

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

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13
14 **Abstract**

15 Our knowledge of the concentrations of bioaerosols in residential micro-environments in low
16 income countries is scanty. The present investigation was conducted to assess the culturable
17 concentration and size distribution of bacteria, gram negative bacteria and fungi in two rural and
18 an urban site in Pakistan. The highest indoor culturable bacteria concentration was found at
19 Rural Site II (14650 CFU/m³) while the outdoor maximum occurred at the urban site (16416
20 CFU/m³). With reference to fungi, both indoor and outdoor concentrations were considerably
21 higher at Rural Site I than the other sites. The size distribution of culturable bacterial at all sites
22 showed greater variability than that of culturable fungi. At all sites more than the half (55 – 93
23 %) the culturable bacterial and fungal counts were observed in the respirable fraction (< 4.7µm)
24 and so had the potential to penetrate into lower respiratory system.

25
26 Capsule abstract

27 *Bioaerosol concentrations up to 14,650 CFU/m³ were measured in the indoor environment*
28 *reflecting the proximity to cattle and poor sanitary conditions. These elevated levels pose a*
29 *significant health risk.*

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33 **Keywords:** Bioaerosols; Pakistan; size distribution

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36 **Introduction**

37 Bioaerosols are ubiquitous in the environment and include viruses, bacteria, fungi, pollen, plant
38 or animal debris, as well as fragments and products of these organisms. They can range in size
39 from ~1 nm to ~100 µm (Grinshpun and Clark, 2005). They are often dispersed attached to other
40 biological or non-biological particles, such as, soil, dust, skin flakes saliva or water droplets. In
41 recent times, airborne microorganisms have received significant attention due to their potential
42 health effects and threat of the bioterrorism. A number of studies have been conducted in a
43 variety of environments to assess levels of bioaerosols and their by products (e.g. endotoxin and
44 1,3-β-d-glucan) (Dong and Yao, 2010). There is a growing concern that bioaerosols may be
45 associated with ill health: allergenicity, toxicity and pathogenicity (Douwes et al., 2003). Today,
46 we spend almost 90% of our time indoors in variety of enclosed micro-environments (Klepeis et
47 al., 2001; Leech et al., 1996). Among the different micro-environments, the residential setting is
48 of vital importance due to amount of time spent there, especially by children and the elderly.
49 Children on a per-body-weight basis tend to inhale relatively more air than adults and elderly
50 persons are more likely to have weak body defence systems. In addition, people with
51 compromised immunity (e.g. pregnant women, post-operative patients) or with existing
52 respiratory conditions, such as allergies and asthma are at increased risk of exposure to
53 bioaerosols and their derivatives. Studies on time-activity patters in rural areas of developing
54 countries indicate that women spend approximately 70% of their time indoors (Ezzati et al.,
55 2000; Zuk et al., 2007). Recently the WHO (2009) published its first indoor air quality guidelines
56 on dampness and mould. It concluded that there is sufficient epidemiological evidence to
57 indicate that the inhabitants of both damp or mouldy houses and public buildings are at increased
58 risk of respiratory symptoms, respiratory infections and exacerbation of asthma.

59

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

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

73
74 In recent years a number of studies have been undertaken to investigate the level of bioaerosols
75 in indoor residential settings in different geographical regions (Pastuszka et al., 2000; Hyvarinen
76 et al., 2001; Gorny and Dutkiewicz, 2002; Green et al., 2003; Lee et al., 2006; Lee and Jo, 2006;
77 Hass et al., 2007; Mentese et al., 2009; Nasir and Colbeck, 2010). The majority of these are from
78 developed countries and the state of knowledge about the biological indoor air pollution in
79 residential environments in low income countries remains relatively narrow and insufficient.
80 Indoor dampness and mould is likely to be wide spread in low income countries with an
81 increasing shortage of affordable houses (WHO, 2009). Due to the geographical, meteorological
82 and socio-economic conditions it is expected that the exposure to bioaerosols in these regions
83 would be different from that in developed countries. In addition, geography, climate and
84 meteorology affect the construction materials and housing types, which also have been noted to
85 influence bioaerosol composition and concentrations (Codina et al., 2008). Different housing
86 types can have different ventilation performance depending on construction material, design and
87 use of housing space which in turn is largely influenced by political, social, environmental and
88 economic factors. Therefore, it is reasonable to assume that concentrations of bioaerosols, both
89 indoors and outdoors may vary according to location. Given the fact that housing conditions can
90 have a considerable impact on bioaerosol exposure there is a need to investigate the
91 concentrations in different residential micro-environments across the globe.

