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1 **Impact of Middle Eastern Dust Storms on Indoor and Outdoor Composition of Bioaerosol**

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21

22 **Abstract**

23

24 The presence of microbes in airborne aerosol particles, especially dust, is a major public  
25 health concern in desert regions. This study is the first of its kind to examine the effect of dust  
26 storms on indoor and outdoor microbial air quality at a hospital on the western side of Iran (city  
27 of Ahvaz), which is notorious for being highly vulnerable to dust emissions. Air samples were  
28 collected inside and outside of the hospital environment for six months, with the unique  
29 advantage of this study being that the region and duration of measurements allow for a clear  
30 comparison between dusty and normal days. On normal days, the average concentrations  
31 (outdoor/indoor) of bacteria and fungi were 423/329 cfu m<sup>-3</sup> and 596/386 cfu m<sup>-3</sup>, respectively,  
32 which increased to 1257/406 cfu m<sup>-3</sup> and 1116/550 cfu m<sup>-3</sup> on dust event days. Indoor/Outdoor  
33 ratios for bacteria and fungi are lower on dust event days (0.26-0.60) versus normal days (0.44-  
34 0.95). *Bacillus spp.*, *Micrococcus spp.*, *Streptomyces spp.*, and *Staphylococcus spp.* were the  
35 dominant bacteria both indoors and outdoors on normal and dust event days. Gram positive  
36 bacteria exhibited higher concentrations than Gram negative bacteria in both outdoor and indoor  
37 air samples as well as during both normal and dust event days. The data suggest that Gram  
38 positive bacteria are more resistant to undesirable outdoor conditions (e.g., high incident solar  
39 radiation) as compared to Gram negative ones. These results have implications for other  
40 populated arid regions where more stringent control of indoor air quality can greatly benefit  
41 public health.

42

43 Key words: Iran; Aerosol; Bacteria; Dust; Indoor Air Quality; Bioaerosol

44

## 45 1. Introduction

46 Bioaerosols are an important class of airborne aerosols that can significantly impact  
47 public health via pathways such as inhalation and ingestion (e.g., Nasir et al., 2012; Faridi et al.,  
48 2015). The impact of these naturally emitted particles extends to climate, cloud formation, and  
49 the hydrologic cycle owing to their ability to interact with solar radiation and activate into  
50 hydrometeors (e.g., Mohler et al., 2007; Ekstrom et al., 2010). Perhaps the two most important  
51 types of bioaerosols are bacteria and fungal spores (e.g., Pastuszka et al., 2000; Mentese et al.,  
52 2012), which typically range in diameter between 1-30  $\mu\text{m}$  and 0.25-8  $\mu\text{m}$ , respectively  
53 (Gregory, 1973; Thompson, 1981; Jones and Harrison, 2004). Bioaerosols promote infectious  
54 diseases, allergy, asthma, and neurological diseases (Chan et al., 2009; Cordeiro et al., 2010).  
55 Soil-dwelling fungi can irritate lungs and some reports indicate a relationship between fungi and  
56 headaches (Sautour et al., 2007). Mycotoxins generated by fungi can negatively impact human  
57 health as they can lead to digestive and respiratory problems (Schlesinger et al., 2006; Sautour et  
58 al., 2007, 2009; Cordeiro et al., 2010). Bioaerosols are emitted to the air through different natural  
59 and man-made sources such as soil, plants, animals, humans, sewage treatment and agricultural  
60 activities (e.g., Hameed et al., 2009); however, the relative importance and characteristics of  
61 bioaerosol emissions from these various sources is uncertain.

62 The impact of a given bioaerosol particle will depend to a large extent on where it  
63 deposits upon inhalation. The deposition region depends on particle size and composition, which  
64 are linked via the property of hygroscopicity (e.g., Sorooshian et al., 2012). Larger aerosol ( $D_p >$   
65 5  $\mu\text{m}$ ) typically impact in the upper parts of the human respiratory system leading potentially to  
66 allergic reactions (e.g., Faridi et al., 2015), while smaller aerosol, especially in the sub-

67 micrometer fraction, can penetrate deeper reaching alveolar regions leading to other adverse  
68 impacts.

69 A better understanding of the composition and concentration of bioaerosols is needed in  
70 different parts of the world to develop strategies to minimize their impact on human health,  
71 especially indoors where humans spend the majority of their time. There are a different set of  
72 factors affecting bioaerosol composition and concentration for indoor versus outdoor  
73 environments. For outdoor air, important variables include geographic location, meteorology  
74 (e.g., temperature, humidity), density and activity of people, seasonally dependent factors, and  
75 plant cover, while for indoor air, important factors include resuspension of dust, indoor pollution  
76 sources (e.g., cooking, restrooms, people), plants, temperature, humidity, ventilation, and air  
77 conditioning (Hargreaves et al., 2003; Fang et al., 2007; Kim et al., 2009; Mehta et al., 2011;  
78 Nasir et al., 2012; Ruzer and Harley, 2012). The relative importance of how each of these factors  
79 influences the concentration and chemical signature of bioaerosols is poorly characterized.

80 Indoor air quality is especially critical in hospital buildings where inhalation of  
81 particulate matter can impact the health of its users, crews, and patients who may have  
82 vulnerable immune systems (Bouza et al., 2002). Hospital environments include an extensive  
83 variety of hazardous materials, such as mixtures of chemical and biological contaminants (e.g.,  
84 Saad, 2003). Although indoor bioaerosols mostly originate from the ambient atmosphere, they  
85 also stem from indoor activities such as with Heating, Ventilation, and Air Conditioning  
86 (HVAC) systems (Pastuszka et al., 2000).

87 A major contributor to reduced indoor air quality in arid and semi-arid regions is dust.  
88 Seasonal dust storm activities in the deserts of Asia are most prevalent between February and  
89 May (Griffin, 2007). Dust storms in Iran often originate from penetration of high pressure

90 systems in southern Iraq and northern Saudi Arabia, and are further promoted by drought in these  
91 regions. Other factors promoting dust storms include destruction of the groves of Abadan and  
92 Khorramshahr in Iran and also the Basra province in Iraq during the Iran-Iraq war (Martiny et al.,  
93 2006). Pathogenic and nonpathogenic species in dust-borne microorganisms can be widely  
94 distributed via aerosol transport pathways that can potentially change over time (Martiny et al.,  
95 2006; Sorooshian et al., 2011; Soleimani et al., 2013; Prabhakar et al., 2014). A classic example  
96 is how dust transports the soil-dwelling fungus *Coccidioides Immitis*, which leads to valley fever  
97 (Maddy, 1965) in some parts of the world such as the southwestern portions of North America.

