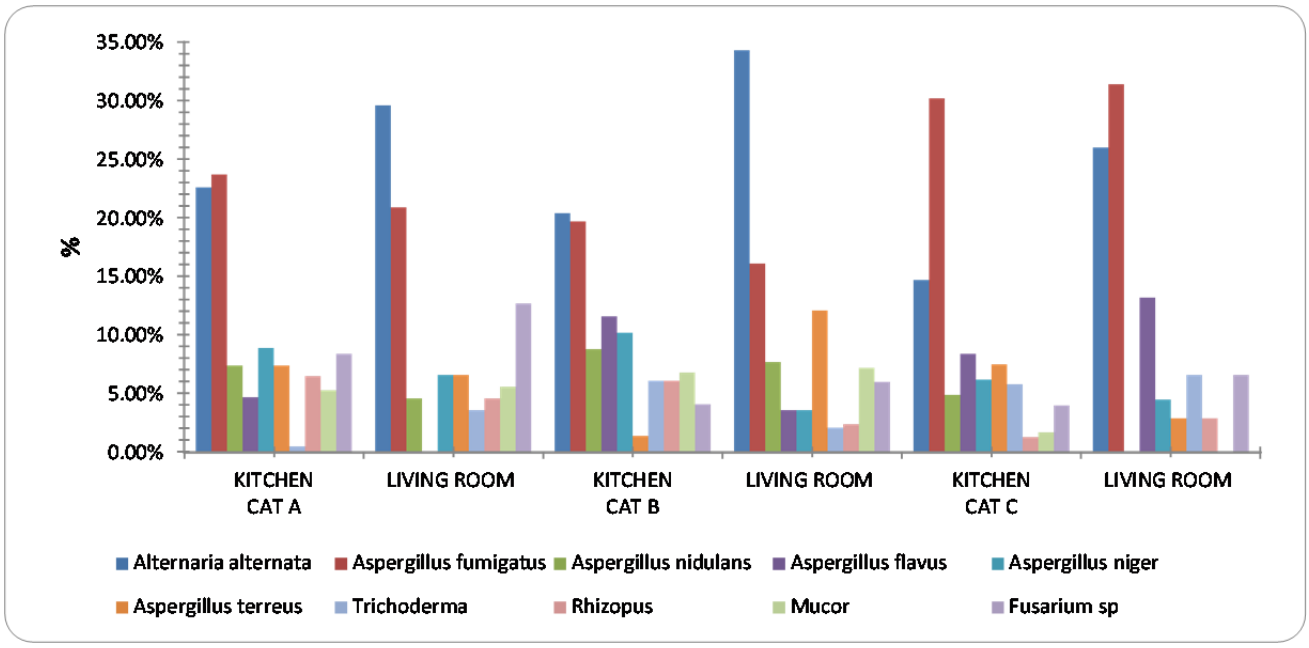

Fig. 3. Percentage of fungal species observed in the kitchens and living rooms of different categories.

Nocardia, *Pseudomonas*, and *Staphylococcus* in residential indoor environment while fungal species included *Aspergillus*, *Penicillium,* and yeasts. Karwowska (2003), Gorny (2004) and Hass *et al.* (2007) observed *Aspergillus* and *Penicillium* to be the most dominant genera in the indoor environment. Similarly Lee and Jo (2006) documented the presence of *Alternaria* and *Cladosporium* in addition to these genera while Reboux *et al.* (2009) also reported higher levels of *Aspergillus*, *Penicillium* and *Cladosporium* in unhealthy houses with visible mold. Recently Joshi and Srivastava (2013) reported the presence of bacterial species *Brevibacillus brevis*, *Arthrobacter* and *Bacillus cereus* and fungal species of *Neosartorya fischeri*, *Aspergillus clavatus* and *Trichoderma* in indoor air.

In order to assess the possible health outcomes of exposure to bioaerosols in the studied sites, the respiratory health of the occupants of the households under observation was also documented. Out of 30 households sixteen had one to two members who had reported allergenic reactions from dust or during the wheat harvesting season. One out of total 19

affected individuals was identified to be an asthma patient while the rest of them reported to be allergic to dust.

