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Ambient concentration of airborne microbes and endotoxins in rural households of southern India

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Abstract

Investigation of microbial communities in the indoor environment is critically important as majority of human life is spent indoors. Airborne microbial flora and their pyrogenic substances can impact human health. They have so far been insufficiently characterized. Here, we quantify bioaerosol concentration and diversity in both indoor and outdoor air of rural households in South India and statistically determine the significant predictor variables influencing them. The median concentration of bacterial and fungal aerosols in indoor air (n = 36 households) was 1031 colony forming units (CFU) m^{-3} and 580 CFU m^{-3}, respectively, and in outdoor air was 742 CFU m^{-3} and 680 CFU m^{-3}, respectively. Bacterial species were comprised of the four major phyla Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes, and fungal species of Ascomycota, Basidiomycota, and Zygomycota, in both indoor and outdoor air. Multivariable linear regression revealed that outdoor bacterial concentration, the number of house inhabitants, indoor temperature, age of the homes and use of biomass (cow dung or firewood) as cooking fuel were significant predictor variables influencing concentrations of bacteria in the indoor air. The significant predictor variables influencing indoor endotoxin concentrations were indoor bacterial concentration, indoor meteorology (temperature and relative humidity), and use of biomass as cooking fuel. Outdoor fungal concentration was the significant predictor variable influencing fungal concentration in indoor air. Endotoxin concentrations in indoor air ranged from 1.23 to 20.7 EU m^{-3}. Quantitative microbial risk assessment (QMRA) model revealed a probability of infection of resident women from B. anthracis to be 0.0009 to 0.010.

Keywords: Bioaerosols, indoor air, risk assessment, multivariable linear regression

1. Introduction

Ninety per cent of human life is estimated to be spent indoors [1,2]. Therefore, the indoor environment plays an important role in influencing a person's overall health. Still, assessment of indoor air quality has received relatively lesser attention compared to outdoor air quality, even though the number of health complaints related to indoor quality is increasing substantially [3,4].

Aerosols are an important constituent of indoor air pollutants. Aerosols comprising of or originating from bacteria, virus, fungi, pollen, faeces, mites, insects are called bioaerosols [5–8]. Up to 34% of indoor aerosols have been found to be bioaerosols [9,10]. Bacteria and fungi are the most important of these bioaerosols. Spores of *Staphylococcus, Cladosporium, Aspergillus* can cause adverse health effects in humans, such as infections and respiratory diseases [5,11–14]. By-products or end-products of bacteria and fungi are also known to cause adverse health effects [15–18]. Collectively termed as pathogen-associated molecular patterns (PAMPs), these include the endotoxins and exotoxins released from bacteria, and mycotoxin released from fungi [19]. Endotoxins are the most significant PAMPs commonly found in the environment [20]. Endotoxin is the cell wall-bound component of gram-negative bacteria and it is released when a bacterial cell is lysed. Upon entering the human respiratory system, endotoxins activate the alveolar macrophages and the epithelial tissue, and induce the inflammatory cytokines [21,22].

The concentration of bioaerosols in an indoor environment is dependent on both indoor and outdoor factors. Outdoor determinants include proximity to anthropogenic activities such as wastewater and sewage treatment plants, composting facilities [14,23–25], agriculture lands, livestock farming [26,27], landfills [28,29], geographic conditions [30,31], outdoor meteorology (temperature, relative humidity, wind velocity and direction) [30,32–36] and vegetation [37,38].

Indoor determinants include the extent of ventilation [39,40], home ecology (presence of pets and/or plants, number of inhabitants) [39,41–46], indoor meteorology (temperature and relative humidity) [47], building design (insufficient circulation of indoor air leading to stagnant zones) [48–50] and building age [51].

Approximately 69% of the Indian population lives in rural areas [52,53]. Largely driven due to the poor socio-economic status of the inhabitants, rural houses are generally overcrowded, ill-ventilated and ill-lit leading to an accumulation of chemical pollutants, vermin and microbes [54]. So far, most of the studies on indoor air pollution in rural India have focused on chemical pollutants such as CO, NOx, SO_2 and residues of fuel used to burn rural stoves [55–57]. In comparison, there have been very few studies assessing indoor bioaerosol contamination and the associated health risks.

Here, we assess the presence and variability of bioaerosols and endotoxins (the most abundant of the PAMPs) in a rural setting in South India. The objectives were to (i) characterize and analyze the richness and abundance of bacteria and fungi in indoor and outdoor air of surveyed households, (ii) estimate the probability of infection caused by bioaerosol inhalation using a Quantitative Microbial Risk Assessment model (QMRA) and (iii) to quantify the concentration of endotoxins in the indoor air.

2. Methodology

2.1 Sampling site

Sampling was carried out in a village Kyasaram (17.8522°N, 78.7108°E) in Sangareddy District, Telangana State, India (Fig. 1). The total area of the village is 788 hectares. The village consists of 752 houses with 2752 inhabitants. The village is surrounded by agriculture lands and

moderate vegetation. About 60% of the houses belong to small and marginal farmers. Thirty-six (about 5% of total) houses were sampled (Supplementary Information-Table S1). Parameters that have been shown to potentially influence indoor bioaerosol concentration or characteristic were noted during sampling [39]. These included home ecology, indoor meteorological parameters (temperature and relative humidity), building parameters (number of rooms in the house, age of the house) and the type of fuel used for cooking [liquefied petroleum gas (LPG) and/or biomass (cow dung and firewood)].

2.2 Sample collection

Bioaerosol samples were collected from the middle of the living rooms. While it has been reported that people spend most of their indoor time in the living room and also in the bedroom [58], sampling of bedrooms was not allowed by the home owners. Sampling height was 1.5 m above the ground level [59]. The living room was occupied by all the inhabitants during the sampling event and no other activity was taking place. The number and status of windows in the living room and door that leads to the main entrance were noted. Fifteen out of the thirty-six sampled houses had a second room, and the door to that room was closed. Door to the main entrance was open during sampling (Table S1). Outdoor sampling was carried out at the entrance of the houses immediately following the indoor sampling.

