Bioaerosols in residential micro-environments in low income countries: A case study from Pakistan

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Abstract

Our knowledge of the concentrations of bioaerosols in residential micro-environments in low income countries is scanty. The present investigation was conducted to assess the culturable concentration and size distribution of bacteria, gram negative bacteria and fungi in two rural and an urban site in Pakistan. The highest indoor culturable bacteria concentration was found at Rural Site II (14650 CFU/m^3) while the outdoor maximum occurred at the urban site (16416 CFU/m^3). With reference to fungi, both indoor and outdoor concentrations were considerably higher at Rural Site I than the other sites. The size distribution of culturable bacterial at all sites showed greater variability than that of culturable fungi. At all sites more than the half (55 – 93 %) the culturable bacterial and fungal counts were observed in the respirable fraction (< 4.7μm) and so had the potential to penetrate into lower respiratory system.

Capsule abstract

Bioaerosol concentrations up to 14,650 CFU/m^3 were measured in the indoor environment reflecting the proximity to cattle and poor sanitary conditions. These elevated levels pose a significant health risk.

Keywords: Bioaerosols; Pakistan; size distribution

Introduction

Bioaerosols are ubiquitous in the environment and include viruses, bacteria, fungi, pollen, plant or animal debris, as well as fragments and products of these organisms. They can range in size from~1 nm to ~100 µm (Grinshpun and Clark, 2005). They are often dispersed attached to other biological or non-biological particles, such as, soil, dust, skin flakes saliva or water droplets. In recent times, airborne microorganisms have received significant attention due to their potential health effects and threat of the bioterrorism. A number of studies have been conducted in a variety of environments to assess levels of bioaerosols and their by products (e.g. endotoxin and 1,3-β-d-glucan) (Dong and Yao, 2010). There is a growing concern that bioaerosols may be associated with ill health: allergenicity, toxicity and pathogenicity (Douwes et al., 2003). Today, we spend almost 90% of our time indoors in variety of enclosed micro-environments (Klepeis et al., 2001; Leech et al., 1996). Among the different micro-environments, the residential setting is of vital importance due to amount of time spent there, especially by children and the elderly. Children on a per-body-weight basis tend to inhale relatively more air than adults and elderly persons are more likely to have weak body defence systems. In addition, people with compromised immunity (e.g. pregnant women, post-operative patients) or with existing respiratory conditions, such as allergies and asthma are at increased risk of exposure to bioaerosols and their derivatives. Studies on time-activity patters in rural areas of developing countries indicate that women spend approximately 70% of their time indoors (Ezzati et al., 2000; Zuk et al., 2007). Recently the WHO (2009) published its first indoor air quality guidelines on dampness and mould. It concluded that there is sufficient epidemiological evidence to indicate that the inhabitants of both damp or mouldy houses and public buildings are at increased risk of respiratory symptoms, respiratory infections and exacerbation of asthma.

Bioaerosols indoors are mainly of outdoor origin (Burge, 1990; Levetin et al., 1995). They enter through a range of avenues: heating ventilation and air conditioning system, doors, windows, cracks in the walls, attached to people and objects and via the potable drinking water system. Once in the indoor environment, a range of abiotic factors (water, humidity, temperature, nutrients, oxygen, and light) determines their growth. Indoor temperature and humidity, age and size of buildings, use of wood stoves and fireplaces, absence of mechanical ventilation, and presence of pets and old wall-to-wall carpeting have shown a positive correlation with indoor

microbial levels (Dharmage et al., 1999; Lawton et al., 1998). Moreover, the reduced ventilation in newly constructed houses, due to a focus on energy conservation, may lead to build up of indoor bioaerosols and conversely the old housing stocks with high ventilation and infiltration rates may facilitate the ingress of outdoor bioaerosols. In terms of exposure routes, along with inhalation, the ingestion and dermal absorption of various derivatives of bioaerosols may have significant health effects.

In recent years a number of studies have been undertaken to investigate the level of bioaerosols in indoor residential settings in different geographical regions (Pastuszka et al., 2000; Hyvarinen et al., 2001; Gorny and Dutkiewicz, 2002; Green et al., 2003; Lee et al., 2006; Lee and Jo, 2006; Hass et al., 2007; Mentese et al., 2009; Nasir and Colbeck, 2010). The majority of these are from developed countries and the state of knowledge about the biological indoor air pollution in residential environments in low income countries remains relatively narrow and insufficient. Indoor dampness and mould is likely to be wide spread in low income countries with an increasing shortage of affordable houses (WHO, 2009). Due to the geographical, meteorological and socio-economic conditions it is expected that the exposure to bioaerosols in these regions would be different from that in developed countries. In addition, geography, climate and meteorology affect the construction materials and housing types, which also have been noted to influence bioaerosol composition and concentrations (Codina et al., 2008). Different housing types can have different ventilation performance depending on construction material, design and use of housing space which in turn is largely influenced by political, social, environmental and economic factors. Therefore, it is reasonable to assume that concentrations of bioaerosols, both indoors and outdoors may vary according to location. Given the fact that housing conditions can have a considerable impact on bioaerosol exposure there is a need to investigate the concentrations in different residential micro-environments across the globe.