92 **Bioaerosols and Pakistan**

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

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

113

114 **Materials and Methods**

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

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

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

134 The sampling was carried out with an Andersen 6 stage viable impactor (Graseby-Andersen,
135 Atlanta, USA). The samples were taken from living rooms and outdoors. The Andersen six stage
136 viable particle sampler is a multi-orifice cascade impactor, which collects and aerodynamically
137 sizes all the particles regardless of their physical size, shape or density and can be related to
138 human lung deposition. The sampler operates at a flow rate of 28.3 l/min with suction provided
139 by a calibrated vacuum pump. The sampled air enters the inlet cone and cascades through the
140 succeeding orifice stages with successively higher orifice velocities from stage 1 to stage 6. The
141 particles were inertially impacted, according to their size, onto agar plates. The aerodynamic
142 sizes of particles collected on each stage are: stage 1 ($7\mu\text{m}$ & above), stage 2 ($4.7\mu\text{m}$ - $7\mu\text{m}$),
143 stage 3 ($3.3\mu\text{m}$ - $4.7\mu\text{m}$), stage 4 ($2.1\mu\text{m}$ - $3.3\mu\text{m}$), stage 5 ($1.1\mu\text{m}$ - $2.1\mu\text{m}$) and stage 6 ($0.65\mu\text{m}$
144 - $1.1\mu\text{m}$). The six stage Andersen viable impactor has been widely used for the investigation of
145 indoor and outdoor bioaerosols over many years due to its high collection efficiency and ability
146 to preserve culturability during sampling (Reponen et al. 1994; Pastuszka et al. 2000; Hyvarinen
147 et al. 2001; Meklin et al. 2002; Kim and Kim 2007). The impactor is designed so that all
148 particles collected, regardless of physical size, shape, or density, are aerodynamically sized and
149 can be directly related to human lung deposition.

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

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

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

163 The number of colonies from each plate was enumerated and the total numbers of culturable
164 colony forming units per cubic meter (CFU/m³) were calculated for each stage and total
165 culturable counts for all the stages made. The data was analyzed in terms of Rural Site I, II and
166 urban site (indoors and outdoors). The normality of the distribution of the concentrations in
167 different settings was checked by the Shapiro–Wilk test. The distributions were lognormal and
168 geometric means and geometric standard deviation were calculated for each size and total
169 concentration for all sites. Furthermore, the geometric mean diameter of each sample and
170 average geometric mean diameter for each setting were determined. The Mann-Whitney U test
171 was used to test the difference between indoor and outdoor concentrations of total bacteria, gram
172 negative bacteria and fungi at all the sites. In addition, the Kruskal-Wallis test was used to test
173 the difference among all these sites and post hoc comparisons were carried out with the Mann-
174 Whitney U tests with Bonferroni adjustments.

175

176 **Results and discussion**

177 **Total concentration of culturable bacterial and fungal aerosol**

178 The mean indoor temperature at Rural Site I, II and the urban site was 20°C (n = 20, ± 4), 26°C
179 (n = 10, ± 3), and 28°C (n = 12, ± 2) as compared to outdoor averages of 23°C (n = 20, ± 3),
180 29°C (n = 10, ± 1), and 31°C (n = 12, ± 1), respectively. The mean indoor relative humidity at
181 these sites were 67% (n = 20, ± 10), 28% (n = 12, ± 6) and 51% (n = 10, ± 7) in contrast to
182 outdoor means of 61% (n = 20, ± 9), 19% (n = 12, ± 2) and 48% (n = 10, ± 16), respectively.
183 Both indoor and outdoor relative humidity levels at Rural Site II were much lower than at the