Viable bioaerosols were collected in 90 mm petri dishes (Tarson USA) placed on a single-stage cascade impactor with 400 holes with a size of 0.25 mm (BioStage-single-stage sampler, SKC, USA) [59]. Air was drawn by using a diaphragm vacuum pump at a flow rate of 28.3 L min^{-1} for 2 min [59,60]. The sampler was cleaned with 70% ethanol before and after each sampling to avoid cross-contamination [61]. Bacterial aerosols were collected on petri dishes containing

Tryptic Soy Agar (TSA) medium and fungal aerosols were collected on petri dishes containing Potato Dextrose Agar (PDA) medium. Each sampling was carried out separately for 2 min [62–64]. After sampling, TSA plates were stored at 37□ and colonies were counted after two days [64]. Similarly, PDA plates were stored at 25□ for 3 days and colonies were examined [65]. Samples were collected in triplicates for both bacteria and fungi, and an average of the triplicate samples was reported as the concentration of the bacterial and fungal aerosol in the air. All the three plates of each sample was considered as one and colonies were subjected to identification. Field blanks (n = 5) were taken to check the sterility of instruments. Field blank samples were collected in the same manner as the bioaerosol samples, but without switching the pump on. Bacterial and fungal aerosol concentrations were reported as colony forming units per cubic meter (CFU m^{-3}) [14,62,66]. Standard deviation was calculated for each triplicate sample. No bacterial or fungal growth was observed in the field blanks.

Particulate matter (PM) for endotoxin analysis were collected by filtration technique using a Whatman filter holder (GE healthcare, US) connected with a diaphragm vacuum pump. PM samples were collected on 47 mm diameter Whatman glass microfiber filter paper [67] and sampling was carried out at the flow rate of 50 L min^{-1} for 30 min. Similar to bioaerosol sampling, PM sampling was carried out in the living room at 1.5 m height from the ground level. Samples were kept in airtight 60 mm petri dishes (Tarson USA) immediately after sampling and were stored in a refrigerator at 4°C until analysis within 24 hours [64]. Similar to bacterial and fungal aerosol sampling, field blanks (n = 5) were taken without switching the pump on. Each filter paper was pre-conditioned in muffle furnace at 400°C for eight hours. Temperature and

relative humidity were monitored throughout the sampling by using a Meco 920-P humidity logger (Meco Instruments, India) with an accuracy of ±3.5% for RH and ±0.5°C for temperature.

2.3 Microbial quantification

2.3.1. DNA extraction

After incubation, colonies from TSA and PDA plates were examined and picked based on the morphology [62]. Colonies were then mixed with 1X Saline for DNA extraction. Commercially available Purefast Bacterial/Fungal DNA extraction kit was used (Helini Biomolecules® India) [62]. 180 µL digestion buffer and 20 µL lysozyme were added to the saline mixture and the mixture was incubated at 37□ for 15 min. Then, 200 µL binding buffer was added followed by 20 µL of Proteinase K. Resulting mixture was incubated at 56□ for 10 min. This was followed by buffer addition and centrifugation at 14,000 rpm for 1 min as per the manufacturer's instructions (Helini Biomolecules® India). Finally, DNA was extracted by adding 100 µL of elution buffer [62].

2.3.2 PCR analysis and sequencing

Eluted DNA was then subjected to individual PCR runs targeting the universal 16s and ITS region of bacteria and fungi [60,62]. The primer sequence used for bacteria was (5′-AGAGTTTGATCCTGGCTCAG-3′, 3′-ACGGCTACCTTGTTACGACTT-5′) and for fungi was (5′- TCCGTAGGTGAACCTGCGG-3′, 3′-TCCTCCGCTTATTGATATGC-5′) [60]. Each PCR reaction was performed in a mixture containing 25 µL of Red Dye master mix, 2 µL of primer mixture with forward and reverse primers of 2 pM/µL each, 20 µL DNAase free water and 3 µL of DNA extracted from the bacterial and fungal colonies [60]. Assays were performed using an

153 Agilent SureCycler 8800 PCR system. Amplification cycle was maintained at 95□ for 5 min for
154 an initial denaturation. Further denaturation was carried out at the same temperature, 1 min for
155 bacteria and 30 seconds for fungi. Similarly, annealing and elongation temperatures were
156 maintained at 58□ and 72□ for bacteria and fungi, respectively, and time was 1 min for bacteria
157 and 30 seconds for fungi. This further denaturation, annealing and elongation cycle was repeated
158 thirty times for bacteria and thirty-five times for fungi [60,62]. Final elongation was carried out
159 at 72□ for 3 min. PCR products were examined using 2% agarose gel electrophoresis. UV
160 transilluminator was used to visualize the gel. Amplified PCR products were purified using
161 Highyield Gel/PCR DNA fragments purification kit (Abzyme Bio-labs, India) and subjected to
162 Sanger sequencing (Eurofins Genomics Pvt. Ltd. Bangalore). The sequences which contained
163 above 95% identity values from NCBI Blast analysis were considered. The nucleotide sequences
164 obtained in the study were given accession numbers MK097320-MK097370 and MN122272-
165 MN122299 for bacteria, MK108375-MK108436 and MN128877-MN128881 for fungi at the
166 NCBI GenBank.