Bioaerosols and Pakistan

Pakistan is the world's sixth most populous country with an estimated population of 173 million in 2010 (Pakistan Economic Survey 2009 -10). Owing to the population explosion the country is facing severe housing issues. The Pakistan Housing Policy (2001) reported that there were 19.3 million housing units in the country and that the present housing stock is rapidly deteriorating.

The housing conditions are overcrowded and average household size is 7.2 persons with 31% of households with only one sleeping room (Sheraz and Zahir, 2008). Although no data on dampness in the housing stock are available it is likely that a vast proportion of households would be suffering due to an abundance of factors favourable to dampness, especially in urban slums. Scattered studies on ambient aeromycological concentrations have been reported from different parts of the country and most of these focused on species composition and used a settling plate exposure method. (Ahmed et al., 1960; Bajwa et al., 1995a & b; Bajwa et al., 1997; Shah, 1995; Farooq et al., 2001; Afzal and Mehdi, 2002; Afzal et al., 2004; Shabbir et al., 2009; Rao et al., 2009; Shah and Bashir, 2008). In addition a few studies have been carried out in the indoor environment (e.g. Zoological Museum (Shabbir et al., 2007), slaughter house (Adeeb and Shooter, 2003), hospitals (Shah et al., 1995; Nasim et al., 1998). To best of our knowledge, apart from a conference paper presented by Colbeck et al. (2008) no studies have been published on indoor bioaerosol concentrations in Pakistan. The present study was carried out to investigate the levels of bioaerosols in rural and urban residential settings of Pakistan. The results will provide an insight into the bioaerosol concentrations in Pakistan and will contribute to our knowledge about bioaerosol in developing countries.

Materials and Methods

The air was sampled at a total of 42 houses comprising two rural sites (20 and 10 houses, respectively) and an urban site (12 houses) during August –October, 2007. The Rural Site I (Village 35/ 2L) was located in District Okara of Punjab province. The site is located south-west of Lahore (Capital of Punjab) and the sampling village (35/2L) was 15 km away from the main urban area of Okara. Rural Site II was a town (Bhaun) 12 km from the Chakwal (Punjab province). Chakwal is 90 km south-east of Islamabad. The urban site was Lahore: the second largest city of Pakistan. Figure 1 shows the locations of the sampling sites within Pakistan.

The houses were of mixed ages and construction materials. At Rural Site I the roofs of the houses were made of a combination of wood and bricks or wood with straw; the brick walls either plastered with cement or mud. In addition, cattle sheds were present either within the courtyard of the house or in close proximity of houses. Manure piles were present at different

sites within the residential areas. At Rural Site II most of houses were roofed with brick and wood or brick with iron; the walls were plastered with cement. Limited livestock was present within the residential area and relatively few houses had cattle sheds within the houses. Streets were bricked with open sanitary lines, often filled with household waste and water. At the urban site the houses consisted of concrete roof and cement walls, except for one house that was roofed with wood and bricks. All the houses were ventilated naturally and the bathrooms were not close to the living rooms. Information on humidity, temperature, water damage, visible mould growth, number of occupants, construction material and presence of livestock was recorded.

The sampling was carried out with an Andersen 6 stage viable impactor (Graseby-Andersen, Atlanta, USA). The samples were taken from living rooms and outdoors. The Andersen six stage viable particle sampler is a multi-orifice cascade impactor, which collects and aerodynamically sizes all the particles regardless of their physical size, shape or density and can be related to human lung deposition. The sampler operates at a flow rate of 28.3 l/min with suction provided by a calibrated vacuum pump. The sampled air enters the inlet cone and cascades through the succeeding orifice stages with successively higher orifice velocities from stage 1 to stage 6. The particles were inertially impacted, according to their size, onto agar plates. The aerodynamic sizes of particles collected on each stage are: stage 1 (7μm & above), stage 2 (4.7μm - 7μm), stage 3 (3.3μm - 4.7μm), stage 4 (2.1μm - 3.3μm), stage 5 (1.1μm - 2.1μm) and stage 6 (0.65μm - 1.1μm). The six stage Andersen viable impactor has been widely used for the investigation of indoor and outdoor bioaerosols over many years due to its high collection efficiency and ability to preserve culturability during sampling (Reponen et al. 1994; Pastuszka et al. 2000; Hyvarinen et al. 2001; Meklin et al. 2002; Kim and Kim 2007). The impactor is designed so that all particles collected, regardless of physical size, shape, or density, are aerodynamically sized and can be directly related to human lung deposition.

The impactor was loaded with six Petri dishes containing Malt Extract Agar (Oxoid, UK), Tryptone Soy Agar (Oxoid, UK), or MacConkey Agar (Oxoid, UK), prior to sampling. The Tryptone Soy Agar was used for the total bacterial counts while, cultivation and enumeration of gram negative bacteria was carried out on the MacConkey agar. One sample was taken at each location and sampling was always carried out around noon at each location at the height of 1 metre. The sampling duration was 2 minutes and after collection the agar plates were incubated

at 25ºC for 48 hours in the case of bacteria and up to 7 days for fungi. The agar plates were incubated at 25ºC to recover the maximum colony forming units.