It should be noted that the airborne micro-flora of the sampling sites was composed of species generally present in the indoor air. These species are known to be opportunistic pathogens and/or allergenic. Among the fungal species, *Aspergillus*, *Alternaria*, *Cladosporium* and *Fusarium* are held to be responsible for hypersensitivity reactions including asthma, sinusitis and rhinitis (Hardin *et al.*, 2003; Kalogerakis *et al.*, 2005). *Aspergillus* species along with *Penicillium* has been reported as pathogenic and can prove to be life-threatening, in particular, to immune-compromised individuals (Vonberg and Gastmeier, 2006; Basilico *et al.*, 2007). A description of the observed microbial species as well as their potential sources and susceptible sites of entry have been summarized in Table 2. Although species of *Cladosporium* and *Penicillium* were not observed in our sampling sites, other species were present in variable numbers and could be a possible cause for the allergic reactions reported from 16 out of 30 sampling sites.

Levels of Fine Particulate Matter

The respective highest and lowest mean levels of $PM_{2.5}$ observed during twenty minutes of bioaerosol sampling were noted to be 700.4 ± 71.8 μg m^{-3} and 40.8 ± 15.3 μg m^{-3} in the kitchen and 809.5 ± 54.5 μg m^{-3} and 39.6 ± 5.6 μg m^{-3} in the living room. The average mass concentration levels of $PM_{2.5}$ were observed to be elevated although there was no significant activity being carried out at the time of sampling. It is possible that these levels were a result of some previous activities that caused the generation and/or re-suspension of particulate matter to a large degree taking time to settle or some outside unidentified source which contributed towards PM levels indoors.

In addition to the elevated 20-min averages of particulate matter, the 24-h means were also high (Tables 1(a) and 1(b)). These values resulted from a variety of household activities being carried out throughout the day. The average $PM_{2.5}$ values were significantly higher in both micro-environments than the WHO limit of 25 μg m^{-3}. Other studies conducted in indoor environments in Pakistan have also reported concentrations to be higher than the prescribed limits (Siddiqui *et al.*, 2005a, b; Akhtar *et al.*, 2007; Colbeck *et al.*, 2008; Siddiqui *et al.*, 2008; Siddiqui *et al.*, 2009; Colbeck *et al.*, 2010b; Janjua *et al.*, 2012; Nasir *et al.*, 2013).

The concentrations of bioaerosols and particulate matter were compared using one-way ANOVA to observe any significant association between them. The results revealed no substantial association between the sampling sites and any of the measured variables. Moreover, no significant correlation was observed to exist between PM levels and bioaerosols. Previous studies conducted by Pastuszka *et al.* (2000) and Hargreaves *et al.* (2003) have also observed lack of any significant association and concluded that bioaerosols in indoor residential settings were not dependent on non-biological particulate matter.

Air Exchange Rate

The ventilation rate of the sampling sites varied largely. In a few semi-open kitchens the ventilation rates ranged from 11 to 15 air changes per hour. Otherwise rates ranged between 2 ACH to 8 ACH (Tables 1(a) and 1(b)). The air change rate was observed to have a significant effect on particulate matter levels indoors. Ventilation, either natural or mechanical, plays an important role in determining the indoor air quality since infiltration of outdoor air as well as the removal rate of pollutants from the indoor air depends on it. It is possible that the reduced ventilation rates may have resulted in inefficient removal of pollutants from the indoor environment causing them to accumulate in the rooms as observed during the course of this study.

Regression Analysis of Monitored Parameters

Regression analysis was carried out to investigate the impact of air change rate (ACH), temperature and relative humidity (RH) (independent variables) on bacteria, fungi and $PM_{2.5}$ (dependent variables). A linear regression model was applied on single and multiple variables. It was observed that temperature had a direct relationship with bacteria and $PM_{2.5}$ but not on fungi. Moreover the ventilation rate was observed to have a significant relation with particulate matter (Tables 3 and 4).

The temperature measured during monitoring ranged from 18°C to 37.8°C in the kitchens and living rooms while the average relative humidity levels ranged from 20% to 75% in both the kitchens and living rooms. According to several studies, bacterial activity is generally reduced at temperatures higher than 24°C (Tang, 2009) and bacteria also require more water activity than the fungi. On the contrary, in the case of fungi, temperature may not be a limiting factor with most fungi growing at 10–35°C but humidity is still considered a critical factor affecting fungal growth since dampness facilitates the growth of fungal spores (Douwes *et al.*, 1999; Nielsen *et al.*, 1999). Relative humidity levels above 90% were found to cause a 30% increase in fungal spore size (Reponen *et al.*, 1996). This increase in spore size was calculated to increase the risk of deposition of spores in the respiratory tract, particularly bronchi by 20%. Moreover the transport of fungal spores is known to be more under the control of meteorological factors such as temperature, relative humidity and air flow. In fact the most suitable RH levels for growth of fungi are 70% and above while the optimum temperature range is between 30 to 40°C (Deguchi and Yoshizawa, 1996). However, variation still exists as similar bacteria may behave differently at different temperatures and relative humidity levels. Our results were in contrast with the above discussion as although temperature had a direct but weak relation with bacteria, relative humidity exhibited no significant association with bacterial and fungal levels.