167 **2.3.3 Microbial diversity**

168 From NCBI-Blast analysis, species level identification was done. It is important to study the
169 diversity of microbes as they determine numerous critical functions essential for the environment
170 such nitrogen fixation, carbon fixation and recycling of inorganic nutrients [68,69]. Therefore, to
171 understand the biodiversity of microbes obtained in our work, Alpha diversity was assessed
172 using Shannon's and Gini-Simpson's indices which characterize the ecological community with
173 respect to species richness and evenness. These indices were calculated separately for bacteria
174 and fungi in indoor and outdoor air for all the thirty-six individual houses and for all houses

175 combined to obtain an overall assessment of indoor and outdoor air. Example calculations are
176 provided in the supplementary information (Sections S1-S4).
177
178 Shannon's diversity index (*H*) for each house was calculated as $H = -\sum[p_i \times \ln(p_i)]$ where p_i is
179 the total number of occurrences of an individual species *i* divided by total number of occurrences
180 of all the individual species in the cultured TSA or PDA plates for each house. The higher *H*
181 value indices indicate a high richness of the community. Shannon's evenness of equitability (E_h)
182 was calculated using the equation $E_h = H/H_{max}$ where $H_{max} = lnS$, and *S* is the total number of
183 species in each house. Gini-Simpson's diversity index (*D*) was calculated as $D = 1 - \sum \frac{n_i(n_i-1)}{N(N-1)}$,
184 where n_i is the number of occurrences of an individual species *i* in the cultured TSA or PDA
185 plates for each house, and *N* is the total number of occurrences of all the individual species in
186 each house [70]. The values of E_h and *D* generally lie between 0 to 1. A value close to 1 indicates
187 a higher diversity and evenness.
188
189 Overall *H* and *S* for the indoor or outdoor air for all the sampled houses were calculated with the
190 same formula where p_i and n_i represent the total number of occurrences of an individual species *i*
191 in the indoor air or in the outdoor air of all sampled houses divided by total number of
192 occurrences of all the individual species in the cultured TSA or PDA plates of indoor air or
193 outdoor air of all sampled houses. Similarly, E_h was calculated as $E_h = H/H_{max}$ where $H_{max} =$
194 lnS, and *S* is the total number of species in the cultured TSA or PDA plates of indoor air or
195 outdoor air of all sampled houses. Relative abundance (%) of species was calculated from the
196 NCBI-Blast results obtained for indoor and outdoor air of each house, and for indoor or outdoor
197 air of all houses combined; by dividing the total number of occurrences of i^{th} species in indoor or

198 outdoor air of each house or all houses combined by the total number of occurrences of all
199 individual species in indoor or outdoor air of each house or all houses combined, respectively,
200 and the result was then multiplied by 100 [71].
201
202
203 **2.4 Endotoxin analysis**

204 Endotoxin concentration in ambient air particulate matter was measured by Limulus Amebocyte
205 Lysate Assay (LAL) (Chromogenic Endotoxin assay kit, Genscript, USA) [71]. All the materials
206 used for this assay were sterilized overnight at 180°C to avoid contamination [71]. Dust particles
207 from filter paper were extracted in endotoxin-free water with 0.05% Tween-20 [72,73] into
208 pyrogen-free sterile tubes. Tubes were rocked at 37°C for 1 hour and then centrifuged at 1000g
209 for 10 minutes [73]. The supernatant was added to an endotoxin-free glass tube and endotoxin
210 concentration was measured as per the manufacturer's instruction at 37°C. The concentration of
211 endotoxin was measured by colorimetric analysis using a spectrophotometer (Lab India
212 analytical, India) at 455 nm wavelength as instructed by the manufacturer (Chromogenic
213 Endotoxin assay kit, Genscript, USA). Endotoxin standards were prepared with LAL assay water
214 (calibration R^2= 0.998). Endotoxin concentration was expressed as endotoxin units per cubic
215 meter (EU m^{-3}). All samples were analyzed in duplicates. Samples with higher concentration
216 were diluted, and the dilution factor was used in the final calculations. Five control filter paper
217 samples were extracted and analyzed as field blanks. The method detection limit (obtained as
218 three times the standard deviation of filter blanks) was 0.2 EU m^{-3}.

219 **2.5 Quantitative Microbial Risk Assessment (QMRA) analysis**

Quantitative Microbial Risk Assessment (QMRA) was conducted for exposure of resident women to the most abundant pathogen found in indoor air based on Sanger sequencing results, *B. anthracis*. These women reported that they spend the majority of their time indoors, and only some time outdoors (two to three hours every day) just outside their house door entrance. QMRA model was designed as previously described [74,75]. Infection caused due to inhalation of *B. anthracis* was considered as a hazard [74]. Exposure to *B. anthracis* was calculated as

$$\boldsymbol{Dose = C \times I \times t} \quad \textbf{(1)}$$

where i) *D*ose is in CFUs, ii) *C* is the concentration of *B. anthracis* present in the air (CFU m^{-3}), iii) *I* is inhalation rate (m^3 h^{-1}), and iv) *t* is exposure time (h). Inhalation rate of average adult Indian women was used for the calculation (Table 1) [76].

The probability of infection (P_r) was calculated using a dose-response relationship [77]:

$$\boldsymbol{P_r = 1 - e^{-k \times Dose}} \quad \textbf{(2)}$$

Where *k* is the survival probability of a single pathogen (Table 1) [77,78].

The women residents did not respond clearly on how much time they spend in the living room. Therefore, we estimated the probability of infection for several exposure durations ranging from 1 hour to 12 hours, which covers a wide range of times that they might spend indoors. We assumed that there is no ventilation, or deposition, that removes *B. anthracis* from indoor air (a worst case scenario), and that there is no variation in its concentration in indoor air during the entire assessment period of one to twelve hour (which may be a worse or better scenario).

2.6 Statistical analysis

All statistical tests were performed using the Statistical Package for Social Sciences (SPSS) and Microsoft Excel 2010. The logarithmic values of obtained concentrations followed a normal distribution (Shapiro Wilk test- $p > 0.05$) [79], indicating that the concentrations were log-normally distributed. Multivariable linear regression was used to understand the association between indoor bacterial, fungal and endotoxin concentrations (dependent variables) with the independent variables indoor and outdoor temperatures, indoor and outdoor relative humidity, and home ecology factors like number of inhabitants, age of the house, number of rooms in the house, presence of pet(s) and type of fuel used for cooking (LPG and/or biomass).