Relative humidity and temperature in different settings was recorded with a Gasprobe IAQ 4 (BW Technologies Ltd, Canada) with a logging interval of 1 minute. The measurements were carried out at each sampling house, for a minimum of half an hour in both living rooms and outdoors, in conjunction with bioaerosol sampling. The mean temperature and relative humidity was calculated for each site for both indoors and outdoors.

The number of colonies from each plate was enumerated and the total numbers of culturable colony forming units per cubic meter (CFU/m^3) were calculated for each stage and total culturable counts for all the stages made. The data was analyzed in terms of Rural Site I, II and urban site (indoors and outdoors). The normality of the distribution of the concentrations in different settings was checked by the Shapiro–Wilk test. The distributions were lognormal and geometric means and geometric standard deviation were calculated for each size and total concentration for all sites. Furthermore, the geometric mean diameter of each sample and average geometric mean diameter for each setting were determined. The Mann-Whitney U test was used to test the difference between indoor and outdoor concentrations of total bacteria, gram negative bacteria and fungi at all the sites. In addition, the Kruskal-Wallis test was used to test the difference among all these sites and post hoc comparisons were carried out with the Mann-Whitney U tests with Boneferroni adjustments.

Results and discussion

Total concentration of culturable bacterial and fungal aerosol

The mean indoor temperature at Rural Site I, II and the urban site was 20°C (n = 20, ± 4), 26°C (n = 10, ± 3), and 28°C (n = 12, ± 2) as compared to outdoor averages of 23°C (n = 20, ± 3), 29°C (n = 10, ± 1), and 31°C (n = 12, ± 1), respectively. The mean indoor relative humidity at these sites were 67% (n = 20, ± 10), 28% (n = 12, ± 6) and 51% (n = 10, ± 7) in contrast to outdoor means of 61% (n = 20, ± 9), 19% (n = 12, ± 2) and 48% (n = 10, ± 16), respectively. Both indoor and outdoor relative humidity levels at Rural Site II were much lower than at the

other sites. This reflects the differences in geographical location of the sites. The temperature at each site was relatively uniform while a large variation was observed in relative humidity among different houses at each site. The relative humidity at all the sites was higher indoors than outdoors. As the living rooms were away from bathrooms and kitchens, it is very likely that indoor humidity was influenced by indoor sources of moisture generation (e.g. presence of people, release from building fabrics) and differences in micro-environmental conditions between indoors and outdoors.

Table 1 summarises the geometric mean concentrations of indoor culturable bacteria, gram negative bacteria and fungi at all the sites. For Rural Site I the levels of bacteria and fungi were slightly higher outdoors than indoors. At Rural Site II the concentrations of both bacterial and fungal aerosols were considerably higher outdoors than indoors except for the total bacteria, which was marginally higher indoors. Apart from fungi, the outdoor levels of bacterial aerosol at the urban site were higher in comparison to those indoors (Table 1).

Generally, the concentrations of both bacterial and fungal aerosols were higher outdoors than indoors at both rural and urban sites (except for the slightly higher indoor fungal concentration at the urban site and total bacteria at Rural Site II). In addition, there was wide variation indoors, at rural sites and outdoors at the urban site, as depicted by the higher geometric standard deviation (Table1). The variation amongst the houses at the same location might be due the role of microclimate, number of people, construction material, ventilation behaviour, daily household activities and outdoor levels. Comparison between indoor and outdoor concentrations of total bacteria, gram negative bacteria and fungi at all sites showed that a statistically significant difference was present for total bacteria at Rural Site I ($Z = -1.652$; $P<0.10$), the urban site ($Z = -1.667$; $P<0.10$) and for fungi at Rural Site II ($Z = -1.964$; $P<0.05$). The higher outdoor concentrations in these settings highlight the diversity of biological emission sources and the complex processes affecting indoor /outdoor relationships of airborne microorganisms. The role of ventilation behaviour cannot be ignored as Rural Site II and the urban site had close plan construction with a well defined indoors and outdoors in comparison with Rural Site I.

There was considerable difference in fungal concentration among the different sites with Rural Site I being highest. Here extensive agricultural activities, irrigated by canal systems, results in favourable environmental conditions for fungal infestation. Agricultural activities and livestock breeding have been associated with high microbial concentrations (Lis et al., 2008; Karwowska, 2005). The decomposition of raw organic materials in cattle sheds is enhanced by wet and humid conditions and results in high concentrations of airborne fungal spores (Adhikari et al., 2004a). With the exception of total and gram negative bacteria outdoors at the urban site, the concentration of bacterial and fungal aerosol was generally lower at the urban site compared with Rural Site I. Higher concentrations in rural rather than urban environments has been documented in previous studies (Pasanen, 1992; Lis et al., 2008). Both studies suggested that the levels in farm houses resulted from the transfer of fungal spores from barns. At Rural Site I the cattle sheds were either within the house or in close proximity, so it is very likely that bioaerosols were transported from these to the indoor living spaces.

Due to the absence of interpretive numerical guidelines for bioaerosols, comparison of indoor to outdoor bioaerosol concentration is commonly used to determine whether an indoor environment is normal or if there is an indoor source. However, the present study showed that outdoor bioaerosol sources can be a cause of high indoor concentrations, especially in rural communities. Hence, the comparative indoor/outdoor bioaerosol concentration is of limited usefulness in rural communities.