98         Microorganisms in outdoor and indoor air have been studied during dust storms and clear  
99 days in numerous regions (Bouza et al., 2002; Griffin et al., 2006; Schlesinger et al., 2006; Chan  
100 et al., 2009), but very few studies have characterized the amount of biological material contained  
101 in dust that influences the arid region of western Iran, specifically the vulnerable city of Ahvaz,  
102 Iran (Goudarzi et al., 2013; Rad et al., 2014). The aim of this study is to, for the first time, survey  
103 and compare the concentration and composition of bioaerosols in the indoor and outdoor air of a  
104 hospital in Ahvaz, Iran during dusty and normal days. The results have implications for other  
105 populated cities in semi-arid and arid regions that cover over a third of the global land area, in  
106 addition to providing a valuable set of bioaerosol characterization data to be contrasted with  
107 other various regions of the world.

108

## 109 **2. Experimental Methods**

### 110 **2.1. Hospital**

111 Measurements were conducted in Golestan Hospital located on the campus of the Ahvaz  
112 Jundishapur University of Medical Sciences (see map in Figure 1). This hospital was constructed  
113 in 1971 and contains 24 wards and 500 beds. The two areas of focus in the hospital include the  
114 Intensive Care Unit (ICU) and selected internal wards, with details for each (e.g., floor area;  
115 number of rooms, patients, beds, crew) shown in Table S1 (Supplement). The internal wards  
116 cover more than twice the floor area of the ICU (~1200 m<sup>2</sup> versus 540 m<sup>2</sup>) with more total rooms  
117 (17) and numbers of beds (40). On a representative day in the middle of the sampling period,  
118 more patients were observed in the internal wards (~34 versus ~15); however, the ICU had more  
119 crew personnel (~25 versus ~22). Patients admitted to this hospital are mainly from Ahvaz and  
120 other cities of the Khuzestan province, which has a population of more than 3.2 million people  
121 and significantly impacted by dust owing to its location. Past work has shown that dust storms  
122 impacting Ahvaz typically originate from the west/northwest/southwest including northern  
123 Africa, Iraq, and the Arabian Peninsula (Soleimani et al., 2015).

## 124 **2.2. Sampling**

125 Air sampling was carried out between September 2010 and March 2011. This sampling  
126 period is divided into two seasons: autumn (September – November) and winter (January –  
127 March). Sampling days are further categorized as either normal days or dust event days, with the  
128 latter based on whether a given sample satisfied any of the conditions for dusty days shown in  
129 Table 1 following the criteria defined by Hoffmann et al. (2008). The ICU and selected internal  
130 wards were chosen for analysis due to their high density of patients, their significance,  
131 hospitalization time period, and higher rates of referrals of people to these wards during dusty  
132 days. Since an objective of the study is to compare the concentration and genus of bioaerosols

133 between indoor and outdoor environments, air samples were collected outdoor of the hospital  
134 building at a distance of 4 m from an entry door. Ventilation in hospital rooms is conducted  
135 naturally by opening the windows and doors but the windows and doors were closed during  
136 sampling.

137 The strategy used for collecting samples was to obtain one sample every six days for  
138 normal days, and one each day on dusty days. Sampling was conducted twice a day at each of the  
139 three targeted spots in and out of the hospital at the following times: morning from 09:00-12:00  
140 (Local Time) and afternoon from 16:00-17:00. Indoor and outdoor air samples were collected  
141 using a single-stage Anderson sampler with 400 holes (0.25-mm diameter) to draw air over a  
142 petri dish containing culture. The air sampling flow rate was  $28.3 \text{ L min}^{-1}$  and sampling time was  
143 two and five minutes for dusty days and five minutes for normal days. The different sampling  
144 frequency on dusty days is due to the fact that excessive colony growth complicated  
145 measurements when sampling for up to five minutes. Air samples were collected at a height of  
146 ~2 m above the ground and a distance of at least 1 m from walls and other obstacles (Sautour et  
147 al., 2009).

148

### 149 **2.3. Characterization of Biomaterials**

150 Trypticase Soy Agar (TSA) and Potato Dextrose Agar (PDA) were used to cultivate  
151 bacteria and fungi, respectively. Cycloheximide and chloramphenicol were added to TSA and  
152 PDA, respectively (Kim et al., 2009). TSA and PDA culture plates were prepared as per the  
153 manufacturer's instruction and kept in a  $37^{\circ}\text{C}$  incubator for 24 hr. After sampling, fungal cultures  
154 were kept at room temperature for 72-96 hrs and bacterial cultures were incubated at  $33\text{-}37^{\circ}\text{C}$  for  
155 48-72 hrs (Kim et al., 2009). The concentration of airborne bacteria and fungi were expressed as



156 the number of colony forming units per cubic meter of air ( $\text{cfu m}^{-3}$ ) by considering the number of  
157 colonies counted on the air sample plates, air flow rate and sampling time, and applying the  
158 positive hole correction method of Anderson (1958). Genera identification of cultured airborne  
159 bacteria was conducted by examination of gram-stained smears and standard biochemical tests  
160 according to Bergey's Manual (Kim et al., 2009). Spore staining and acid-fast staining were used  
161 to identify *Bacillus* and *Nocardia* genera, respectively. Enterobacteriaceae were identified using  
162 an Analytical Profile Index (API) kit (Fang et al., 2007).

163

#### 164 **2.4. Statistical Analyses**

165 The one sample Kolmogorov-Smirnov test is utilized here to examine the normality of  
166 the data. Analysis of variance (ANOVA) is used to compare the results obtained for different  
167 places on dusty days. The nonparametric equivalence of ANOVA (i.e., Kruskal Wallis test) is  
168 employed for the comparison of bioaerosols in different places on normal days. The Mann-  
169 Whitney U test and t-test are employed in this study to determine significant differences of  
170 bioaerosol concentration between normal and dusty days, and also between seasons. The  
171 Spearman's rank correlation coefficient (i.e., a nonparametric measure) is used to quantify the  
172 correlation between indoor and outdoor airborne bioaerosol levels during normal and dust event  
173 days.