3. Results and discussions

3.1. Bioaerosol concentration in the air

The median concentration of bacteria present in the indoor air was 1031 CFU m^{-3} with a minimum and maximum concentration of 578 CFU m^{-3} to 1708 CFU m^{-3}, respectively. The median concentration of fungi in the indoor air was 580 CFU m^{-3} with a minimum and maximum concentration of 342 CFU m^{-3} to 878 CFU m^{-3}, respectively (Fig. 2). Relative standard deviation of all samples was less than 1% (Fig. S1). The median concentration of bacteria present in the outdoor air was 742 CFU m^{-3} with a minimum and maximum concentration of 430 CFU m^{-3} to 1578 CFU m^{-3}. The median concentration of fungi in the outdoor air was 680 CFU m^{-3} with a minimum and maximum concentration of 371 CFU m^{-3} to 1225 CFU m^{-3}.

Bacterial concentration was higher in indoor air than outdoor air ($p < 0.001$, one-way ANOVA). However, fungal concentration was higher in outdoor air than indoor air ($p < 0.001$, one-way ANOVA). We found that observed bioaerosol concentrations in this study are lower than study reported in UK and higher than the studies reported in USA and Poland [80–82] (Table 2), possibly due to variations in geographical and climatic conditions [83–86]. The transport of microbes is primarily controlled by their hydrodynamics and kinetics, and their fate is determined by their chemical composition and the meteorological conditions to which they are exposed [83,87].

3.2. Parameters influencing indoor bacterial and fungal concentration

Multivariable linear regression analysis considering all variables showed that outdoor bacterial concentration, indoor temperature, number of inhabitant, age of home and type of fuel used for cooking were significant predictor variables for indoor bacterial concentration at $p < 0.05$ or better. The adjusted R^2 was 0.70 suggesting the model is able to capture a substantial portion of the variability in the observations (Table 3). Outdoor air contributes to indoor air microbial burden [88]. Likewise, the higher the number of inhabitants in a house and higher the time they spend indoors, higher will the potential for them to shed skin, saliva and gut microbiota cells colonized by bacteria to the indoor air [39,88]. The positive association with indoor temperature is consistent with previous works [30,89]. The indoor air temperature in our work ranged between 30□ and 33.6□ which may have been the range of optimum or near optimum growth for the bacteria, and higher temperature promoted higher growth [90]. Bacterial concentration was positively influenced by age of the building. The accumulated dust in old homes may have

285 provided more growth substrate for the growth of gram-negative bacteria [91,92]. Use of
286 biomass cow dung positively influenced bacterial concentration compared to use of LPG.
287 Biomass burning increases the particulate matter (PM) concentration in the air, and consequently
288 the concentration of bioaerosol attached to the PM [89,93]. In contrast with previous studies
289 [30,94], relative humidity did not have any significant role in influencing indoor bacterial
290 concentration.
291
292 Fungal outdoor concentration was the single significant predictor of indoor fungal concentration
293 ($p < 0.001$; adjusted $R^2 = 0.73$) (Table 3). This is consistent with reports that indoor fungi
294 primarily originate from outdoor sources [30,39,95]. In contrast to previous studies, home
295 ecology and meteorological factors were not significant for indoor fungal concentration [96,97].
296 This could be because fungi found in those works such as *Penicillium* and *Cladosporium* whose
297 growth is favored in high relative humidity and cooler environment [98], were not found
298 dominant in our study.
299
300 **3.3. Microbial community composition in the air**
301
302 A total of 3748 viable bacterial colonies were counted from all the households, which
303 contributed to 79 different bacterial species comprising the major phyla *Actinobacteria,*
304 *Firmicutes, Proteobacteria,* and *Bacteroidetes*. Both indoor and outdoor environment were
305 dominated by *Firmicutes* (70% in indoor and 70% in outdoor) compared to the other phyla
306 contributed in the airborne bacteria and subsequently in the atmospheric bioaerosol burden. This
307 is followed by the dominance of *Proteobacteria* (18% and 22% in indoor and outdoor,

308 respectively) and *Actinobacteria* (11% in indoor and 6% in outdoor). Remaining was contributed
309 by *Bacteroidetes* (1% and 2% in indoor and outdoor, respectively).
310
311 The dominant families of bacterial species combining both indoor and outdoor air of all houses
312 included, in decreasing order, *Bacillaceae, Staphylococcaceae, Pseudomonadaceae,*
313 *Microbacteriaceae, Planococcaceae, Nocardiaceae, Oxalobacteraceae, Enterobacteriaceae,*
314 *Acetobacteraceae, Burkholderiaceae, Gordoniaceae, Enterococeae, Cytophagaceae,*
315 *Paenibacillaceae, Promicromonosporaceae, Brevibacteriaceae* and *Lactobacillaceae*
316 (Supporting Information, Fig. S2a,b).
317
318 The dominant bacterial genera found in the study region were *Bacillus, Staphylococcus* and
319 *Micrococcus,* consistent with previous studies on indoor air [12,99,100]. Soil dust carried from
320 outside due to the wind and human activity could be the source for indoor *Bacilli* [99,101,102].
321 Human skin harbors a wide range of microbes, and it has been reported-*Staphylococcus* and
322 *Micrococcus* are the common genera found in human skin microbiome [37,103]. Thus, indoor
323 bacterial composition was influenced by both outdoor environment and human presence indoors.
324
325 There were certain bacterial species which were found either exclusively in indoor, or
326 exclusively in outdoor. For example, *Enterobacter cloacae* was found only in indoor air. This is
327 a common bacteria present in human body [104] and may be released to the indoor air by the
328 resident inhabitants. In contrast, *Bacillus aryabhattai* has been previously isolated from soil of
329 agricultural field [105] and was found only in the outdoor air in our work.
330