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

191
192 Table 1 summarises the geometric mean concentrations of indoor culturable bacteria, gram
193 negative bacteria and fungi at all the sites. For Rural Site I the levels of bacteria and fungi were
194 slightly higher outdoors than indoors. At Rural Site II the concentrations of both bacterial and
195 fungal aerosols were considerably higher outdoors than indoors except for the total bacteria,
196 which was marginally higher indoors. Apart from fungi, the outdoor levels of bacterial aerosol at
197 the urban site were higher in comparison to those indoors (Table 1).

198
199 Generally, the concentrations of both bacterial and fungal aerosols were higher outdoors than
200 indoors at both rural and urban sites (except for the slightly higher indoor fungal concentration at
201 the urban site and total bacteria at Rural Site II). In addition, there was wide variation indoors, at
202 rural sites and outdoors at the urban site, as depicted by the higher geometric standard deviation
203 (Table1). The variation amongst the houses at the same location might be due the role of
204 microclimate, number of people, construction material, ventilation behaviour, daily household
205 activities and outdoor levels. Comparison between indoor and outdoor concentrations of total
206 bacteria, gram negative bacteria and fungi at all sites showed that a statistically significant
207 difference was present for total bacteria at Rural Site I ($Z = -1.652$; $P < 0.10$), the urban site ($Z = -$
208 1.667 ; $P < 0.10$) and for fungi at Rural Site II ($Z = -1.964$; $P < 0.05$). The higher outdoor
209 concentrations in these settings highlight the diversity of biological emission sources and the
210 complex processes affecting indoor /outdoor relationships of airborne microorganisms. The role
211 of ventilation behaviour cannot be ignored as Rural Site II and the urban site had close plan
212 construction with a well defined indoors and outdoors in comparison with Rural Site I.

213

214 There was considerable difference in fungal concentration among the different sites with Rural
215 Site I being highest. Here extensive agricultural activities, irrigated by canal systems, results in
216 favourable environmental conditions for fungal infestation. Agricultural activities and livestock
217 breeding have been associated with high microbial concentrations (Lis et al., 2008; Karwowska,
218 2005). The decomposition of raw organic materials in cattle sheds is enhanced by wet and humid
219 conditions and results in high concentrations of airborne fungal spores (Adhikari et al., 2004a).
220 With the exception of total and gram negative bacteria outdoors at the urban site, the
221 concentration of bacterial and fungal aerosol was generally lower at the urban site compared with
222 Rural Site I. Higher concentrations in rural rather than urban environments has been documented
223 in previous studies (Pasanen, 1992; Lis et al., 2008). Both studies suggested that the levels in
224 farm houses resulted from the transfer of fungal spores from barns. At Rural Site I the cattle
225 sheds were either within the house or in close proximity, so it is very likely that bioaerosols were
226 transported from these to the indoor living spaces.

227
228 Due to the absence of interpretive numerical guidelines for bioaerosols, comparison of indoor to
229 outdoor bioaerosol concentration is commonly used to determine whether an indoor environment
230 is normal or if there is an indoor source. However, the present study showed that outdoor
231 bioaerosol sources can be a cause of high indoor concentrations, especially in rural communities.
232 Hence, the comparative indoor/outdoor bioaerosol concentration is of limited usefulness in rural
233 communities.

234
235 Another striking feature was the elevated concentration of gram negative bacteria outdoors at
236 Rural Site II. Although there was very limited farming and animal breeding, the sanitary
237 conditions were very poor with household sewage/waste standing in uncovered lines in streets.
238 These conditions could be a possible reason for the observed concentrations of gram negative
239 bacteria. The levels of bioaerosols in the present investigation are higher than those reported
240 from other studies. Adhikari et al. (2004a) carried out an investigation on airborne fungi in two
241 sections of rural cattle sheds for 2 consecutive years in West Bungal, India and found that the
242 average monthly concentration of viable colony-forming units ranged between 165 and 2225
243 CFU/m³. The highest mean monthly concentration was more than 3 times lower than in the
244 present study. This might be due to differences in the sampling environment as their study was