Seasonal Variation in Bioaerosol Levels

Seasons have previously been documented to have a marked impact upon bioaerosol levels as concluded by Ahmed (2007). Frankel *et al.* (2012) also observed a distinct seasonal variation in bioaerosol levels. In our study, since the sampling was carried out during different seasons, a one way ANOVA was applied to determine the impact of

Table 2. Sources and health hazards posed by the bacterial and fungal species observed in this study (Source: Kowalski, 2006).

BACTERIA	Description	Sources	Diseases caused	Susceptible site of entry
Staphylococcus spp.	Gram positive bacteria, non-communicable, opportunistic pathogen	Humans, sewage, nosocomial.	Staphylococcal pneumonia, opportunistic infections	Upper Respiratory Tract
Micrococcus spp.	Gram positive bacteria, non-communicable, opportunistic pathogen	Skin of humans and other animals and in soil, marine and fresh water, plants, dust and air	Pneumonia, septic arthritis, endocarditis, bacteremia and meningitis	Upper Respiratory Tract, skin
Serratia spp.	Gram negative bacteria, opportunistic pathogen	Environmental, indoor growth in potable water, nosocomial.	Opportunistic infections, bacteremia, endocarditis, pneumonia.	Upper Respiratory Tract, wounds, eyes, urinary tract
		FUNGI		
Aspergillus spp.	Non-communicable, causes Aspergillosis, also associated with sick building syndrome	Environmental, nosocomial, indoor growth on insulation and coils.	Aspergillosis, alveolitis, asthma, allergic fungal sinusitis, ODTS, toxic reactions, pneumonia possible	Upper respiratory tract
Alternaria alternata	Non-pathogenic, non-communicable, common indoor contaminant, can cause opportunistic allergic reactions and contribute to sick building syndrome	Environmental, indoor growth on paint, dust, filters and cooling coils.	Allergic alveolitis, rhinitis, sinusitis, asthma, toxic reactions	Upper respiratory tract
Mucor	Non-communicable, opportunistic pathogen	Environmental, sewage, dead plant material, horse dung, fruits.	mucormycosis, rhinitis, pneumonia	Upper respiratory tract
Trichoderma	Non-communicable, allergenic	Environmental, soil, wood, decaying vegetation.	allergic alveolitis, toxic reactions, MVOCs	Upper respiratory tract
Rhizopus	Non-communicable, opportunistic pathogen	Environmental, decaying fruit and vegetables, compost.	Zygomycosis, allergic reactions, pneumonia, mucormycosis.	Upper Respiratory Tract, sinus, skin eyes
Fusarium spp.	Non-communicable, allergenic	Environmental, indoor growth on floor dust filters and in humidifiers.	allergic alveolitis, allergic fungal sinusitis, toxic reactions, MVOCs	Upper respiratory tract, skin, eyes

Table 3. Regression modeling of different parameters in kitchens (α = 0.05) with significant results shown in bold.

(a) Bacteria (CFU m^{-3}).

	r^2	F	P-value
Temperature	**0.341**	**14.465**	**0.001**
Relative Humidity	0.002	0.051	0.823
Air change per hour	0.035	1.018	0.322
Temperature and RH	**0.361**	**7.628**	**0.002**
Temp., RH and ACH	**0.396**	**5.676**	**0.004**

(b) Fungi (CFU m^{-3}).

	r^2	F	P-value
Temperature	0.043	1.263	0.271
Relative Humidity	0.002	0.048	0.828
Air change per hour	0.038	1.105	0.302
Temperature and RH	0.043	0.609	0.551
Temp., RH and ACH	0.088	0.841	0.484

(c) $PM_{2.5}$ (μg m^{-3}).

	r^2	F	P-value
Temperature	**0.146**	**4.773**	**0.037**
Relative Humidity	0.000	0.013	0.909
Air change per hour	0.123	3.943	0.057
Temperature and RH	0.153	2.443	0.106
Temp., RH and ACH	**0.256**	**2.977**	**0.050**

Table 4. Regression modeling of different parameters in living rooms (α = 0.05) with significant results shown in bold.