Another striking feature was the elevated concentration of gram negative bacteria outdoors at Rural Site II. Although there was very limited farming and animal breeding, the sanitary conditions were very poor with household sewage/waste standing in uncovered lines in streets. These conditions could be a possible reason for the observed concentrations of gram negative bacteria. The levels of bioaerosols in the present investigation are higher than those reported from other studies. Adhikari et al. (2004a) carried out an investigation on airborne fungi in two sections of rural cattle sheds for 2 consecutive years in West Bungal, India and found that the average monthly concentration of viable colony-forming units ranged between 165 and 2225 CFU/m^3. The highest mean monthly concentration was more than 3 times lower than in the present study. This might be due to differences in the sampling environment as their study was

carried out in cattle shed with ventilation, drainage and sanitary systems in operation. Similarly, airborne viable and non-viable fungi were assessed in five outdoor sites, for two years, in a rural agricultural area of India by Adhikari et al. (2004b). The concentration of viable fungi during the first and second year ranged from 72–1796 CFU/m^3 and 155–1256 CFU/m^3, respectively. The outdoor fungal concentration in our study was more than four times higher than Adhikari et al. (2004b). This again may be due to differences in sampling locations (e.g. agricultural intensity, sampling distance from bioaerosol sources)

In terms of difference in bioaerosol concentrations among all the sites, the results of the Kruskal-Wallis test depicted that a statistically significant difference was only present in the concentration of fungi (χ^2 = 20.609; $P<0.05$). In order to carry out post hoc comparisons, a Mann-Whitney U test was used and this showed that the indoor fungal concentrations at rural I differed significantly ($P<0.05$)from both Rural Site II and the urban site. Similarly there was statistically significant difference ($P<0.05$) between Rural Site I and the urban site for fungi outdoors.

The season has been reported to influence the concentration of bioaerosols (Shelton et al., 2002; Ren et al., 1999) with fungal levels highest in fall and summer. According to several studies, the moisture content of building materials, relative humidity and temperature (Pasanen et al., 2000; Ritchkoff et al., 2000), outdoor concentrations, air exchange rates (Kulmala et al., 1999), human activities (Buttner and Stetzenbach, 1993) and number of people and pets (ACGIH 1999) significantly affect the levels of indoor bioaerosols. In addition, housing conditions, the activities and life style of occupants can contribute to the varying concentrations. These factors fluctuate to a great degree between various housing types and geographic location. The present investigation was carried out during the summer and indoor spaces were well ventilated. Furthermore, Rural Site I was an extensive agricultural region with almost every household having some livestock normally close to residential areas.

Size distribution of bacteria and fungi

The maximum number of indoor culturable total bacteria, gram negative bacteria and fungi at Rural Site I were isolated from stage 3 (3.3-4.7µm) (Figure 2), whereas the size distribution

outdoors was completely different with the highest number present in the size range 7µm and above (Stage 1), 0.65-1.1µm (stage 6) and 3.3-4.7µm (stage 3), respectively (Figure 3). A shift in the size distribution of bacterial aerosol outdoors highlights the differences in the indoor/outdoor environment. Outdoor spaces at the rural sites either had a large number of livestock or open sewage lines in the streets.

At Rural Site II, stage 4 (2.1-3.3µm) was dominant for indoor total culturable bacteria and gram negative bacteria, while the highest number of indoor fungi was in the size range of 7µm and above (Figure 4). Outdoors the maximum number of total bacteria, gram negative bacteria and fungi was isolated from stage 1 (7µm & above), 2 (4.7-7µm) and 5 (1.1-2.1µm), respectively. (Figure 5) The size distribution at Rural Site II was considerably different between indoors and outdoors, particularly for fungi and gram negative bacteria. It is of note that 93% of indoor gram negative was in the respirable fraction (< 4.7µm) while outdoors it was 55%. This shows indoor and outdoor bioaerosol assemblages are different and likely to have diverse sources.

The size distribution of culturable indoor bacterial, gram negative bacteria and fungi at the urban site was dominated by stages 3 (3.3-4.7µm), 1 (7µm & above) and 4 (2.1-3.3µm), respectively (Figure 6). Outdoors, the maximum number of CFUs were present in the size range 2.1-3.3µm (stage 4) for both gram negative bacteria and fungi and 7µm and above (Stage 1) for total bacteria (Figure 7). The size distribution of fungi indoors and outdoors is comparable, suggesting no indoor sources for fungi at the urban site. However, there may be indoor sources for bacteria due to the resultant differences in the indoor and outdoor size distributions.

The culturable bacterial and fungal aerosol had different size distributions at both rural and urban sites. However, the size distribution of fungal aerosol was less variable, except at Rural Site II. In terms of indoors and outdoors concentrations Rural Site II showed highest variability. Additionally, outdoors, the peak concentration of total bacteria was observed on stage 1((>7 µm) for all sites. The concentration and size distributions, not only vary with geographical location, but also depend on a wide range of biotic and abiotic factors. The observed differences in the size distribution of bioaerosols among the sites could be due to differences in the local micro-climate and housing conditions. The micro-organism species (Reponen et al., 1996), age of the

spore and nutrient medium (Ellis, 1981), relative humidity of surrounding air (Pasanen et al., 1991), differences in aggregation rates of the spores (Gorny et al., 1999), type of particles they are associated with such as mist or dust (Dowd and Maier, 2000) and hygroscopic growth of bioaerosols (Liao et al., 2004) are among the factors that may affect the size distribution.