245 carried out in cattle shed with ventilation, drainage and sanitary systems in operation. Similarly,
246 airborne viable and non-viable fungi were assessed in five outdoor sites, for two years, in a rural
247 agricultural area of India by Adhikari et al. (2004b). The concentration of viable fungi during the
248 first and second year ranged from 72–1796 CFU/m³ and 155–1256 CFU/m³, respectively. The
249 outdoor fungal concentration in our study was more than four times higher than Adhikari et al.
250 (2004b). This again may be due to differences in sampling locations (e.g. agricultural intensity,
251 sampling distance from bioaerosol sources)

252
253 In terms of difference in bioaerosol concentrations among all the sites, the results of the Kruskal-
254 Wallis test depicted that a statistically significant difference was only present in the
255 concentration of fungi ($\chi^2 = 20.609$; $P < 0.05$). In order to carry out post hoc comparisons, a
256 Mann-Whitney U test was used and this showed that the indoor fungal concentrations at rural I
257 differed significantly ($P < 0.05$) from both Rural Site II and the urban site. Similarly there was
258 statistically significant difference ($P < 0.05$) between Rural Site I and the urban site for fungi
259 outdoors.

260
261 The season has been reported to influence the concentration of bioaerosols (Shelton et al., 2002;
262 Ren et al., 1999) with fungal levels highest in fall and summer. According to several studies, the
263 moisture content of building materials, relative humidity and temperature (Pasanen et al., 2000;
264 Ritchkoff et al., 2000), outdoor concentrations, air exchange rates (Kulmala et al., 1999), human
265 activities (Buttner and Stetzenbach, 1993) and number of people and pets (ACGIH 1999)
266 significantly affect the levels of indoor bioaerosols. In addition, housing conditions, the activities
267 and life style of occupants can contribute to the varying concentrations. These factors fluctuate to
268 a great degree between various housing types and geographic location. The present investigation
269 was carried out during the summer and indoor spaces were well ventilated. Furthermore, Rural
270 Site I was an extensive agricultural region with almost every household having some livestock
271 normally close to residential areas.

272

273 **Size distribution of bacteria and fungi**

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

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

281
282 At Rural Site II, stage 4 (2.1-3.3 μ m) was dominant for indoor total culturable bacteria and gram
283 negative bacteria, while the highest number of indoor fungi was in the size range of 7 μ m and
284 above (Figure 4). Outdoors the maximum number of total bacteria, gram negative bacteria and
285 fungi was isolated from stage 1 (7 μ m & above), 2 (4.7-7 μ m) and 5 (1.1-2.1 μ m), respectively.
286 (Figure 5) The size distribution at Rural Site II was considerably different between indoors and
287 outdoors, particularly for fungi and gram negative bacteria. It is of note that 93% of indoor gram
288 negative was in the respirable fraction (< 4.7 μ m) while outdoors it was 55%. This shows indoor
289 and outdoor bioaerosol assemblages are different and likely to have diverse sources.

290
291 The size distribution of culturable indoor bacterial, gram negative bacteria and fungi at the urban
292 site was dominated by stages 3 (3.3-4.7 μ m), 1 (7 μ m & above) and 4 (2.1-3.3 μ m), respectively
293 (Figure 6). Outdoors, the maximum number of CFUs were present in the size range 2.1-3.3 μ m
294 (stage 4) for both gram negative bacteria and fungi and 7 μ m and above (Stage 1) for total
295 bacteria (Figure 7). The size distribution of fungi indoors and outdoors is comparable, suggesting
296 no indoor sources for fungi at the urban site. However, there may be indoor sources for bacteria
297 due to the resultant differences in the indoor and outdoor size distributions.