174

#### 175 **3. Results**

176 The daily mean  $\text{PM}_{10}$  mass concentration on dust event days in autumn (September -  
177 November) and winter (January - March) seasons ranged from 500-1541  $\mu\text{g m}^{-3}$  and 1121-2734

178  $\mu\text{g m}^{-3}$ , respectively. As compared to autumn, higher levels between January and March are  
179 partly attributed to a combination of shallower mixing heights (in the earlier part of the winter),  
180 and higher wind speeds and more likely transport from upwind desert regions in the latter part of  
181 the winter (e.g., Crosbie et al., 2014). Table 2 shows the average concentration of total and  
182 dominant bacteria including *Bacillus spp.*, *Micrococcus spp.*, *Staphylococcus spp.*, and  
183 *Streptomyces spp.* on normal and dusty days. These isolated genera from indoor and outdoor air  
184 can cause opportunistic infections and sometimes death (ACGIH 1989). Discussion of data in the  
185 two categories of days (normal and dusty) follows below.

186

### 187 **3.1. Normal Days**

188 Total bacterial concentrations in the outdoor environment were significantly higher than  
189 those in the ICU and internal wards during both seasons ( $p < 0.05$ ) (Table 2). Bacterial  
190 concentrations were higher in the internal wards as compared to the ICU. Generally, bacterial  
191 concentrations in autumn were higher than winter in all sampling areas. There was a significant  
192 difference between bacterial concentrations in outdoor air in autumn (average =  $458.32 \text{ cfu m}^{-3}$ )  
193 as compared to the winter (average =  $392.28 \text{ cfu m}^{-3}$ ) ( $p < 0.05$ ). However, a significant  
194 difference was not observed in indoor air samples when the results of autumn and winter were  
195 compared ( $p > 0.05$ ). A significant difference was observed between *Staphylococcus spp.* and  
196 *Streptomyces spp.* concentrations in autumn and winter ( $p = 0.042$  and  $p = 0.03$ , respectively).  
197 There was also a significant difference between *Streptomyces spp.* concentration in both seasons  
198 in indoor air ( $p < 0.05$ ). Panagopoulou et al. (2002) reported summer and autumn as the seasons  
199 with the highest concentration of airborne fungi in their studied hospitals in Greece. Similar

200 results have been reported for airborne bacteria and fungi in Beijing outdoor air and a hospital in  
201 France (Kim et al., 2009; Fang et al., 2007).

202 Table 3 shows the frequency of the identified bacteria on normal and dust event days in  
203 all three sampling locations. For normal days, frequencies of detection for dominant bacteria  
204 (*Bacillus spp.*, *Micrococcus spp.*, *Streptomyces spp.*, *Staphylococcus spp.*) in indoor and outdoor  
205 air were 50.8-93.1% and 82.8-100%, respectively. The frequency that other bacteria in Table 3  
206 were detected in indoor and outdoor air was 0-52.5% and 3.4-71.2%, respectively.

207 *Staphylococcus spp.* and *Bacillus spp.* were the most frequent dominant bacteria in indoor air.  
208 *Bacillus spp.* and *Streptomyces spp.* were the most frequent ones in outdoor air. Our results are  
209 consistent with those of Fang et al. (2007) and Augustowska and Dutkiewicz (2006), who  
210 identified *Bacillus*, *Staphylococcus*, and *Micrococcus* as dominant bacteria in China and Poland,  
211 respectively. In addition to those bacteria, our study shows that *Streptomyces spp.* also was  
212 among the dominant bacteria.

213 The concentration of fungi in indoor and outdoor environments was higher than the  
214 concentration of bacteria on normal days, with the difference being largest outdoors (596.62 cfu  
215  $\text{m}^{-3}$  versus 423.02 cfu  $\text{m}^{-3}$ ) (Table 4). In contrast, the opposite result was obtained in Egypt by  
216 Hameed et al. (2009). One of the reasons for higher concentrations of fungi in outdoor air can be  
217 the existence of plants and trees on the hospital campus, in addition to other unpaved areas and  
218 the exterior surface of the aged hospital that is over 40 years old. Windows of sampled indoor  
219 wards were opened and exposed to the outdoor campus environment with a 4 m distance from  
220 trees and plants, and thus a higher concentration of total fungi compared to total bacteria in the  
221 indoor air could be expected due to transport.

222 The average amount of bacteria (328 cfu m<sup>-3</sup>) and fungi (386 cfu m<sup>-3</sup>) in the two indoor  
223 hospital areas on normal days exceeded values reported at other sites. For example, estimated  
224 airborne fungi levels ranged from 2 to 26 cfu m<sup>-3</sup> in a French hospital (Fang et al., 2007) and  
225 levels of airborne bacteria of 100 cfu m<sup>-3</sup> were reported in a Polish hospital ward (ACGIH 1989).  
226 Our measured concentrations of fungi in outdoor samples (up to 2420 cfu m<sup>-3</sup>) are higher than  
227 those obtained by Sautour et al. (2009) for France (7-23 cfu m<sup>-3</sup>). In this study, the  
228 indoor/outdoor (I/O) ratios for bacteria exceed those for fungi when considering internal wards  
229 (0.95 versus 0.86) and the ICU (0.60 versus 0.44).

230

### 231 3.2. Dust Event Days

232 In contrast to normal days, total bacterial concentrations in all three locations were higher  
233 in winter as compared to autumn during dust event days (Table 2). A significant difference ( $p <$   
234 0.05) was observed between total bacterial concentration outdoors in autumn versus winter with  
235 averages of 812.1 cfu m<sup>-3</sup> and 1850.5 cfu m<sup>-3</sup>, respectively. But a significant difference was not  
236 observed in indoor air. *Streptomyces spp.* concentrations in both indoor and outdoor  
237 environments were higher in the winter than the autumn, but with only a significant difference  
238 for outdoor air ( $p < 0.05$ ).