The average geometric mean diameter varied both indoors and outdoors among the different sites. For fungal spores it was similar to that reported by Reponen et al. (1994), Meklin et al. (2002) and Zuraimi et al. (2009). The results shown that more than the half of bacterial and fungal aerosols at all the sites were respirable (< 4.7μm) which highlights the higher exposure of inhabitants as these particles have the potential to deposit either in tracheal, bronchial or alveolar region of lungs. The observed differences in the size distribution of bacterial and fungal aerosol clearly indicate their importance in understanding the respiratory exposure of inhabitants and their fate and airborne behaviour Moreover, a significant proportion was recovered from stages 1 (>7 μm) and 2 (4.7–7 μm) but single cells are usually smaller than the observed size. It can be speculated that aggregation of bioaerosol cells or rafting (Moschandreas et al., 2003; Pastuszka et al., 2000) are possible mechanisms for the observed size distributions.

Relatively few publications have considered the respirable fraction of bioaerosols in residential settings. Li and Kuo (1993) found that, in Taiwanese houses, more than 80% of fungi were in the respirable fraction. Similarly, in American homes, around 55% of total bacteria and 80% of total fungi were respirable (DeKoster and Thorne, 1995). According to Pastuszka et al. (2000), 48% of total bacteria and 77% of total fungi were in the respirable fraction in non mouldy Polish homes. For farm houses and urban dwellings in Southern Poland, Lis et al. (2008) reported that 55% of bacteria and 77 % of fungi were respirable in the farms compared with 66% and 82% in urban houses. More recently, Nasir and Colbeck (2010) assessed the levels of bioaerosols in three different types of houses in South East England and found respirable fractions in the range 56 to 88% for bacteria and 56 to 81% for fungi.

Table 2 shows the levels of airborne bacteria and fungi in residential environments in different countries. It is not possible to make direct comparison among various studies due to differences in housing types, household conditions, climatic and geographical parameters. Most of these

studies have been carried out in the developed world and factors influencing the bioaerosols may not be same as in the present investigation. The levels of bacteria in this study are far greater than reported from different parts of the world. The overcrowding and poor living conditions might be responsible for high bacterial load as the average household size in Pakistan is almost 7. However the concentration of fungi is comparable to the reported summer concentration from Taiwan (Pei-Chih et al., 2000).

Due to the absence of established dose response relationships it is not possible to estimate the health risk associated with elevated bioaerosol concentrations in residential micro-environments. At present there are no established threshold limit values for bioaerosols in residential indoor settings. Some organizations have provided guidelines on the levels of indoor bioaerosols. The American Conference of Governmental Industrial Hygienists (ACGIH 1999) does not provide any numerical guideline to interpret the environmental measurements.

Conclusion

The present study was carried out to investigate the levels of airborne bacteria and fungi in rural and urban residential micro-environments in Pakistan. The results showed that concentrations at these sites were highly variable, especially for fungi. This is the first detailed study on levels of indoor bioaerosols in Pakistan and draws attention to the possible increased respiratory exposure of inhabitants to bioaerosols in both rural and urban areas. Farming activities and livestock rearing can be associated with the higher bioaerosol concentration in the rural areas. Nonetheless the levels at the urban sites, especially for bacteria, were not significantly lower than those for rural areas. The poor sanitation conditions might make a considerable contribution to elevated levels in both rural and urban areas. However, the size distribution profile of bacteria, indoors and outdoors, suggested the presence of indoor sources, especially at Rural site II and the urban site.

The present study highlights the limited usefulness of a standard approach to compare indoor/outdoor bioaerosol concentrations to determine if indoor microbial air quality is typical or atypical in rural communities. In addition, knowledge of the size distribution profile of bioaerosols at different locations is not only important with regard to their airborne behaviour

and deposition in the human respiratory system but can also improve our understanding of bioaerosol sources. It is of note that the present study employed culture based method. Due to the specific incubation temperature and medium used to culture the bioaerosols, it is very likely that it would not recover a large number of the viable but not culturable bioaerosols. Hence the exposure risk could be far greater than expected. Furthermore, this investigation was carried out in one province of Pakistan and the concentration in other geographical regions may well vary. There is a need for detailed studies from different regions of the country keeping in view the household conditions and socioeconomic differences.

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568

Table 1 .Geometric mean (GM), geometric standard deviation (GSD), range, average geometric mean diameter (dg_{ave}) and % < 4.7μm of total culturable bacteria, gram negative bacteria and fungal aerosol at urban and rural sites in Pakistan.