298
299 The culturable bacterial and fungal aerosol had different size distributions at both rural and urban
300 sites. However, the size distribution of fungal aerosol was less variable, except at Rural Site II. In
301 terms of indoors and outdoors concentrations Rural Site II showed highest variability.
302 Additionally, outdoors, the peak concentration of total bacteria was observed on stage 1 (>7 μ m)
303 for all sites. The concentration and size distributions, not only vary with geographical location,
304 but also depend on a wide range of biotic and abiotic factors. The observed differences in the
305 size distribution of bioaerosols among the sites could be due to differences in the local micro-
306 climate and housing conditions. The micro-organism species (Reponen et al., 1996), age of the

307 spore and nutrient medium (Ellis, 1981), relative humidity of surrounding air (Pasanen et al.,
308 1991), differences in aggregation rates of the spores (Gorny et al., 1999), type of particles they
309 are associated with such as mist or dust (Dowd and Maier, 2000) and hygroscopic growth of
310 bioaerosols (Liao et al., 2004) are among the factors that may affect the size distribution.

311

312 The average geometric mean diameter varied both indoors and outdoors among the different
313 sites. For fungal spores it was similar to that reported by Reponen et al. (1994), Meklin et al.
314 (2002) and Zuraimi et al. (2009). The results shown that more than the half of bacterial and
315 fungal aerosols at all the sites were respirable ($< 4.7\mu\text{m}$) which highlights the higher exposure of
316 inhabitants as these particles have the potential to deposit either in tracheal, bronchial or alveolar
317 region of lungs. The observed differences in the size distribution of bacterial and fungal aerosol
318 clearly indicate their importance in understanding the respiratory exposure of inhabitants and
319 their fate and airborne behaviour. Moreover, a significant proportion was recovered from stages
320 1 ($>7\mu\text{m}$) and 2 ($4.7\text{--}7\mu\text{m}$) but single cells are usually smaller than the observed size. It can be
321 speculated that aggregation of bioaerosol cells or rafting (Moschandreas et al., 2003; Pastuszka
322 et al., 2000) are possible mechanisms for the observed size distributions.

323

324 Relatively few publications have considered the respirable fraction of bioaerosols in residential
325 settings. Li and Kuo (1993) found that, in Taiwanese houses, more than 80% of fungi were in the
326 respirable fraction. Similarly, in American homes, around 55% of total bacteria and 80% of total
327 fungi were respirable (DeKoster and Thorne, 1995). According to Pastuszka et al. (2000), 48%
328 of total bacteria and 77% of total fungi were in the respirable fraction in non mouldy Polish
329 homes. For farm houses and urban dwellings in Southern Poland, Lis et al. (2008) reported that
330 55% of bacteria and 77 % of fungi were respirable in the farms compared with 66% and 82% in
331 urban houses. More recently, Nasir and Colbeck (2010) assessed the levels of bioaerosols in
332 three different types of houses in South East England and found respirable fractions in the range
333 56 to 88% for bacteria and 56 to 81% for fungi.

334

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

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

344

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

351

352 **Conclusion**

353

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

365

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

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

378

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380

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568

569 Table 1 .Geometric mean (GM), geometric standard deviation (GSD), range, average geometric
 570 mean diameter (dg_{ave}) and % < 4.7 μ m of total culturable bacteria, gram negative bacteria and
 571 fungal aerosol at urban and rural sites in Pakistan.
 572

	<i>Bacteria</i>		<i>Gram negative Bacteria</i>		<i>Fungi</i>	
	<i>Indoor</i>	<i>Outdoor</i>	<i>Indoor</i>	<i>Outdoor</i>	<i>Indoor</i>	<i>Outdoor</i>
<i>Rural Site I (n = 20)</i>						
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} (μ m)	3.14	3.62	2.58	2.43	2.80	2.33
% < 4.7 μ m	67	60	76	83	75	81
<i>Rural Site II (n = 10)</i>						
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} (μ m)	2.96	3.51	2.32	2.99	3.54	2.94
% < 4.7 μ m	71	60	93	55	64	76
<i>Urban site (n = 12)</i>						
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} (μ m)	3.73	2.73	2.35	2.34	3.02	2.61
% < 4.7 μ m	61	68	76	88	79	80

573 n = (Number of houses sampled)