239 The dominant bacteria (*Bacillus spp.*, *Micrococcus spp.*, *Streptomyces spp.*,  
240 *Staphylococcus spp.*) in indoor and outdoor air were measured at frequencies ranging between  
241 78.6-92.9% and 78.6-100%, respectively, which is in the range reported for normal days (Table  
242 3). The frequency that other bacteria in Table 3 were detected in indoor and outdoor air was 0-  
243 57.1% and 14.3-64.3%, respectively, which is also similar to normal days. The most frequently  
244 detected bacteria in indoor (*Staphylococcus spp.* and *Streptomyces spp.*) and outdoor (*Bacillus*

245 *spp.* and *Streptomyces spp.*) air are similar to normal days, with the exception of *Streptomyces*  
246 *spp.* replacing *Bacillus spp.* for indoor air. Average concentrations of fungi in indoor air were  
247 higher than bacteria while the opposite was observed outdoors (Table 4). The I/O ratio for fungi  
248 in the internal wards were higher than the ratio for bacteria (0.60 versus 0.38), while the I/O ratio  
249 for bacteria in the ICU was lower than that for fungi (0.26 versus 0.39).

250

### 251 3.3 Diurnal Profiles of PM<sub>10</sub> and Bioaerosol

252 Diurnal variations of PM<sub>10</sub> concentration in the study period for Ahvaz are presented in  
253 Figure S1 (Supplement) based on daily collected between September 2010 and March 2011.  
254 There is some diurnal structure with minimal concentrations in the early morning (03:00, 199.8  
255  $\mu\text{g m}^{-3}$ ) and late afternoon (17:00, 183. 1  $\mu\text{g m}^{-3}$ ). Maximum levels are reached in the early  
256 (~02:00, 236.3  $\mu\text{g m}^{-3}$ ) and late morning (~09:00, 241.0  $\mu\text{g m}^{-3}$ ). The lack of a smooth  
257 concentration profile in the very early morning is likely due to episodic short time scale events.

258 During five dust event days, diurnal data are shown for both PM<sub>10</sub> and bacteria  
259 concentration in Table 5. Regardless of whether PM<sub>10</sub> is higher during the first or second half of  
260 the day, bacteria is also enhanced at that same half of the day in both the Outdoor and Internal  
261 Ward areas. The ICU area in a couple cases shows higher bacteria concentrations when PM<sub>10</sub>  
262 was not at its highest; generally speaking based on results already shown in Table 4, the Internal  
263 Wards were more reflective of the outdoor air as compared to the ICU. The diurnal data provide  
264 support that dust plays an important role to carry and transfer microorganisms between the  
265 outdoor and indoor environment. Subsequent discussion provides more support for the link  
266 between dust and bioaerosols.

## 267 4. Discussion

### 268 4.1 Comparison between Normal and Dust Event Days

269 Compared to normal days, bacteria and fungi concentrations in indoor and outdoor  
270 environments on dusty days were significantly higher ( $p < 0.05$ ). On dust event days, bacteria  
271 and fungi concentrations in outdoor air samples were higher by factors of approximately three  
272 and two, respectively, than those on normal days (Table 4). All four of the I/O ratios for bacteria  
273 and fungi reported in Table 4 are lower on dust event days (0.26-0.60) versus normal days (0.44-  
274 0.95), due most likely to the overwhelmingly higher concentrations outside on dust event days.  
275 For comparison with another city in Iran, specifically Tehran, I/O ratios for bacteria and fungal  
276 spores were 1.77 and 1.23 for a retirement home, respectively, and 1.44 and 1.08 for a school  
277 dormitory (Faridi et al., 2015).

278 Figure 2 shows the percentage of concentration of dominant bacteria on normal and dusty  
279 days. On normal days, *Staphylococcus spp.* exhibited the highest concentration of bacteria in the  
280 ICU (21.2%) and internal wards (30.4%), while *Bacillus spp.* was the most abundant one in  
281 outdoor samples (34%). On dusty days, *Bacillus spp.* and *Streptomyces spp.* were significantly  
282 enhanced in concentration in both indoor and outdoor air samples ( $p < 0.05$ ); *Bacillus spp.*  
283 exhibited the highest concentration in the ICU, internal wards, and outdoor samples, accounting  
284 for 21%, 26.8% and 39.1%, respectively.

285 Figure 3 shows the association between bacterial concentration in both the air of the  
286 internal and ICU wards versus that of outdoor air on normal and dust event days. On normal  
287 days, the correlation between outdoor bacterial concentrations was higher with internal ward  
288 concentrations ( $r = 0.67$ ,  $n = 42$ ) as compared to with the ICU ( $r = 0.41$ ,  $n = 42$ ) (Figure 3).  
289 Conversely, for dust event days, outdoor bacterial concentrations were better correlated with the

290 ICU ( $r = 0.58$ ,  $n = 14$ ) as compared to internal wards ( $r = 0.42$ ,  $n = 14$ ). The Figure 3 results,  
291 especially for normal days, suggest that indoor air quality can be affected by outdoor air quality  
292 with regard to bioaerosols. To further support this thought, the highest indoor microbial  
293 concentration was recorded in the last month of winter (March) coincident with a dust event  
294 lasting two days, showing that dust can affect the concentration and genus of bioaerosols in both  
295 indoor and outdoor air. This can partly be explained by the increase in number of patients  
296 admitted to the hospital on dusty days, and thus a greater frequency of opened doors and  
297 windows to allow for dust to come indoors in addition to patients transporting dust themselves.  
298 Thus, the occurrence of dust storms in Ahvaz can affect the indoor air quality of hospital wards  
299 both directly, by entrance of dust particles in wards, and indirectly by increase in the patients  
300 admitted to hospital.

301 The number of identified colonies on normal and dusty days is presented in Figure 4. The  
302 concentration of bacteria in the most of samples on both normal and dust event days was higher  
303 than ACGIH (American Conference of Governmental Industrial Hygienists) standards for indoor  
304 air bioaerosols ( $200 \text{ cfu m}^{-3}$ ). During normal days, Gram-positive cocci and Gram-positive rods  
305 contributed the most in indoor samples with contributions of 44% and 39%, respectively. In  
306 outdoor air, Gram-positive cocci and Gram-positive rods contributed 36% and 42%, respectively.  
307 On dust event days, Gram-positive cocci and Gram-positive rods contributed 31% and 34% to  
308 indoor samples, respectively, and 28% and 39% to outdoor samples, respectively. Gram positive  
309 bacteria exhibited the highest concentration in both outdoor and indoor air samples as well as  
310 during both normal and dust event days.