	Bacteria		*Gram negative Bacteria*		*Fungi*	
	Indoor	*Outdoor*	*Indoor*	*Outdoor*	*Indoor*	*Outdoor*
Rural Site I (n = 20)						
Total GM (CFU/m^3)	11616[a]	15790[a]	2498	2625	7576	8031
Range (CFU/m^3)	5318-16607	11819 - 29169	318-13710	671 - 7261	1607-32756	3657 – 12526
GSD	1.56	1.37	2.51	2.63	2.17	1.51
dg_{ave} (μm)	3.14	3.62	2.58	2.43	2.80	2.33
% < 4.7μm	67	60	76	83	75	81
Rural Site II (n = 10)						
Total GM (CFU/m^3)	14650	13638	1053	5608	2123[b]	5909[b]
Range (CFU/m^3)	6873 – 24876	12897 - 14611	300 - 5512	5141 - 6325	1042 - 3445	5477 – 6272
GSD	1.95	1.06	4.46	1.11	1.87	1.07
dg_{ave} (μm)	2.96	3.51	2.32	2.99	3.54	2.94
% < 4.7μm	71	60	93	55	64	76
Urban site (n = 12)						
Total GM (CFU/m^3)	9408[c]	16416[c]	1693	2721	3137	2788
Range (CFU/m^3)	6113 – 13922	13003 - 20724	442 - 4717	1431 - 5176	1590 - 5300	1201 – 5494
GSD	1.42	1.39	2.35	2.48	1.53	2.16
dg_{ave} (μm)	3.73	2.73	2.35	2.34	3.02	2.61
% < 4.7μm	61	68	76	88	79	80

n = (Number of houses sampled)

% < 4.7μm = Respirable fraction of culturable bacteria and fungi

a,b,c. The means with the same superscript were significantly different at 0.05 (a) and 0.10 (b,c) level of significance

579 Table 2. Levels of airborne bacteria and fungi in residential environments in different countries
580

581

Reference	Country	Location	Concentration (CFU/m^3)		Comments
			Bacteria	**Fungi**	
Present study	Pakistan	Rural Site I (Living room) Rural Site II (Living room) Urban Site (Living room)	11616 14650 9408	7576 2123 3137	Geometric mean, Single stage Andersen impactor
Nasir and Colbeck, (2010)	UK	Housing Type I Housing Type II Housing Type III	1557 2403 5036	925 813 2124	Geometric mean, Six stage Andersen impactor
Lis et al., (2008)	Poland	Living rooms Farm houses Living rooms Urban	 3235 1792	 838 375	Mean, Six stage Andersen impactor
Hass et al.,(2007)	Austria	Apartments – Visible mould Apartments- without mould		260 (MEA) 350 (DG18) 1500 (MEA) 1700 (DG18)	Median, One-stage MAS-100 air sampler.
Lee et al., (2006)	USA	Child activity room		88	Geometric mean, Button Personal Inhalable Aerosol Sampler
Lee and Jo, (2006)	Korea	Low-rise apartment (Winter) High-rise apartment (Winter) Low-rise apartment (Summer) High-rise apartment (Summer)	 280 288 331 319	 93 112 456 476	Geometric mean, Single-stage Andersen Samplers
Schleibinger et al., (2005)	Germany	Apartments mouldy Apartments non mouldy		3200 180	2-stage Andersen cascade impactors
Hargreaves et al., (2003)	Australia	Living room normal ventilation Bed room normal ventilation Living room Min. Ventilation Bathroom Min. ventilation		 810 692 453 499	Average, Reuter centrifugal air sampler
Green et al., (2003)	USA	Room central to house	369	369	Mean, Andersen two-stage viable microbial particle sizing sampler
Pastuszka et al., (2000)	Poland	Living room- healthy Living room- mouldy	1021 980	225(Summer) 59 (Winter) 834(Summer) 256(Winter)	Geometric mean, Andersen 6-stage impactor
Pei-Chih et al., (2000)	Taiwan	Urban homes Winter		 9099	Geometric mean, Burkard sampler

		Summer Suburban homes Winter Summer		3608 8333 7302	
Ren et al., (1999)	USA	Winter Living room Bed room Basement Spring Living room Bed room Basement Summer Living room Bed room Basement Fall Living room Bed room Basement		 431.8 313.6 1657.6 834.1 790.9 1165 1036.4 970.5 987.5 7.6.8 704.5 1242.1	Mean, Burkard portable air sampler
Rosas et al., (1997)	Mexico City	Homes Dry season Wet season		 460 141	Geometric mean, Andersen 2 stage impactor
Garrett et al., (1997)	Australia	Bedroom, living room and kitchen		812	Median, Single stage Andersen impactor
Strachan et al., (1990)	UK	Living room Childs bedroom Kitchen		0 – 41,000	Range, Single-stage Andersen Samplers