574 % < 4.7 μ m = Respirable fraction of culturable bacteria and fungi

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

576 level of significance

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578

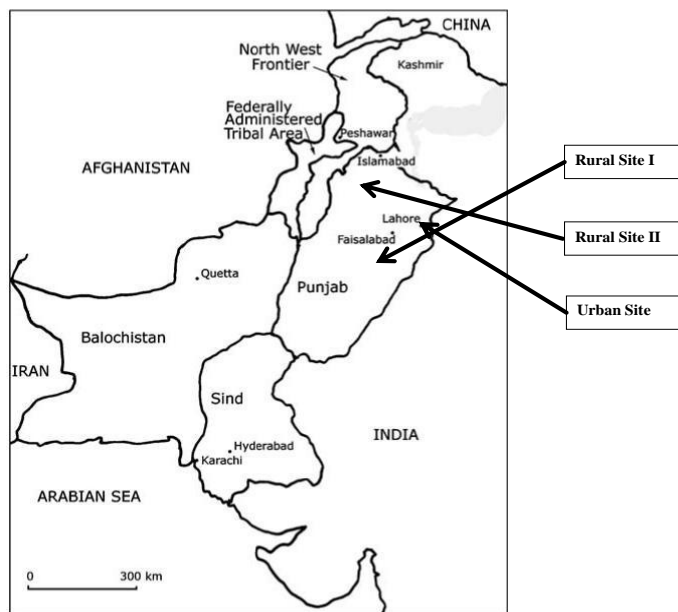
579 Table 2. Levels of airborne bacteria and fungi in residential environments in different countries
 580

581

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

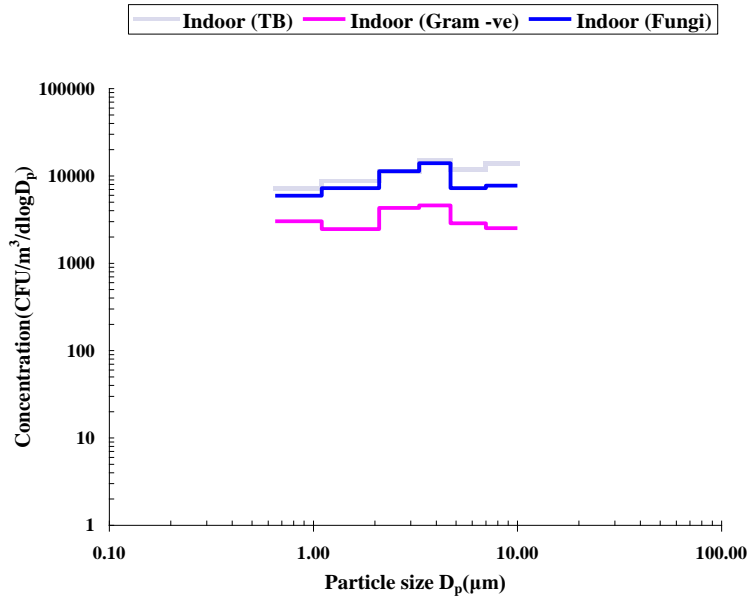
		Summer Suburban homes Winter Summer		3608 8333 7302	
Ren et al., (1999)	USA	Winter Living room Bed room Basement Spring Living room Bed room Basement Summer Living room Bed room Basement Fall Living room Bed room Basement		431.8 313.6 1657.6 834.1 790.9 1165 1036.4 970.5 987.5 7.6.8 704.5 1242.1	Mean, 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