311

## 312 **4.2 Influence of Ambient Environmental Factors**

313 According to previous investigations, prevailing westerly winds and the fact that Ahvaz  
314 is downwind of dust-rich regions such as Iraq, Saudi Arabia and the Sahara Desert, qualify the  
315 study region as a major hot-spot for dust storms in the Middle East region (Soleimani et al.,  
316 2013; Goudarzi et al., 2014). For example, Soleimani et al. (2015) confirmed with HYSPLIT  
317 back-trajectory model data analysis that Ahvaz dust originates from Saudi Arabia, Iraq, and  
318 North Africa. High humidity and mild temperature in autumn make the air in Ahvaz a suitable  
319 media for bacteria to grow. Gram positive bacteria, which originate from the soil of the  
320 aforementioned upwind areas, exhibited the highest percentage ( $> 90\%$ ) in both indoor and  
321 outdoor air on both normal and dusty days, which is similar to the result of other studies (Acea et  
322 al., 1988; Augustowska and Dutkiewicz, 2006; Kim et al., 2009). Relative to Gram positive  
323 bacteria on normal days, Gram negative bacteria exhibited a higher concentration in indoor air as  
324 compared to outdoor air. A likely explanation is that Gram positive bacteria are more resistant to  
325 undesirable outdoor conditions (e.g., high incident solar radiation) as compared to Gram negative  
326 ones (Zhu et al., 2003). Our results indicate that *Bacillus spp.* exhibits the highest outdoor  
327 concentration on both normal and dusty days likely due to their high resistance to undesirable  
328 environmental conditions.

329

## 330 5. Conclusions

331 Dust storms pose a threat for Middle East communities, particularly in Ahvaz on the  
332 western side of Iran, which is highly vulnerable to dust transport from upwind regions.  
333 Bioaerosols are associated with these dust events and can promote infectious diseases, allergy,  
334 asthma, and neurological diseases among vulnerable populations. The focus of this study was to  
335 examine bioaerosol concentrations and composition for samples collected inside and outside of



336 an Ahvaz hospital between September 2010 and March 2011. A unique aspect of this work is the  
337 comparison of indoor and outdoor microbial air quality in one of the dustiest regions of the  
338 planet with field data spanning multiple months, which allows for a comparison between dusty  
339 and normal days.

340 The data clearly show that bioaerosol (bacteria and fungi) concentrations increased in  
341 both indoor and outdoor areas of the hospital atmosphere during dust event days. The dominant  
342 bacterial constituents were *Bacillus spp.*, *Micrococcus spp.*, *Streptomyces spp.*, and  
343 *Staphylococcus spp.* I/O ratios for bacteria and fungi are lower on dust event days (0.26-0.60)  
344 versus normal days (0.44-0.95), due most likely to the overwhelmingly higher concentrations  
345 outside on dust event days. The correlation between outdoor and indoor bacterial concentrations  
346 was stronger on normal days. But a two day dust event case study in the last month of winter  
347 (March) proved that dust can affect the concentration and genus of biomaterials in both indoor  
348 and outdoor air. During such dust events, there is an increase in number of patients admitted to  
349 hospitals, and thus a greater frequency of opened doors and windows to allow for dust to come  
350 indoors in addition to patients transporting dust themselves. Gram positive bacteria exhibited  
351 higher concentrations than Gram negative bacteria in both outdoor and indoor air samples as well  
352 as during both normal and dust event days. The results also suggest that Gram positive bacteria  
353 are more resistant to undesirable outdoor conditions (e.g., solar radiation) as compared to Gram  
354 negative ones.

355 The results of this study motivate artificial ventilation and air conditioning systems  
356 instead of natural ventilation in hospitals in dusty regions such as Iran. Using HEPA, ULPA, and  
357 HVAC systems to improve indoor air quality in different hospital wards is recommended.  
358 Making such changes in hospitals can impact the lives of patients and employees not just in the

359 study region, but many others such as those in other arid regions that cumulatively cover over a  
360 third of the global land area.

361

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365

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521 **Table 1.** Dust storm classification as based on the work of Hoffmann et al. (2008).

522

Category	Visibility (m)	Wind speed (m s <sup>-1</sup> )	Hourly averaged PM <sub>10</sub> (µg m <sup>-3</sup> )
Dusty air	> 2000	-	50-200
Light dust storm (DS1)	< 2000	-	200-500
Dust storm (DS2)	< 1000	> 17	500-2000
Strong dust storm (DS3)	< 200	> 20	2000-5000
Serious strong DS (DS4)	< 50	> 25	> 5000

523

524

525 **Table 2.** Seasonal average concentration (cfu m<sup>-3</sup>) of four airborne bacteria and total bacteria in  
 526 outdoor air and indoor units. The number of total samples collected for normal and dust event  
 527 days was 59 and 14, respectively.

528

		Autumn (Sep - Nov)		Winter (Jan - Mar)	
		Normal	Dust	Normal	Dust
Internal	Bacillus spp.	112.92	120.10	87.61	122.11
Wards	Micrococcus spp.	92.30	101.15	72.70	92.00
	Staphylococcus spp.	133.54	99.41	113.21	97.23
	Streptomyces spp.	47.00	85.30	36.75	96.00
	Other	67.41	64.29	50.76	92.36
	Total bacteria	453.17	470.25	361.03	499.70
ICU	Bacillus spp.	45.00	64.71	47.00	75.00
	Micrococcus spp.	38.70	48.30	56.01	65.00
	Staphylococcus spp.	61.31	72.62	46.23	73.60
	Streptomyces spp.	38.00	48.30	20.10	56.00
	Other	99.38	70.21	56.45	91.23
	Total bacteria	282.39	304.14	225.79	360.83
Outdoor	Bacillus spp.	157.21	325.30	132.40	713.50
	Micrococcus spp.	110.80	143.41	101.91	269.20
	Staphylococcus spp.	45.20	81.40	35.93	90.81
	Streptomyces spp.	80.81	175.60	61.33	364.15
	Other	64.30	86.40	60.71	412.83
	Total bacteria	458.32	812.11	392.28	1850.51

529

530 **Table 3.** Frequency of bacterial genera identification in both indoor and outdoor areas. The  
 531 number of total samples collected for normal and dust event days was 59 and 14, respectively.