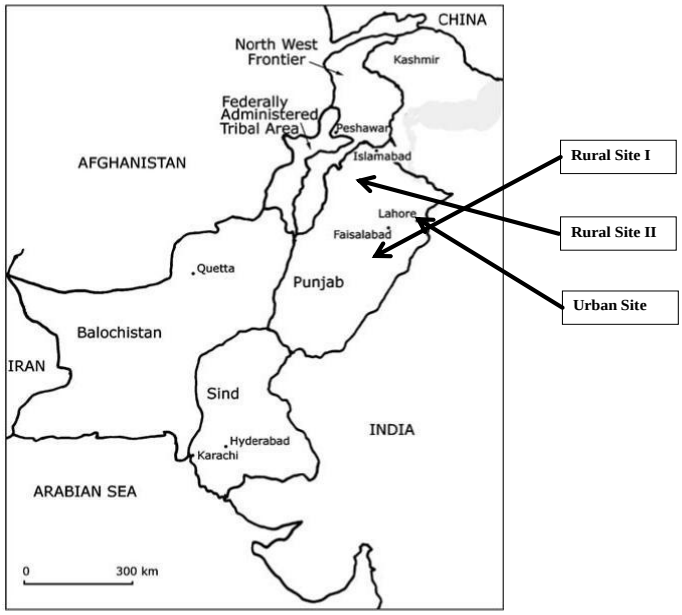
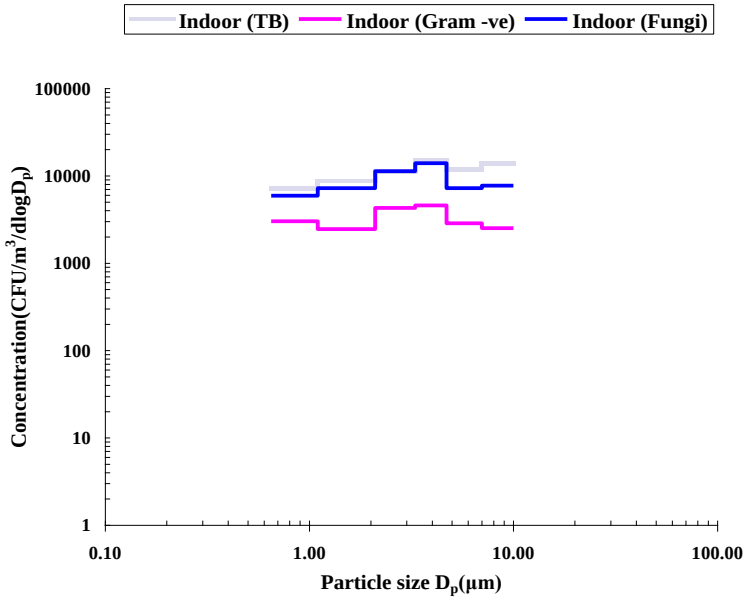
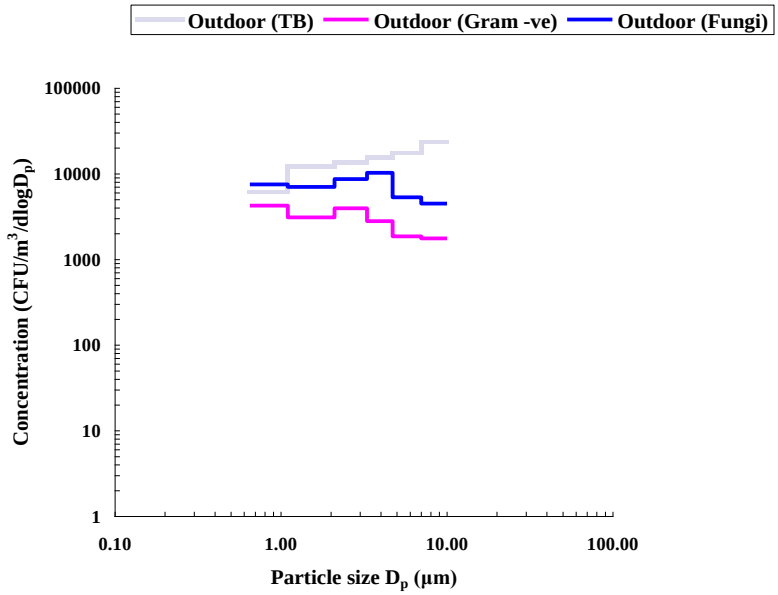


Figure 1. Map of Pakistan showing the sampling sites.

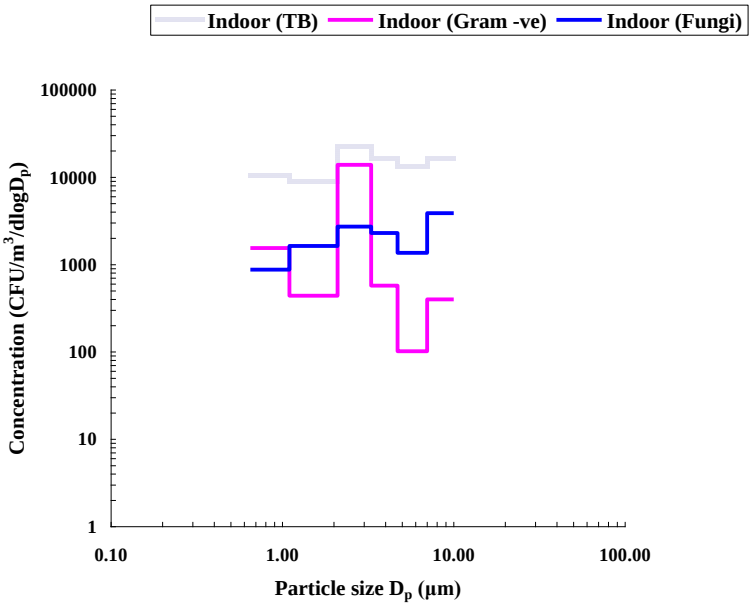


588

589 Figure 2. Size distribution of culturable indoor total bacterial (TB), gram negative bacteria
590 (Gram -ve) and fungi at Rural Site I.