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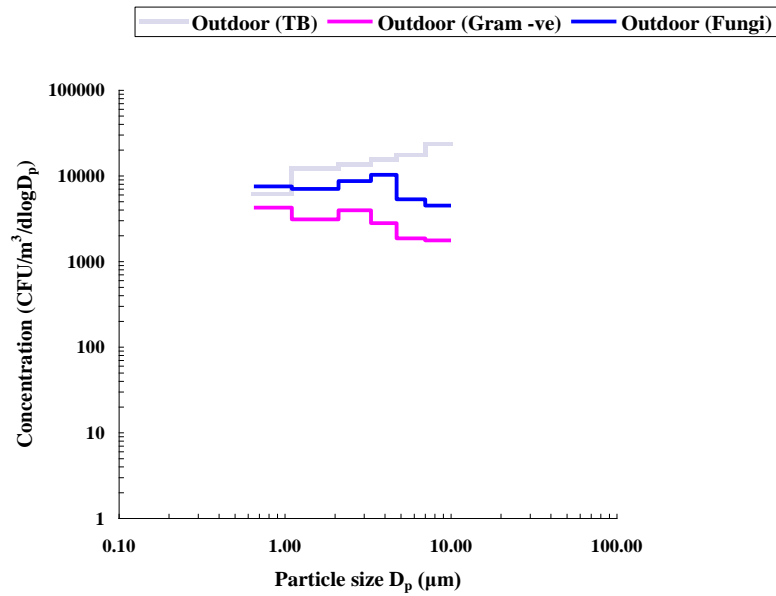
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Figure 1. Map of Pakistan showing the sampling sites.



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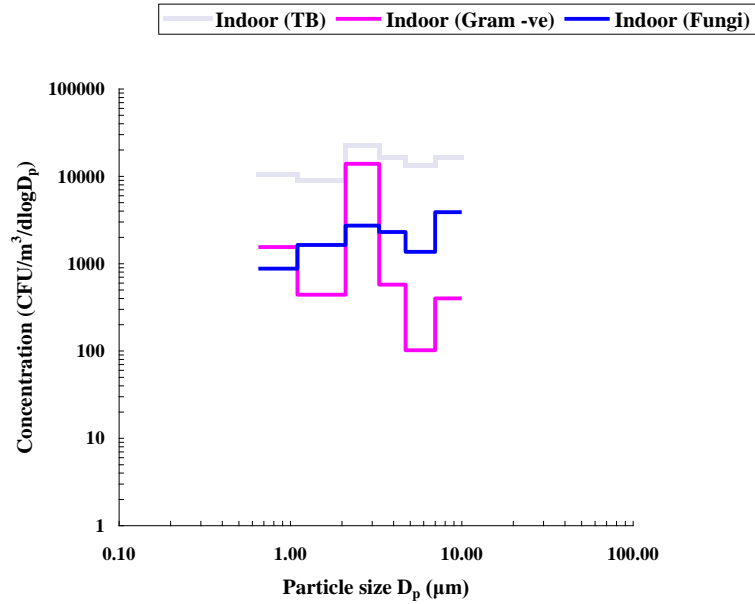
589 Figure 2. Size distribution of culturable indoor total bacterial (TB), gram negative bacteria
 590 (Gram -ve) and fungi at Rural Site I.



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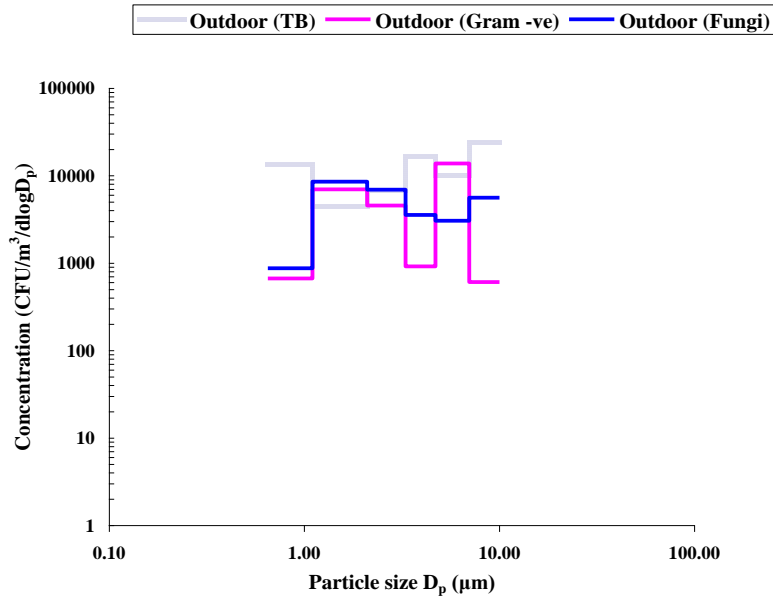
592 Figure 3. Size distribution of culturable outdoor total bacterial (TB), gram negative bacteria
 593 (Gram -ve) and fungi at Rural Site I