A total of 2681 viable fungal bioaerosols were counted from all the households, which contributed to 67 fungal species comprising of major phyla *Ascomycota* (92% in indoor and 90% in outdoor), *Basidiomycota* (3% in indoor and 4% in outdoor) and *Zygomycota* (5% in indoor and 6% in outdoor). Similar to the bacterial diversity distribution, we found high fungal diversity in indoor and outdoor environments with dominant families of *Trichocomaceae, Hypocreaceae, Sordariaceae, Pleosporaceae, Mucoraceae, Chaetomiaceae, Davidiellaceae, Hypoxylaceae, Xylariaceae, Hydnodontaceae, Polyporacea* and *Nectriaceae* (Fig. S2c,d). *Trichocomaceae* was found to be the most dominant in both indoor and outdoor environments followed by *Pleosporaceae*. The *Chaetomiaceae* family which has many plant pathogens was found more dominant in the outdoor air compared to the indoor air [106].The *Xylariaceae* family, which belongs to small ascomycetous fungi, was found only in the outdoor environment.

Aspergillus was the dominant genus found in both indoor and outdoor air. In contrast to the previous studies, this study did not find *Penicillium* or *Alternaria* as a dominant genus in the indoor air [59,107]. It has been reported that the concentration of *Penicillium* increases with the presence of mould patches [108] and no mould patches were observed in this study during the sampling period.

The indoor air of our sampled houses was composed of common microbial species, but some of these species have been reported as opportunistic pathogens and/or allergens. For example, various hypersensitivity reactions like asthma and sinusitis have been caused by some of the fungal species from the genus *Aspergillus* [109,110]. Thus, higher concentration and abundance of *Aspergillus* may have adverse implications on human health. Though *Micrococcus and*

354 *Staphylococcus* are commonly found bacterial genera, they have also been reported as
355 opportunistic pathogens [111] and continuous exposure to them may pose a risk to human health.
356
357 Out of thirty-six houses, bacterial communities in the indoor air of nineteen houses were found to
358 be more diverse than the outdoor air (Table S11a). In the overall, bacterial communities were
359 found to be more dominant (H = 1.86) in indoor air than the outdoor air (H = 1.73). The
360 calculated evenness of the indoor bacterial community was higher (E_h = 0.66) compared to the
361 outdoor community (E_h = 0.61) which suggests that the bacterial communities are more evenly
362 distributed in indoor air than the outdoor air. Although, the Gini-Simpson's bacterial diversity
363 index (D) suggested diversity is same in both the indoor and outdoor air (D = 0.69).
364
365 In contrast to bacterial communities, fungal communities were more dominant in the outdoor air
366 of twenty-four individual houses than the indoor air (Table S11b). In the overall, fungal
367 communities were found to be more dominant (H = 1.73) in the outdoor air than the indoor air
368 (H = 1.42). The calculated evenness of the outdoor fungal community (E_h = 0.70) was higher
369 than indoor fungal community (E_h = 0.60) which suggests that fungal communities are more
370 evenly distributed in outdoor air than in indoor air. The Gini-Simpson's diversity index (D) also
371 suggested that fungal communities are more diverse in outdoor air (D = 0.69) than in indoor air
372 (D = 0.60).
373
374 **3.4. Microbial risk assessment for pathogenic bacteria *B. anthracis***
375

376 *B. anthracis* was the most abundant pathogen in our work, and was isolated from the indoor air
377 of eight houses. Its concentration in the indoor air ranged from 17 CFU m^{-3} to 141 CFU m^{-3}.
378 Calculated probability of risk in the eight houses ranged from 0.0009 to 0.010 (Fig. 3). A
379 maximum probability of infection of 1% exists when a person inhales more than 100 spores
380 [112]. It has also been reported that more than 7000 spores are required to cause infection to 50%
381 of the population being exposed to this pathogen (Infective dose-ID_{50}) [112]. The calculated dose
382 in this study is smaller. As *B. anthracis* is primarily a soil bacterium, regular cleaning of houses
383 may reduce the infection probability. Likewise, resident women may also try to spend more time
384 outdoors where the concentrations were lower (seven of the eight houses did not have *B.*
385 *anthracis* in outdoor air and one had them in much lower concentration, 18 CFU m^{-3} about half
386 the indoor concentration).
387
388 **3.5. Airborne endotoxin concentration**
389
390 The median concentration of endotoxin on indoor particulate matter was 4.68 EU m^{-3} with a
391 minimum and maximum concentration of 1.23 EU m^{-3} and 20.7 EU m^{-3}, respectively.
392 Multivariable linear regression analysis showed that indoor bacterial concentration, indoor
393 temperature, relative humidity and type of fuel used for cooking were significant predictor
394 variables for indoor bacterial concentration at $p < 0.05$ or better (Table 4; adjusted $R^2 = 0.91$).
395 The positive influence of indoor bacterial concentration on endotoxin concentration, also
396 observed previously [47], is intuitive as gram-negative bacteria are the source of endotoxins.
397 These bacteria under increasing temperature and decreasing humidity (drier) environment may
398 potentially produce more endotoxins [80,113–116]. Biomass burning increases the PM