(a) Bacteria (CFU m^{-3}).

	r^2	F	P-value
Temperature	**0.324**	**13.407**	**0.001**
Relative Humidity	0.003	0.073	0.789
Air change per hour	0.095	2.941	0.097
Temperature and RH	**0.332**	**6.711**	**0.004**
Temp., RH and ACH	**0.338**	**4.434**	**0.012**

(b) Fungi (CFU m^{-3}).

	r^2	F	P-value
Temperature	0.064	1.921	0.177
Relative Humidity	0.032	0.914	0.347
Air change per hour	0.000	0.006	0.940
Temperature and RH	0.125	1.933	0.164
Temp., RH and ACH	0.166	1.729	0.186

(c) $PM_{2.5}$ (μg m^{-3}).

	r^2	F	P-value
Temperature	**0.205**	**7.242**	**0.012**
Relative Humidity	0.007	0.207	0.653
Air change per hour	**0.235**	**8.600**	**0.007**
Temperature and RH	**0.206**	**3.506**	**0.044**
Temp., RH and ACH	**0.340**	**4.472**	**0.012**

season upon bacterial and fungal levels in indoor environments. A significant impact of season was observed upon bacterial and fungal levels in the kitchens (p = 0.035 and p = 0.045 respectively) while in the living rooms, the effect upon bacterial and fungal levels was not pronounced (p = 0.53 and p = 0.60 respectively).

This unusual outcome can be possibly due to the limitations of gravitational sampling. Gravitational sampling is not considered suitable for quantitative studies and can only give an approximation of the micro-flora present in the air. Although exact quantitative analysis is not possible with passive sampling, data collected by the sedimentation method allow the drawing of correct conclusions on types of micro-organisms present thus giving an approximation of microbial levels present in the surrounding air (Stryjakowska-Sekulska *et al.*, 2007). In Pakistan there are very few studies encompassing bioaerosol levels in residential settings. Studies carried out by Colbeck *et al.* (2008) and Nasir *et al.* (2012) observed concentrations in residential settings of Pakistan to be higher than those reported here, except maximum indoor bacterial concentration- those were comparable to our maximum bacterial levels recorded in living rooms. They also found that 55 to 93 % of the bacteria and fungi observed were respirable and could penetrate into the respiratory system.

So far there are no established standards set by the Pakistan Environmental Protection Agency for the permissible levels of microbes in the indoor environment. For reference, standards set by other countries were consulted. According to USA Occupational Safety and Health Administration, air is considered contaminated in the presence of 1000 viable colony-forming units in a cubic meter of air. The American Industrial Hygiene Association (AIHA) (2001) proposed the levels of fungal spores to not exceed 500 cfu m^{-3} in residential buildings. The Swedish and Singaporean standards set the limit for bacteria to be not more than 500 cfu m^{-3} and 300 cfu m^{-3} for fungi. Keeping in mind these limits, it is evident that the observed microbial levels in this study were elevated as was also observed by Colbeck *et al.* (2008) and Nasir *et al.* (2012). Although the respiratory health of the occupants was evaluated through direct questioning and no serious health issue except dust allergy was observed, monitoring of indoor air quality is necessary to assess the exposure risk of the occupants.

In the absence of any legislation in the country to monitor and control the levels of contaminants in residential settings and also lack of any baseline data, it is essential to conduct more studies regarding composition of air in indoor micro-environments. The current study is an attempt to record the levels of air borne bacteria and fungi in urban residential areas of Lahore and their association with fine particulate matter. However, the data available so far is insufficient and there is still need of more detailed analysis to identify the sources of these contaminants and to consider the related health risks posed by prolonged exposure to them so that suitable measures may be undertaken in the best interest of health of the general public.

CONCLUSIONS

The air quality of monitored indoor environments was found to be poor. The levels of airborne microbial fauna as well as $PM_{2.5}$ exhibited levels higher than the prescribed limits. The microbial composition of air consisted of common species of both bacteria and fungi which are also opportunistic pathogens and can affect the exposed populations. The lack of

any guidelines for maintenance and/or monitoring of indoor air quality in Pakistan requires much work to be carried out in this context as the general public as well as policy makers in Pakistan are ill-informed of the health risks faced by prolonged exposure to such elevated concentrations of indoor airborne pollutants.

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