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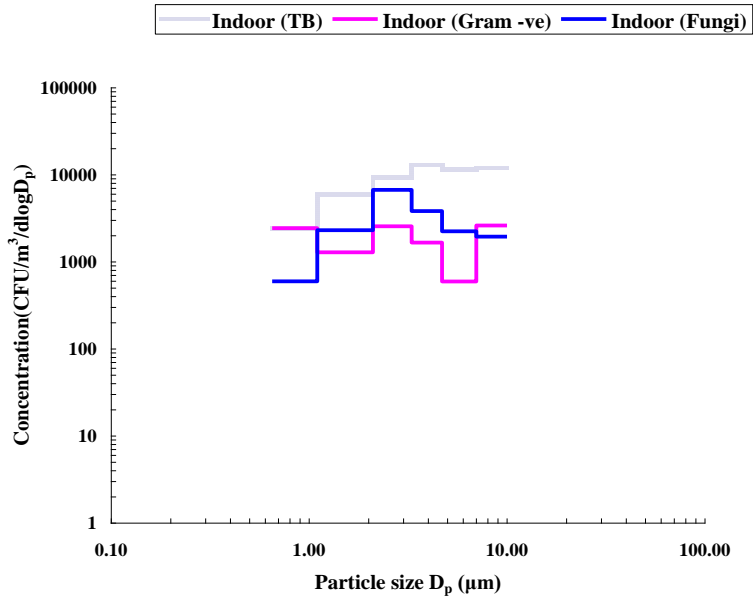
596 Figure 4. Size distribution of culturable indoor total bacterial (TB), gram negative bacteria
 597 (Gram -ve) and fungi at Rural Site II.



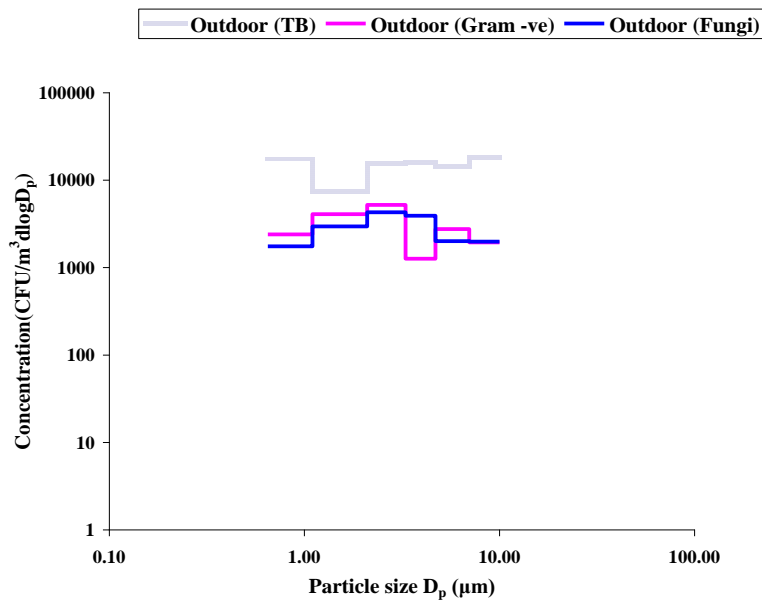
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599 Figure 5. Size distribution of culturable outdoor total bacterial (TB), gram negative bacteria
 600 (Gram -ve) and fungi at Rural Site II

601



602
 603 Figure 6. Size distribution of culturable indoor total bacterial (TB), gram negative bacteria
 604 (Gram -ve) and fungi at urban site



605
 606 Figure 7. Size distribution of culturable outdoor total bacterial (TB), gram negative bacteria
 607 (Gram -ve) and fungi at urban site
 608