Bacteria	Air Type	Frequency (%)		
		Internal Wards	ICU	Outdoor
Gram-Positive Bacillus sp.	Normal	93.1	86.2	100.0
	Dust	85.7	78.6	100.0
Micrococcus spp.	Normal	72.9	50.8	94.9
	Dust	78.6	85.7	92.8
Staphylococcus sp.	Normal	93.1	91.4	82.8
	Dust	85.7	92.9	78.6
Streptomyces spp.	Normal	86.4	81.4	98.3
	Dust	92.8	78.6	92.9
Corynebacterium spp.	Normal	47.5	42.4	50.8
	Dust	57.1	42.8	50.0
Rhodococcus spp.	Normal	13.6	6.8	13.5
	Dust	0.0	0.0	35.7
Micro bacterium spp.	Normal	15.2	8.5	20.3
	Dust	35.7	28.6	50.0
Stomatococcus spp.	Normal	10.2	8.5	18.6
	Dust	42.9	35.7	50.0
Arcanobacterium spp.	Normal	0.0	0.0	6.8
	Dust	21.4	0.0	42.8
Dermabacter spp.	Normal	5.1	0.0	10.2
	Dust	28.6	0.0	42.9
Brevibacterium spp.	Normal	11.9	0.0	15.3
	Dust	35.7	0.0	42.9
Deinococcus spp.	Normal	5.1	0.0	13.6
	Dust	14.3	0.0	28.6
Arthobacter spp.	Normal	8.5	3.4	10.3
	Dust	35.7	14.3	42.9
Bacillus cereus	Normal	3.4	0.0	8.5
	Dust	21.4	14.3	28.6
Cellulomonas spp.	Normal	3.4	0.0	8.6
	Dust	21.4	21.4	35.7
Nocardiaspp.	Normal	8.4	0.0	13.6
	Dust	28.6	7.1	42.8
No identification	Normal	50.8	52.5	71.2
	Dust	42.9	50.0	64.3
Gram-Negative Acinetobacter	Normal	0.0	0.0	5.2
	Dust	0.0	0.0	14.3
Achromobacter spp.	Normal	8.5	6.8	16.9
	Dust	21.4	0.0	35.7
Bacillus pseudomonas sp.	Normal	6.8	13.8	20.6
	Dust	3.4	0.0	21.4
Enterobacter spp.	Normal	15.3	3.4	6.9
	Dust	28.6	14.3	28.6
Seratia spp.	Normal	0.0	0.0	5.1
	Dust	14.3	0.0	21.4
Klebsiella pneumonias	Normal	0.0	0.0	8.5
	Dust	7.1	0.0	28.6
No identification	Normal	5.1	0.0	3.4
	Dust	14.3	0.0	21.4
No identification	Normal	25.4	28.8	20.3
	Dust	42.8	35.7	50.0

532

533 **Table 4.** Comparison of average concentration and indoor/outdoor (I/O) ratio range for bacteria  
 534 and fungi (cfu m<sup>-3</sup>) in both indoor and outdoor air between normal and dust event days. For rows  
 535 with average concentration data, the range is shown in parenthesis. The number of total samples  
 536 collected for normal and dust event days was 177 and 42, respectively.

537

		Normal Days	Dust Event Days	p value
Internal	Total Bacteria	403.92 (40-1667)	482.87 (278-808)	0.024
Wards	Total Fungi	510.7 (0-1560)	668.80 (234-988)	0.005
	In/out Bacteria (Fungi)	0.95 (0.86)	0.38 (0.6)	-
ICU	Total Bacteria	252.14 (27-605)	328.44 (148-587)	
	Total Fungi	261.21 (0-998)	431.80 (200-728)	0.035
	In/out Bacteria (Fungi)	0.6 (0.44)	0.26 (0.39)	-
Outdoor	Total Bacteria	423.02 (47-2000)	1257.14 (523-5000)	< 0.0001
	Total Fungi	596.62 (60-2420)	1115.61 (0-1560)	< 0.0001

538

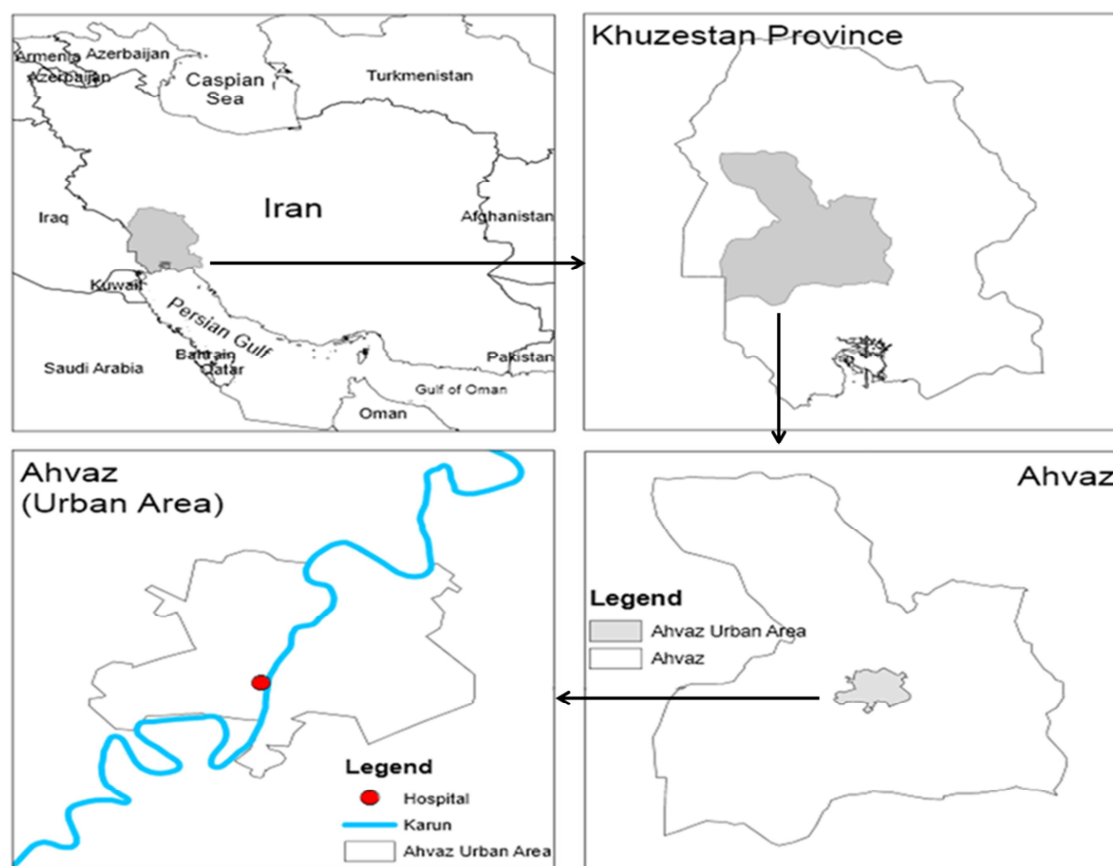
539 **Table 5.** Diurnal variation of PM<sub>10</sub> and bacteria during dust event days for outdoor air and  
 540 indoor hospital units.

Date	Time	PM <sub>10</sub> ( $\mu\text{g m}^{-3}$ )	Bacteria (CFU m <sup>-3</sup> )		
			Internal Wards	ICU	Outdoor
11/3/2010	AM	123	400	223	785
	PM	536	580	375	938
12/14/2010	AM	562	808	500	1220
	PM	200	380	513	754
12/16/2010	AM	139	560	300	1000
	PM	418	574	176	1530
2/12/2011	AM	277	410	316	923
	PM	427	616	587	5000
3/10/2011	AM	200	560	375	1558
	PM	155	278	411	1092

541

542

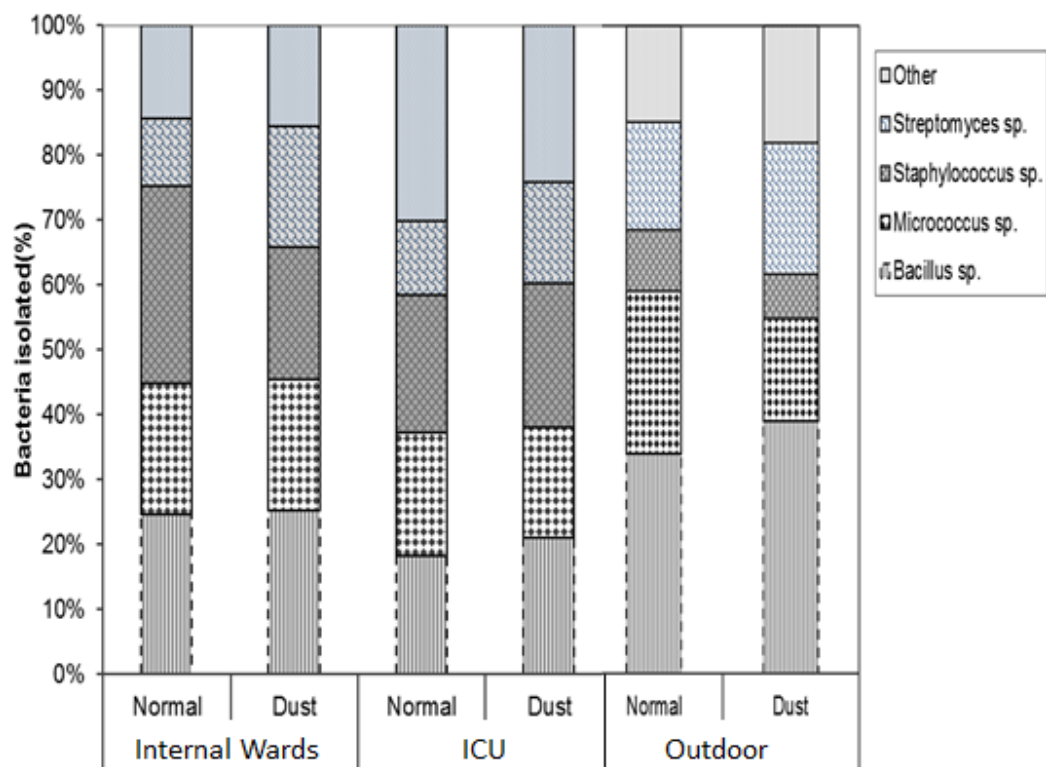
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544

545 **Figure 1.** Location of the study area and sampling station. In the bottom left panel, “Karun”

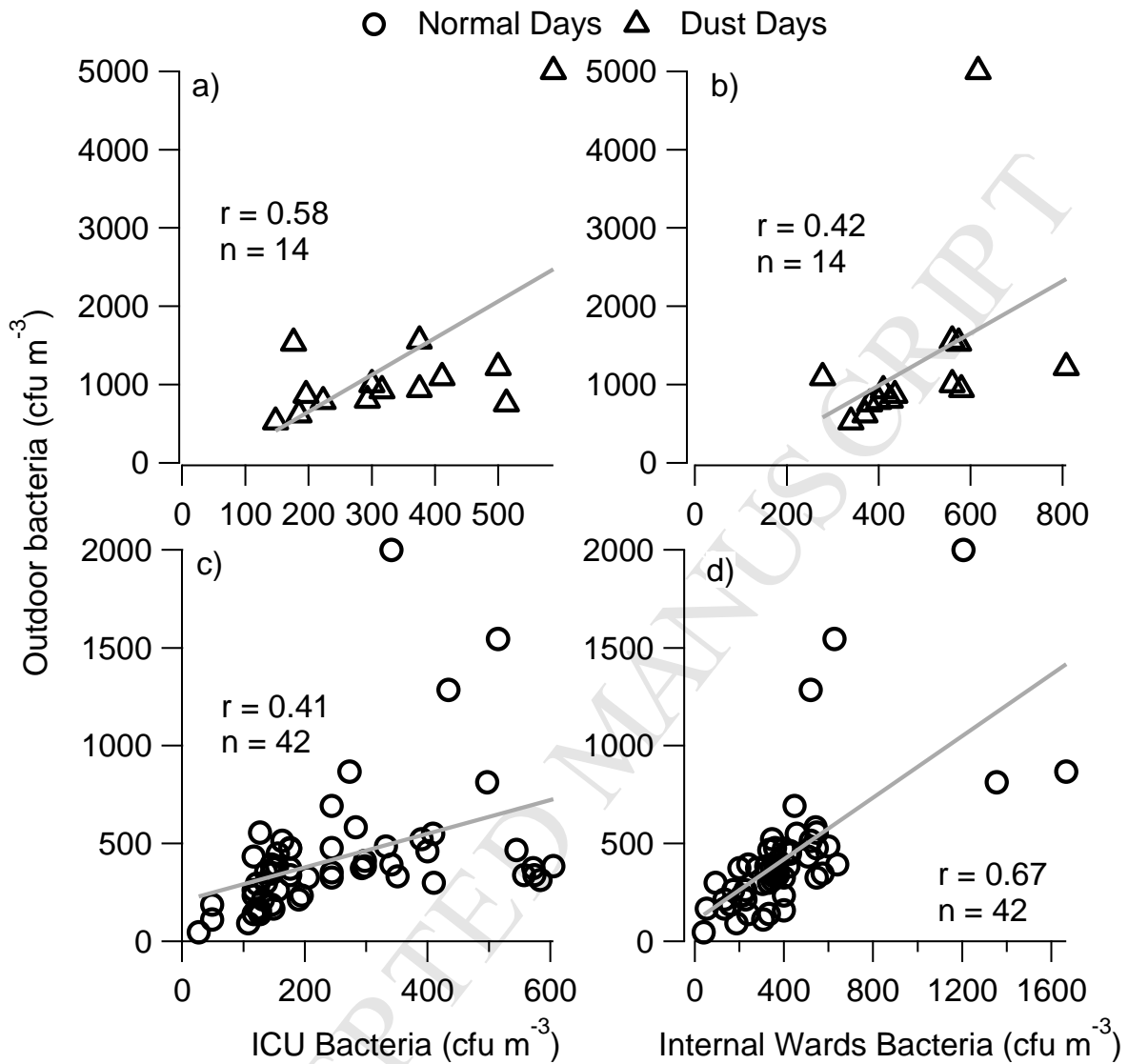
546 refers to a major river.



547

548 **Figure 2.** Percentage contribution of different bacterial species in indoor and outdoor air during  
 549 normal and dust event days.

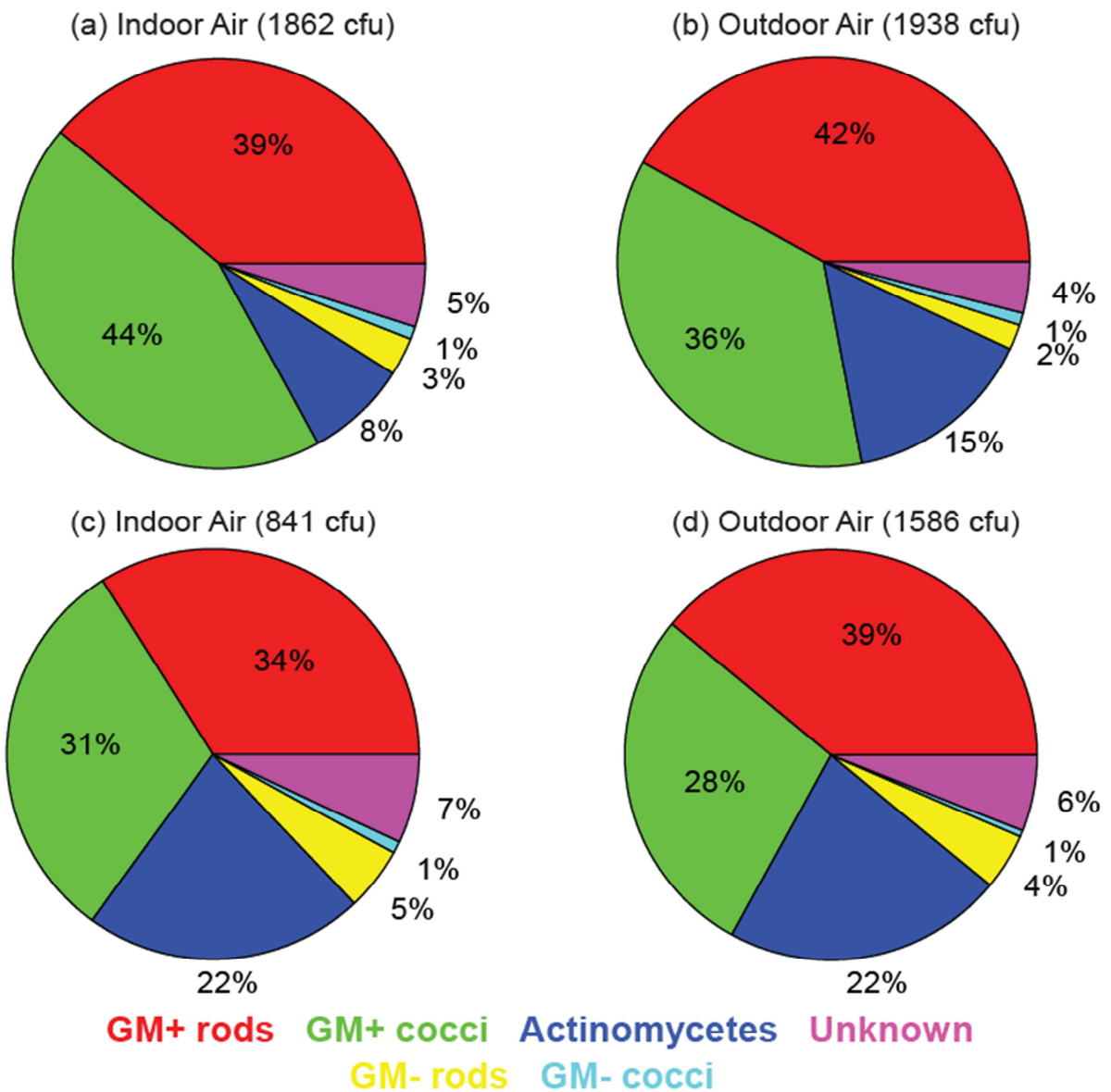




550

551 **Figure 3.** Scatterplot comparing indoor and outdoor airborne bacteria concentration in internal  
 552 and ICU wards during (a-b) dust event and (c-d) normal days. Gray lines represent best fit lines  
 553 with respective correlation coefficients ( $r$ ) and sample number ( $n$ ) shown for each panel.

554



555

556 **Figure 4.** Composition of culturable bacteria in indoor hospital areas and outdoor air during (a-  
 557 **b)** normal and (c-d) dust event days. “GM+” and “GM-” refer to Gram positive and Gram  
 558 negative, respectively.

**Highlights:**

- Bioaerosol composition measurements in and outside of a hospital in Ahvaz, Iran
- Outdoor bioaerosol are shown to impact indoor hospital air quality
- Bacteria and fungi concentrations higher on dusty days in and out of hospital
- Indoor:outdoor ratios of bioaerosol lower on dusty days ( $\leq 0.60$ ) versus normal days
- Gram positive bacteria exhibit higher concentrations than Gram negative bacteria