399 concentration in indoor air; the more the indoor PM concentration the more will be likelihood of
400 endotoxins to attach to PM and remain suspended in air [117].
401
402 **4. Conclusions**
403
404 We have presented data on indoor bioaerosols concentration and composition from a rural setting
405 in India, which are still scarce. We also report the richness and abundance of bacterial and fungal
406 aerosol and their health impacts in different household settings. In line with previous studies,
407 bacterial aerosols were richer and more abundant in all indoor and outdoor locations compared to
408 fungal aerosols. Also, the concentration of bacterial aerosols in indoor air was higher compared
409 to outdoor air. The larger aerodynamic diameter of fungi leads them to settle faster compared to
410 bacteria [12,39,99,118,119], Also, the sources of bacteria in indoor environments may be
411 stronger than the sources of fungi [41,120]. These could be the reasons that our indoor fungal
412 concentrations are lower than the indoor bacterial concentrations ($p < 0.001$, one-way ANOVA).
413 Significant predictor variables for indoor air bacterial concentration were outdoor air bacterial
414 concentration, indoor temperature, number of indoor inhabitants, age of homes, and the use of
415 biomass as fuel for cooking. Indoor air fungal concentration was almost entirely influenced by
416 outdoor air fungal concentration. Indoor air endotoxin concentration was influenced by indoor
417 bacterial concentration, indoor temperature, indoor relative humidity and the use of biomass as a
418 fuel for cooking. Multivariable regression analysis revealed that the considered predictor
419 variables were able to explain 70–91%; thus, other variables such as quantitative ventilation rates
420 [121] are also important. In line with previous studies, *Bacillus* and *Staphylococcus* were the
421 most dominant bacterial genera in both indoor and outdoor air. In contrast with other reports,

Aspergillus was the most dominant fungal genera, followed by *Trichoderma.* QMRA for exposure to *B anthracis* suggested probability of infection may be up to 1% in certain households when residents are exposed to them for 12 hours continuously. Novel information has been presented about the concentration, diversity and richness of the indoor bioaerosol, factors influencing their concentration and also the health impacts of pathogens in different households from a rural region. Such studies are important as they provide the information on indoor air quality where people spend most of their time.

Conflicts of interest

The authors declare no competing interests

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830 **Figure Captions**

831 Figure 1. Study area and sampling locations, Kyasaram village in Sangareddy district, Telangana
832 state, India.

833 Figure 2. Indoor and outdoor bacterial and fungal concentration.

834 Figure 3. Probability of infection as a function of exposure time (H = house number).

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Table 1. Input parameters used in Dose-Response model

Parameter	value	Reference
Inhalation rate (I, m^3 h^{-1})	0.342	[76]
Survival probability of single pathogen (k)	1.65×10^{-5}	[77,78]

Table 2. Concentration of airborne bacteria and fungi in different residential sectors of different countries

Country and type of locality	**Location**	**Sampling area**	**Number of samples**	**Concentration (CFU m^{-3})**						**Reference**
				Bacteria			**Fungi**			
				Mean	**Min**	**Max**	**Mean**	**Min**	**Max**	
Present Study (rural)	Village homes	Living room	36	1073	578	1708	582	342	878	
UK (sub-urban)	Housing Type1	Living room	5	1557*	1102	2438	925*	296	2452	[80]
	Housing Type 2	Living Room	5	2403*	1456	6332	813*	240	3236	[80]
	Housing type 3	Living room	5	5036*	2594	9780	2124*	1689	2671	[80]
USA (urban)	Single family residence	Room central to house	39	369	51	1158	369	83	1245	[81]
Poland (urban)	Story building	Living room	24[b] 27[f]	1021*	182	7745	225*	36	2494	[82]

858 *Type 1- Single room in shared accommodation, Type 2- Single bed room flat, Type 3- Two or*
859 *three bed room houses, *-Geometric mean, b: bacteria, f: fungi*

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Table 3. Results of multivariable linear regressions of log10 bioaerosol concentration (CFU m^{-3}) with potential predictor variables (*B:* unstandardized coefficients, *CI*: confidence interval, *SE:* standard error, *β:* standardized coefficients)

Predictor variable		Model 1: Indoor bacterial concentration				Model 2: Indoor fungal concentration			
R^2		0.784				0.806			
Adjusted R^2		0.697				0.729			
		B	**95% CI**	***SE***	***β***	***B***	**95% CI**	***SE***	***β***
Outdoor bacterial concentration (CFU m^{-3})		0.405 [b]	0.112 to 0.698	0.142	0.347	--	--	--	--
Outdoor fungal concentration (CFU m^{-3})		--	--	--	--	0.810 [a]	0.560 to 1.059	0.121	0.819
Outdoor relative humidity (%)		-0.269	-1.327 to 0.789	0.514	-0.079	-0.306	-1.286 to 0.674	0.476	-0.092
Outdoor temperature (□)		-0.844	-3.121 to 1.433	1.106	-0.108	-1.405	-3.602 to 0.792	1.067	-0.184
Indoor relative humidity (%)		0.032	-1.548 to 1.613	0.768	0.006	-0.153	-1.572 to 1.267	0.689	-0.027
Indoor temperature (□)		3.738 [b]	1.037 to 5.332	1.154	0.390	1.060	-1.212 to 3.332	1.103	0.113
Number of inhabitants		0.262 [b]	0.067 to 0.457	0.095	0.293	0.006	-0.035 to 0.065	0.087	0.007
Number of rooms	2 and above	Referent							
	1 and below	-0.020	-0.074 to 0.034	-0.075	-0.763	0.019	-0.035 to 0.073	0.026	0.73
Indoor pets	Absent	Referent							
	Present	0.021	-0.033 to 0.076	0.026	0.080	0.015	-0.035 to 0.065	0.024	0.058
Age of homes (years)		0.072 [c]	0.000 to 0.143	0.035	0.213	0.003	-0.064 to 0.069	0.032	0.008
Type of cooking fuel	LPG	Referent							
	Biomass	0.066 [c]	0.005 to 0.127	0.030	0.237	0.029	-0.028 to 0.087	0.028	0.109

[a] $p < 0.001$
[b] $p < 0.01$
[c] $p < 0.05$

LPG: liquefied petroleum gas; Biomass: cow dung and firewood

Table 4. Results of multivariable linear regressions of log10 endotoxin concentration (EU m^{-3}) with potential predictor variables (*B:* unstandardized coefficients, *CI*: confidence interval, *SE:* standard error, *β:* standardized coefficients)