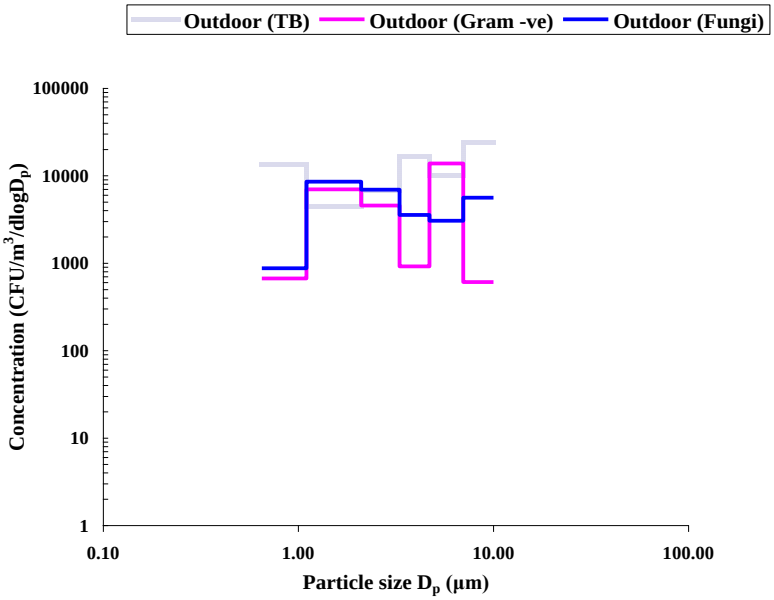


591
592 Figure 3. Size distribution of culturable outdoor total bacterial (TB), gram negative bacteria
593 (Gram -ve) and fungi at Rural Site I
594



595

596 Figure 4. Size distribution of culturable indoor total bacterial (TB), gram negative bacteria
597 (Gram -ve) and fungi at Rural Site II.



598
599 Figure 5. Size distribution of culturable outdoor total bacterial (TB), gram negative bacteria
600 (Gram -ve) and fungi at Rural Site II
601

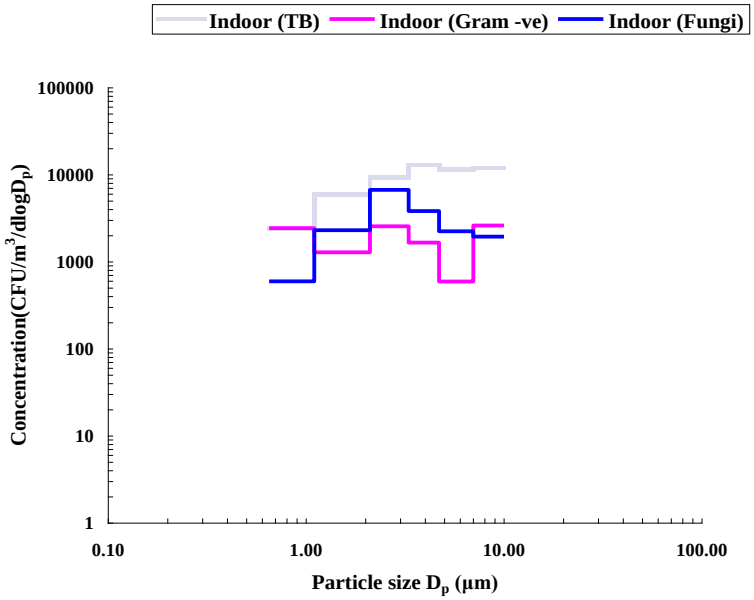


Figure 6. Size distribution of culturable indoor total bacterial (TB), gram negative bacteria (Gram -ve) and fungi at urban site

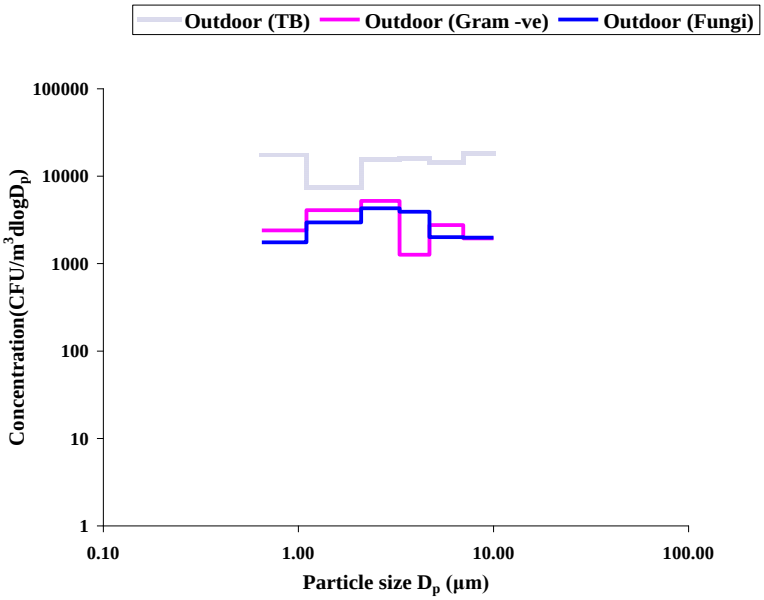


Figure 7. Size distribution of culturable outdoor total bacterial (TB), gram negative bacteria (Gram -ve) and fungi at urban site