Predictor variable		Model: Indoor endotoxin concentration			
R^2		0.941			
Adjusted R^2		0.914			
		B	**95% CI**	***SE***	***β***
Outdoor bacterial concentration (CFU m^{-3})		-0.074	-0.496 to 0.349	0.205	-0.027
Outdoor relative humidity (%)		0.276	-1.056 to 1.609	0.646	0.035
Outdoor temperature (□)		-0.725	-3.611 to 2.161	1.398	-0.039
Indoor bacterial concentration (CFU m^{-3})		1.823 [a]	1.307 to 2.338	0.250	0.775
Indoor relative humidity (%)		-2.283 [b]	-4.263 to -0.302	0.960	-0.170
Indoor temperature (□)		3.941 [b]	0.393 to 7.488	1.719	0.175
Number of inhabitants		0.003	-0.277 to 0.282	0.135	0.001
Number of rooms	2 and above	Referent			
	1 and below	-0.053	-0.121 to 0.016	0.033	-0.085
Indoor pets	Absent	Referent			
	Present	-0.021	-0.090 to 0.048	0.033	-0.033
Age of homes (years)		0.006	-0.091 to 0.103	0.047	0.007
Type of cooking fuel	LPG	Referent			
	Biomass	0.083 [b]	-0.001 to 0.166	0.040	0.127

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949 [a] $p < 0.001$
950 [b] $p < 0.05$
951 LPG: liquefied petroleum gas; Biomass: cow dung and firewood
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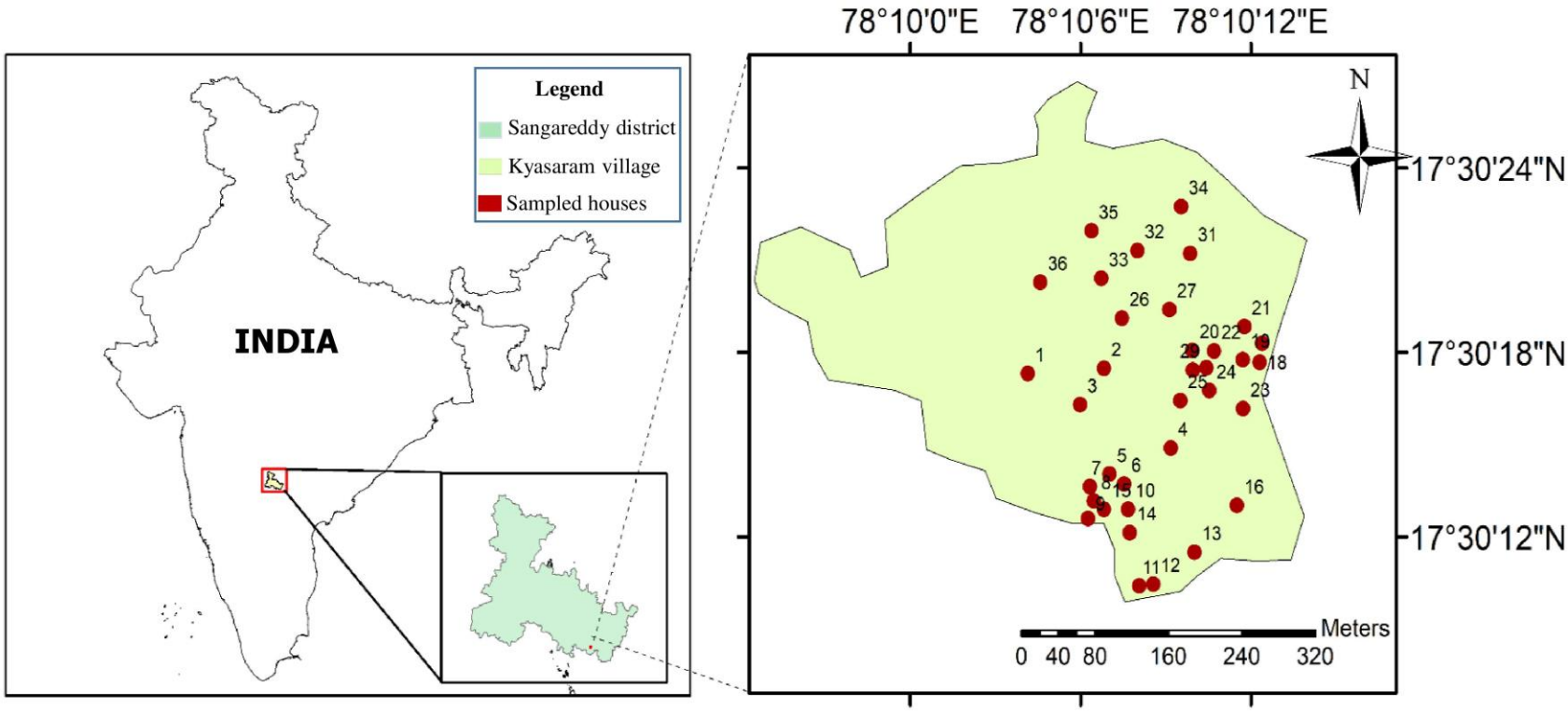

Figure 1. Study area and sampling locations, Kyasaram village in Sangareddy district, Telangana state, India

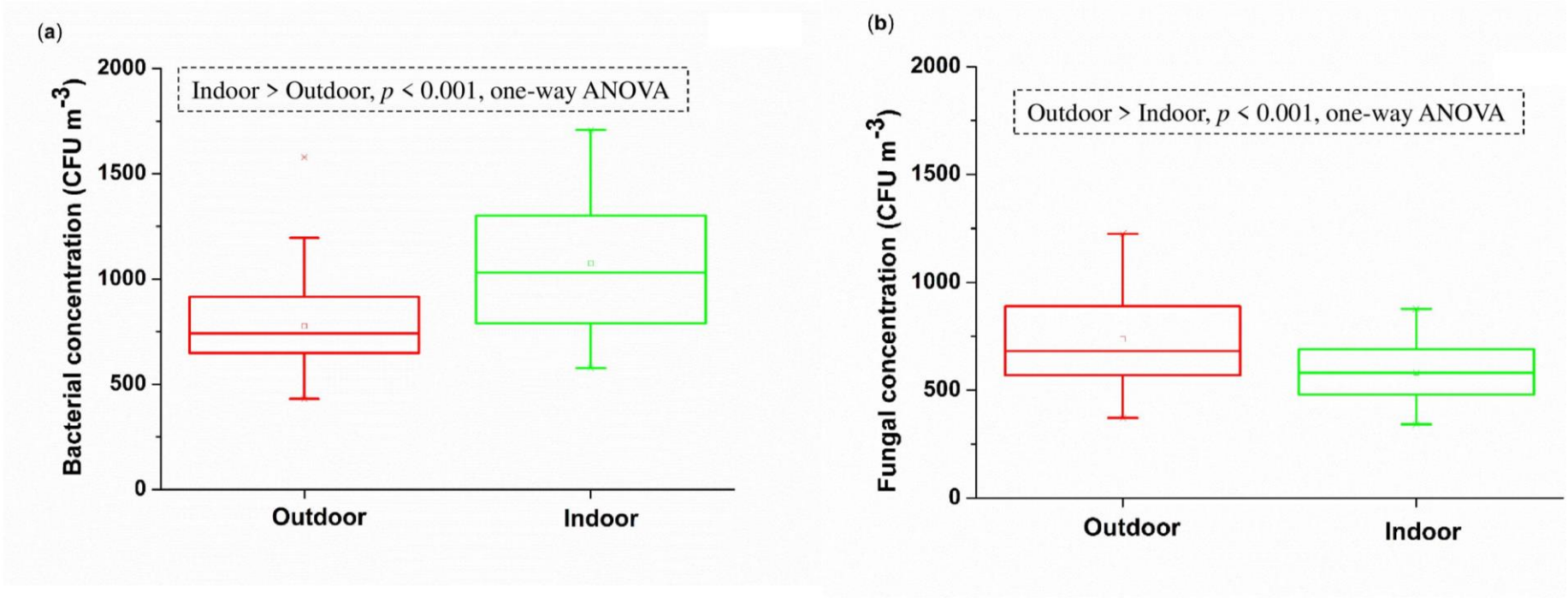

Figure 2. Indoor and outdoor bacterial and fungal concentration

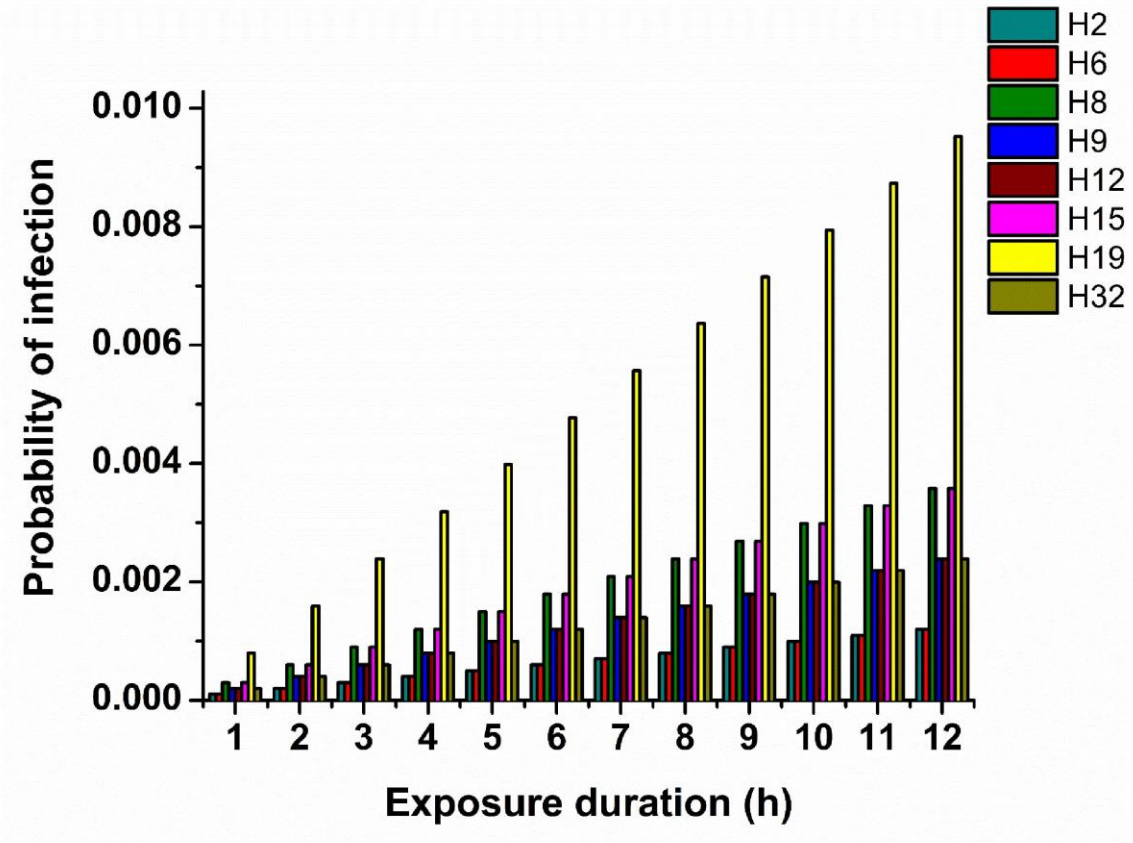

Figure 3. Probability of infection as a function of exposure time (H = house number)

Highlights

- Bacterial, fungal and endotoxin concentrations were determined in indoor and outdoor of rural homes in India
- Bacterial concentrations in indoor air were higher than in outdoor air. Fungal concentrations in outdoor air were higher than in indoor air.
- Biomass burning was found to be important variable influencing the indoor bacterial and endotoxin concentration.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: