



Indoor and outdoor bioaerosol distributions and concentration profiles during different seasons and pollution events in Qingdao city

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ABSTRACTS

Most people spend more than 10 h indoors daily, accompanied by the deposition of pathogenic microbes in the respiratory system. This health risk has not been fully studied across different seasons and pollution events. We investigated bioaerosol samples from offices, laboratories, and outdoor environments at three universities in a typical coastal city. Using fluorescence microscopy with 4',6-diamidino-2-phenylindole (DAPI) staining and live/dead BacLight™ staining, the concentrations of viable bacteria (VBs), nonviable bacteria (NVBs), and total airborne microbes (TAMs) were in the range of $(3.21\text{--}6.02) \times 10^3$ cells/m³, $(7.09\text{--}19.08) \times 10^4$ cells/m³, and $(1.52\text{--}5.78) \times 10^5$ cells/m³, respectively. The outdoor levels of bioaerosols were consistent across the universities. The outdoor bacterial viability (1.8–14.4 %) was slightly lower than that at offices (2.71–15.94 %) and laboratories (2.12–19.34 %). Correlation analysis and indoor/outdoor (I/O) ratio comparisons revealed that outdoor sources exerted a dominant influence on indoor microbial concentrations. Notably, pollution events significantly altered both indoor and outdoor bioaerosol levels and their size distributions. During pollution events, indoor bioaerosol concentrations were 1.24–2.12 times higher than those on clean days, influenced primarily by relative humidity (RH) and particulate matter (PM) levels. The deposition characteristics of VBs from fine particles in the human respiratory tract during haze and dust events generally indicated higher levels indoors than outdoors, and higher in females than in males. The highest health risk index value was 0.752 during dust events. Our findings strongly suggested controlling indoor RH and PM levels, especially during pollution events, to reduce the health risks associated with pathogenic microbes.

1. Introduction

Bioaerosols are ubiquitous in human living environments, and the outbreak of COVID-19 has significantly increased academic interest in effects of bioaerosols on the human health [1–3]. Bioaerosols are suspended particles that may contain various pathogenic or nonpathogenic microbes, such as bacteria, fungi, and viruses [4,5]. Outdoors, bioaerosols are associated with a wide range of sources, including natural sources such as water bodies, soil, plants and animals, waste, and feces [6–8], as well as direct releases at various locations due to human activities, including farms, landfills, wastewater treatment plants, and kitchen chimneys [9]. Humans spend most of their time indoors, where heating, ventilation, and air-conditioning (HVAC) systems, emissions

from plants, animals, and human activities (such as walking, breathing, or sneezing) can influence indoor microbial abundance levels [10–13]. Bioaerosols are disseminated via airborne transmission and droplet dispersion, and fine particles can be inhaled and deposited in various parts of the human respiratory tract [14]. Exposure to airborne microbes can pose significant threats to life and well-being [4,15,16], causing illnesses such as the some viral infections, tuberculosis [17], severe acute respiratory syndrome (SARS), influenza, COVID-19 [18], respiratory conditions, and even neoplasms. Therefore, investigating the characteristics of bioaerosols in indoor and outdoor environments is important for human health.

Extensive research has focused on indoor bioaerosol levels in settings such as kindergartens [19,20], daycare centers, sanatoriums [21,22],

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hospitals [23], and residential areas [24], with an emphasis on the sensitivity of elderly individuals, children, and patients. According to our investigation (Fig. S1), 86.86 % of university faculty and students remained indoors for more than 10 h, and 62.37 % remained indoors for more than 16 h. Priyamvada et al. reported higher fungal and bacterial levels in university labs than in student areas [25], while the highest microbial concentrations in Iranian student dorms, followed by classrooms and laboratories [26]. Although indoor bioaerosols have been widely studied, research on microbial concentrations in universities is limited. In addition, the limited studies have concentrated on culturable fungi and bacteria in atmospheric bioaerosols [27–30]. However, these culturable microbes constitute less than 1 % of the total airborne microbes (TAMs, including culturable and nonculturable microbes) in the atmosphere [31]. Therefore, further investigations into the concentration patterns and sources in universities are needed.

For sources influencing indoor bioaerosols, occupants and outdoor air are considered key factors [32,33]. Bioaerosols from outdoor environments can penetrate indoor spaces via natural ventilation (e.g., windows and doors) [34], infiltration [35], and mechanical ventilation systems (e.g. HVAC systems) [36,37]. Occupants can impact indoor microbial concentrations through respiratory behaviors [38], shedding from the human body [39], and resuspension induced by direct or indirect contact with surfaces [40]. The characteristics of outdoor bioaerosol concentrations are influenced by meteorological factors and weather conditions, often resulting in seasonal, interannual, and regional differences. For example, outdoor microbial concentrations peak in winter and decline in summer in various years [41–43]. However, the distribution of fungal and bacterial concentrations in indoor bioaerosols varies notably due to factors such as seasonal fluctuations, varying floor elevations [44], diverse indoor environments [45], and diurnal variations [46]. There is a lack of comparative studies on the distributions of indoor and outdoor microbes, especially TAMs and viable bacteria. Thus, further studies are needed to clarify the microbial level and sources, enhancing our understanding of the influence of outdoor microbes on indoor microbes.

In this study, bioaerosol samples were collected from offices, laboratories, and outdoor environments across three universities in Qingdao during seasons, as well as during pollution events from 2023 to 2024. Our research focused on three aspects: (1) analyzing the effects of seasonal variations and (2) pollution events on the concentrations and size distributions of TAMs, viable bacteria (VBs), and nonviable bacteria (NVBs) both indoors and outdoors and (3) assessing the health risks associated with inhalable microbes. The results can provide scientific evidence on the dynamics and factors controlling bioaerosols and enhance our understanding of the health implications of bioaerosols.

2. Materials and methods

2.1. Sampling locations and environments

As indicated by the China Meteorological Administration (<http://www.cma.gov.cn/>), Qingdao is located in southeastern Shandong Province, China, along the Yellow Sea, with geographical coordinates between 35°35' and 37°09' N latitude and 119°30' to 121°00' E longitude. Qingdao has a temperate monsoon climate with distinct seasons, and the annual average humidity reaches 73 %. Southeasterly and northwesterly winds prevail in spring and winter, respectively. The sample collection in this study was divided into two stages on the basis of different research objectives. In the first stage, we established long-term monitoring from March 2023 to April 2024 at Ocean University of China (OUC), an independent site for analyzing bioaerosol distribution patterns under seasonal and pollution events. Owing to the frequent dust events in spring and the common occurrence of haze events in winter in Qingdao, sample collection was mainly concentrated in spring and winter. In the second stage, a multi-university comparison from March to April 2024 was conducted based on preliminary findings from

the long-term monitoring, aiming to validate the spatial generalizability of these patterns. We synchronized sampled across three universities in Qingdao, including OUC, (36°10'10.851"N, 120°30'17.713"E), Shandong University (SDU, 36°22'14.300"N, 120°41'38.598"E), and Qingdao University (QDU, 36°04'20.01"N, 120°25'20.73"E). The detailed locations are shown in Fig. 1.

Outdoor sampling environments at the three universities varied in green and water areas, traffic and pedestrian flow and road materials. The offices and laboratory environments at the three universities varied in their area and purpose, which had different anthropogenic activities. The surrounding environment (Fig. 2), indoor area, usage, and other specific information on the sampling points are provided in Table S1. Indoor sampling was performed at two diagonal points within each room, with simultaneous indoor and outdoor sampling, and the distribution of the indoor sampling points is shown in Fig. 3.

2.2. Sample collection and measurement

To investigate the variations between indoor and outdoor environments, from March 2023 to April 2024, 249 bioaerosol samples were collected at OUC, including 93 samples of TAMs, 78 samples of VBs, and 78 samples of NVBs. From March to April 2024, two groups of simultaneous indoor and outdoor bioaerosol samples were collected at the three universities in Qingdao due to limitations on the actual sampling conditions. Group 1 represented simultaneous sampling at SDU and OUC in March. Group 2 represented simultaneous sampling at QDU and OUC in April.

The fog, haze, dust events, sunny and cloudy days were identified on the basis of the weather phenomena from the Meteorological Information Comprehensive Analysis and Process System (MICAPS) of the China Meteorological Administration. Samples collected on sunny/cloudy days with $PM_{2.5} < 35 \mu\text{g}/\text{m}^3$ and $PM_{10} < 50 \mu\text{g}/\text{m}^3$ were classified as clean day samples, based on the Grade 1 Ambient Air Quality Standard of China (GB 3095–2012). And we classified the samples collected during fog, haze and dust events as polluted events samples.

Haze events occurred frequently in winter, and dust was transported to Qingdao in spring. Therefore, sample collection was mainly concentrated in winter and spring. According to the seasonal classification standards issued by the China Meteorological Administration, spring was defined as from March to May, and winter was from December to February in this study. The indoor and outdoor meteorological parameters in different seasons and pollution events are provided in Table S2–3.

During sampling, any interior doors and windows remained closed, and outdoor meteorological parameters (particulate matter with an aerodynamic diameter smaller than $2.5 \mu\text{m}$ ($PM_{2.5}$), coarse particulate matter (PM_{10}), relative humidity (RH), wind speed (WS), and visibility) and indoor environmental parameters (temperature, RH, particle concentration, and population density) were recorded. Bioaerosol samples were collected using a six-stage microbial FA-1 cascade impactor (Liaoyang Application Technology Research Institute, China) at a flow rate of 28.3 L/min for 30 min on sterile polycarbonate membranes ($0.22\text{-}\mu\text{m}$ pore size; 80-mm diameter). All the membranes and materials were sterilized at 121°C for 15 min before use. The six-stage particle size ranges were $> 7.0 \mu\text{m}$, $4.7\text{--}7.0 \mu\text{m}$, $3.3\text{--}4.7 \mu\text{m}$, $2.1\text{--}3.3 \mu\text{m}$, $1.1\text{--}2.1 \mu\text{m}$, and $0.65\text{--}1.1 \mu\text{m}$. For further analysis, particles smaller than $2.1 \mu\text{m}$ were classified as fine particles, and those larger than $2.1 \mu\text{m}$ as coarse particles.

After collection, the sample membranes were employed to prepare microbial suspensions. The samples were stained with 4',6-diamidino-2-phenylindole (DAPI) in the dark for 8 min. Additionally, 10 mL of each suspension was stained with a BacLight™ Bacterial Viability Kit (L-13,152, Thermo Fisher Scientific, USA) containing 36 nM SYTO-9 and 180 nM propidium iodide (PI) for 15 min in the dark. After staining, the samples were filtered through $0.2\text{-}\mu\text{m}$ black polycarbonate filters (Whatman Inc., USA) to prepare microbial slides, which were observed

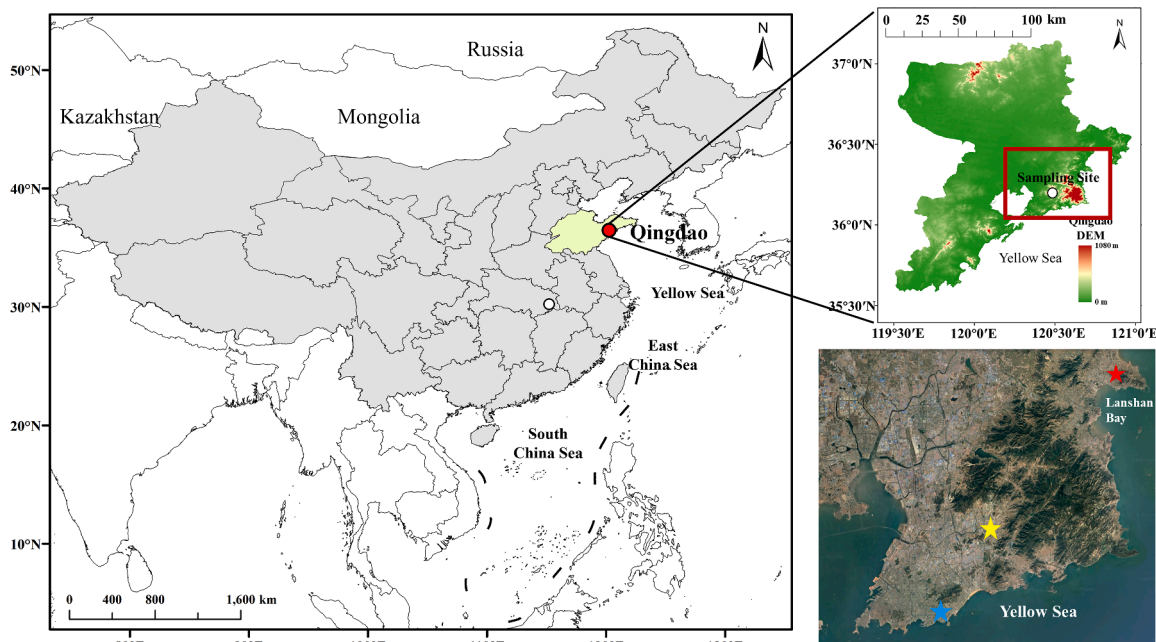


Fig. 1. Map of the sampling sites (red—SDU; yellow—OUC; blue—QDU).



Fig. 2. Sampling environments at the three universities (OUC—Ocean University of China; SDU—Shandong University; QDU—Qingdao University).

and counted using an epifluorescence microscope, with twenty random fields of view counted [47,48]. Bacteria were distinguished from mineral particles by identifying spherical particles with diameters smaller than 1 μm [49]. Additional details can be found in Text SII.

2.3. Modeling of deposition in the respiratory system and health risks

Via the use of the multiple-path particle dosimetry (MPPD) model

(version 3.04) and actual measured bioaerosol data, we calculated the deposition dose (DD) of bioaerosols in the human respiratory tract. By adjusting the respiratory parameters, we estimated the PM deposition fraction in the respiratory tract [50,51]. The deposition efficiency calculated from the model was used to calculate the bioaerosol DD according to the equation $DD = DF \times BC \times TV \times F \times T\#(1)$, where DD is the DD of inhalable microbes in the human respiratory tract (cells/h), DF is the deposition efficiency of inhalable microbes in the

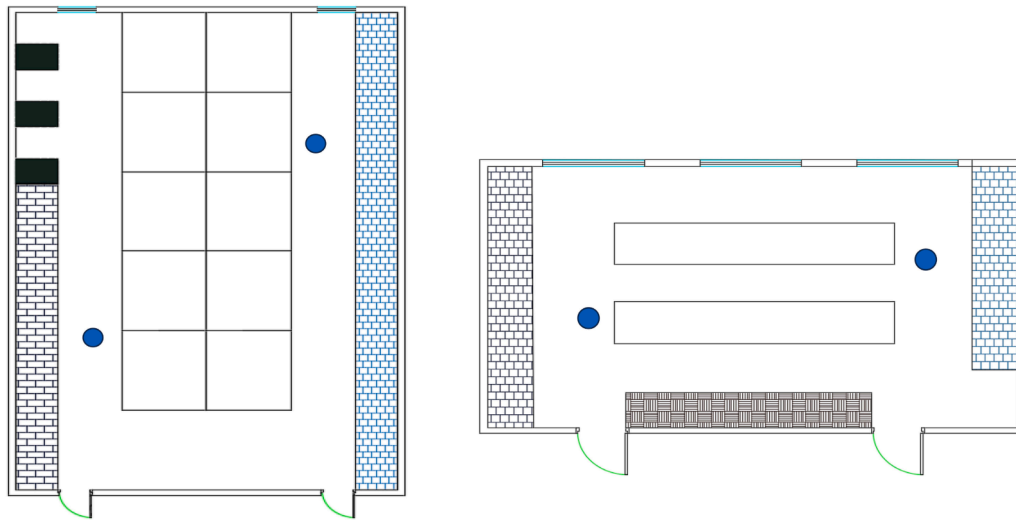


Fig. 3. Diagram of the sampling sites in office (left) and laboratory (right).

human respiratory tract, BC is the concentration of inhalable microbes (cells/m^3), TV is the tidal volume (m^3), F is the breathing frequency (min^{-1}), and T is the exposure time (60 min/h). The inhalation risk of bioaerosols was assessed using a U.S. EPA-recommended model [50,52]. On the basis of the DD, we calculated the exposure DD (EDD) for different populations over specific exposure times. The hazard quotient (HQ) was the ratio of the total DD to the maximum acceptable daily DD (reference dose, RfD). $\text{HQ} > 1$ indicates potential adverse health risks. Additional details regarding the model parameters and formulas can be found in the supplementary materials.

2.4. Statistical analysis

The outdoor meteorological parameters (T, RH, WD, WS, and visibility) and environmental indices ($\text{PM}_{2.5}$, PM_{10} , SO_2 , NO_2 , CO, and O_3) were obtained from the Qingdao Meteorological Bureau (<http://qdx.qingdao.gov.cn/>) and the Qingdao Environmental Protection Bureau (<http://www.qepb.gov.cn/m2/>), respectively. The indoor $\text{PM}_{1.0}$, $\text{PM}_{2.5}$, PM_{10} , and total suspended particle (TSP) concentrations were measured via an online dust monitor (OPM-6303; Cubic Junray Instrument Co.), whereas the temperature and RH were recorded with a digital thermometer-hygrometer (HTC-1, Xinwei Electronic Technology Co., LTD). The indoor population density was calculated as the number of people per unit area (persons per square meter, ppsm). The average values of the indoor and outdoor environmental parameters during the sampling period are listed in Tables S2 and S3.

Redundancy analysis (RDA) was conducted with the RDA plugin (v1.10) in OriginPro (v2024), and Spearman correlation analysis was performed to examine the relationships between microbes and meteorological factors. One-way analysis of variance (ANOVA) was performed to test for statistically significant differences in concentrations and proportions, and a linear regression model was adopted to assess the relationship between indoor and outdoor particulate concentrations. A p -value > 0.05 was considered nonsignificant, whereas $p < 0.05$ was considered statistically significant.

3. Results

3.1. Concentrations and bacterial viability

3.1.1. Indoor and outdoor concentrations of bioaerosols in spring and winter

To better understand the seasonal variations in the indoor and outdoor concentrations of bioaerosols, we collected 249 bioaerosol samples

continuously in the outdoor, office and laboratory environments at the OUC from March 2023 to April 2024. The concentrations of TAMs in both the indoor and outdoor environments decreased in the following order: outdoors ($3.33 \pm 3.64 \times 10^5 \text{ cells}/\text{m}^3$) $>$ office ($2.29 \pm 1.74 \times 10^5 \text{ cells}/\text{m}^3$) \approx laboratory ($2.26 \pm 1.42 \times 10^5 \text{ cells}/\text{m}^3$). In the indoor and outdoor environments, bacteria accounted for 2.5 % to 72.0 % of TAMs, which was consistent with the literature [52–54].

The average concentrations of microbes in bioaerosols in the indoor and outdoor environments in spring and winter are listed in Table 1. The seasonal distributions of the outdoor VB concentrations were high in winter and low in spring. The seasonal concentrations of NVBs and TAMs were the opposite to those of VBs. This agreed with the results obtained in coastal cities from 2007 to 2008 and 2013–2014 [48,55].

In the indoor environment, the seasonal distribution trends of TAMs, VBs and NVBs were the same as that outdoors, with high VB concentrations in winter and low ones in spring, whereas the opposite was observed for NVBs and TAMs. Overall, in both spring and winter, the outdoor microbial concentrations were generally higher than those indoors.

3.1.2. Indoor and outdoor concentrations of bioaerosols during pollution events

Table 2 provides the average bioaerosol concentrations in the outdoor, office and laboratory environments during pollution events. The findings indicated that the outdoor concentrations of TAMs, VBs and

Table 1

Indoor and outdoor bioaerosol concentrations (mean \pm standard deviation) at the OUC in spring and winter.

Sample Site (AVG \pm RSD)	^a VBs ($\times 10^4 \text{ cells}/\text{m}^3$)		^b NVBs ($\times 10^4 \text{ cells}/\text{m}^3$)		^c TAMs ($\times 10^5 \text{ cells}/\text{m}^3$)	
	Winter	Spring	Winter	Spring	Winter	Spring
Outdoor ($n^d=20$)	0.62 ± 0.49	0.48 ± 0.11	9.00 ± 3.26	17.45 ± 16.88	2.18 ± 0.43	5.81 ± 6.36
Office ($n = 20$)	0.45 ± 0.37	0.40 ± 0.11	5.84 ± 1.89	14.62 ± 16.04	1.65 ± 0.32	3.46 ± 2.95
Laboratory ($n = 20$)	0.42 ± 0.39	0.41 ± 0.14	6.00 ± 1.66	12.15 ± 10.76	1.68 ± 0.32	3.32 ± 2.27
F-	1.334	0.779	11.218	0.256	13.691	0.972
P-	0.271	0.477	0.000**	0.777	0.000**	0.393

* $P < 0.05$ ** $P < 0.01$.

^a viable bacteria.

^b nonviable bacteria.

^c total airborne microbes.

^d number of sampling days.

Table 2

Indoor and outdoor bioaerosol concentrations (mean \pm standard deviation) at the OUC during pollution events.

Pollution events (cells/m ³)		Clean (n ^d = 13)	Dust (n = 4)	Haze (n = 8)	Fog (n = 4)	F	P
VBs $\times 10^4$	Outdoor	0.51 \pm 0.44	0.49 \pm 0.11	1.06 \pm 0.56	0.48 \pm 0.11	3.334	0.036*
	Office	0.40 \pm 0.15	0.48 \pm 0.14	0.66 \pm 0.49	0.36 \pm 0.20	1.544	0.228
	Laboratory	0.35 \pm 0.09	0.48 \pm 0.14	0.61 \pm 0.57	0.35 \pm 0.10	1.23	0.319
NVBs $\times 10^4$	Outdoor	9.65 \pm 3.67	15.40 \pm 1.73	11.20 \pm 4.18	7.55 \pm 1.07	2.968	0.053
	Office	8.13 \pm 4.22	11.15 \pm 4.20	5.96 \pm 2.07	6.02 \pm 3.03	1.674	0.2
	Laboratory	7.67 \pm 4.22	9.57 \pm 2.67	6.44 \pm 2.42	6.23 \pm 2.12	0.679	0.574
TAMs $\times 10^5$	Outdoor	2.12 \pm 0.31	8.35 \pm 8.53	2.40 \pm 0.39	2.35 \pm 1.03	4.786	0.009*
	Office	1.79 \pm 0.36	3.80 \pm 2.52	1.54 \pm 0.23	1.80 \pm 0.58	4.684	0.010*
	Laboratory	1.71 \pm 0.36	3.78 \pm 2.12	1.65 \pm 0.22	1.90 \pm 0.86	5.263	0.006*

* $P < 0.05$ ** $P < 0.01$.

^dnumber of sampling days.

NVBs during pollution events were 1.59–3.94 times greater than those on clean days. The TAM concentration reached as high as $(8.35 \pm 8.53) \times 10^5$ cells/m³ during dust events, which was significantly greater than that during other periods (Fig. 4-c2). The outdoor VB concentration was highest $((1.06 \pm 0.56) \times 10^3$ cells/m³) during haze events ($P < 0.05$), followed by dust events, whereas the outdoor VB concentration was low

on clean and foggy days.

The indoor concentrations of TAMs, VBs and NVBs were 1.24–2.12 times higher on polluted days than on clean days. The TAM concentrations ranged from $(1.66\text{--}6.32) \times 10^5$ cells/m³ during dust events, which were significantly higher than those during haze and fog events ($P < 0.05$) (Fig. 4-b2). The indoor VB concentrations conformed with the

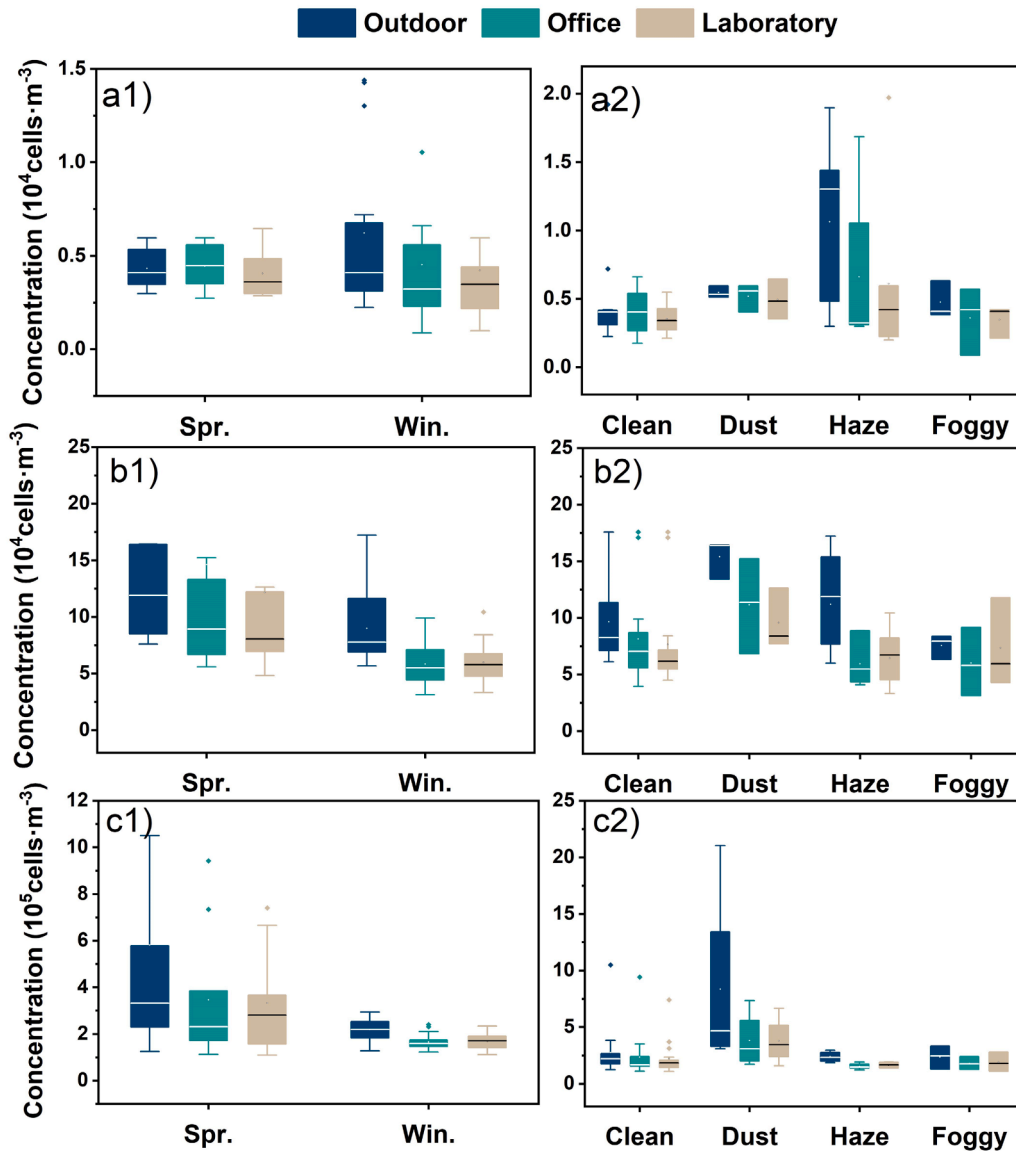


Fig. 4. Indoor and outdoor concentrations of VBs (a1, a2), NVBs (b1, b2), and TAMs (c1, c2) during different seasons (1) and pollution events (2). The box frames denote the upper and lower quartiles, the line denotes the median, and the whiskers denote the range.

outdoor trends, with the highest concentration of $(0.04\text{--}1.15) \times 10^4$ cells/m³ occurring during haze events and the lowest concentration of $(1.6\text{--}35.61) \times 10^3$ cells/m³ occurring on foggy days.

The outdoor concentrations of NVBs and TAMs were significantly greater than the indoor concentrations, especially during haze events ($P < 0.05$).

3.1.3. Comparison of microbial concentrations between universities

To validate the spatial generalizability of the concentration patterns, two groups of simultaneous comparison experiments were conducted from March to April 2024 at the three universities in Qingdao. The concentrations and particle size distributions of TAMs, VBs, and NVBs in bioaerosols at OUC, SDU, and QDU are shown in Fig. 5.

During the observation period, the outdoor levels of TAMs, VBs, and NVBs at the three universities were similar, with TAM, VB, and NVB concentrations ranging from 1.52×10^5 to 5.79×10^5 cells/m³, from 3.20×10^3 to 6.01×10^3 cells/m³, and from 7.09×10^4 to 1.91×10^5 cells/m³, respectively. These findings conformed with previous studies in the coastal region of Qingdao and the southwestern coast of Japan [47,49,56]. Although the indoor environments differed, the indoor concentrations of TAMs, VBs, and NVBs were similar across the universities, with the TAM concentration ranging from 1.17×10^5 to 3.84×10^5 cells/m³, the VB concentration ranging from 2.4×10^3 to 5.7×10^3 cells/m³, and the NVB concentration ranging from 4.82×10^4 to 1.54×10^5 cells/m³. Overall, in the comparative experiments, the outdoor microbial concentrations were higher than those indoors. The bioaerosol size distributions were similar across the universities, with most microbes occurring in coarse particles (61–80 %), which agrees with extensive findings on the size distribution [41,43].

Whether for indoor or outdoor microbes, the concentrations and size distributions were consistent across the three universities, with no significant difference, despite variations in the sampling environments and human activities.

3.1.4. Indoor and outdoor bacterial viability

Bacterial viability (BV), the proportion of viable bacteria to the total bacterial concentration, was considered to be closely associated with cardiovascular diseases, respiratory diseases, and other health conditions. In this study, the BV among the three universities showed consistency, with the value ranging from 2.98 % to 3.76 % in outdoor environments, 3.07 % to 5.43 % in office, and 3.50 % to 4.47 % in laboratory. The outdoor BV (1.8–14.4 %) was slightly lower than that in the offices (2.71–15.94 %) and laboratories (2.12–19.34 %) at the OUC from March 2023 to April 2024.

Affected by seasonal changes and pollution events, the BV in different indoor and outdoor environments was also different. The average bacterial viabilities in the outdoor, office and laboratory environments in winter were 6.06 ± 2.89 %, 6.62 ± 3.15 % and 6.12 ± 3.50 %, respectively, which were always higher than those in spring (3.69 ± 0.81 %, 4.73 ± 1.58 % and 4.54 ± 1.08 %, respectively).

The BV in the outdoor, office and laboratory environments was the highest during haze events, with mean values of 8.53 ± 3.18 %, 9.01 ± 3.33 % and 9.02 ± 6.40 %, respectively, whereas it was lowest during dust events, with mean values of 3.06 ± 0.40 %, 4.12 ± 0.65 % and 4.98 ± 1.12 %, respectively. During haze and dust events, the indoor BV was higher than outdoor ones. However, indoor BV were similar to outdoor ones on clean days. On foggy days, the outdoor BV (6.20 ± 2.09 %) was higher than office (5.40 ± 2.77 %) and laboratory environments (5.17 ± 1.49 %).

3.2. Indoor and outdoor size distributions of bioaerosols

3.2.1. Indoor and outdoor size distributions of bioaerosols in spring and winter

Fig. 6 shows the indoor and outdoor size distributions of bioaerosols at the OUC in spring and winter. Table S6 details the proportions of VBs, NVBs, and TAMs in fine and coarse particles. In spring, the outdoor VBs exhibited a distribution peaking at $3.3\text{--}4.7$ μm (26.46 %). The indoor VBs showed a valley value at $2.1\text{--}3.3$ μm (13.60–14.97 %) and higher

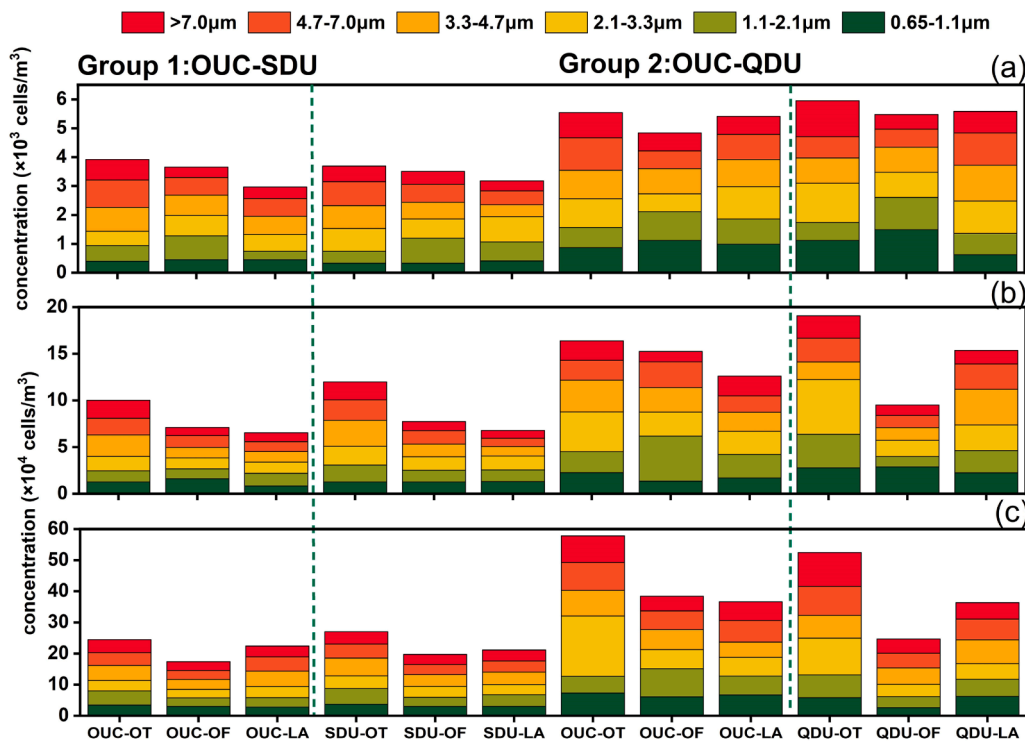


Fig. 5. Concentrations and size distributions of VBs (a), NVBs (b) and TAMs (c) in the two groups of indoor and outdoor samples from the SDU, QDU and OUC (Group 1: simultaneous sampling at the SDU and OUC; Group 2: simultaneous sampling at the QDU and OUC. OT—outdoor; OF—office; LA—laboratory).

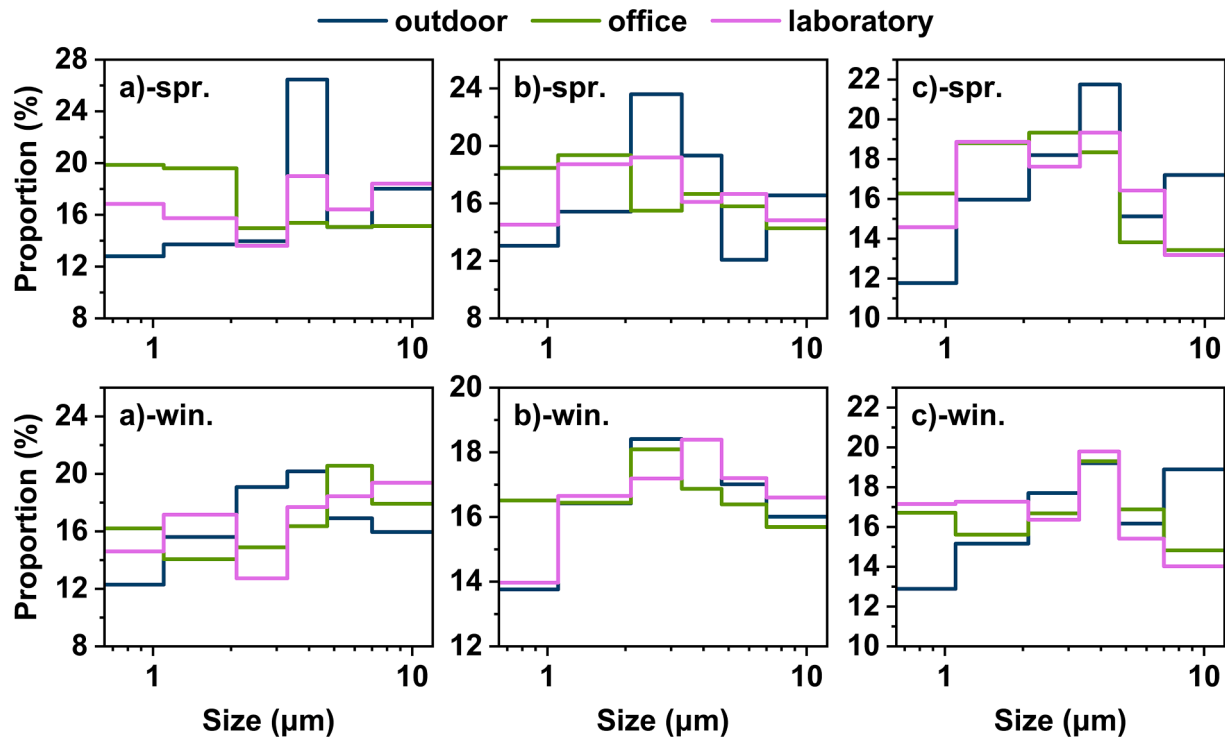


Fig. 6. Proportions of the VB (a), NVB (b) and TAM (c) concentrations within different particle size ranges in the indoor and outdoor environments in spring and winter.

proportions (33.45–35.07 %) in the 0.65–2.1 μm range than outdoors (27.73 %). In winter, outdoor VBs had a high proportion at 2.1–4.7 μm (19.08–20.17 %), while a high proportion of VBs exhibited sizes of 4.7–7.0 μm (20.56 %) and >7.0 μm (19.38 %) in the office and laboratory environments, respectively.

In spring, the outdoor size distribution of TAMs was similar to VBs, peaking at 3.3–4.7 μm (21.75 %). The size of a high proportion (17.62–19.33 %) of TAMs ranged from 1.1 to 4.7 μm in both the office and laboratory environments. In winter, TAMs exhibited similar size distributions in the indoor and outdoor environments, with peaks ranging from 3.3 to 4.7 μm and proportions ranging from 19.20 to 19.79 %.

Overall, VBs, NVBs and TAMs primarily comprised coarse particles in spring and winter, with proportions ranging from 60.54 % to 73.49 %. Notably, in the outdoor environment, the proportion of microbes in fine particles was greater in winter (27.89–30.18 %) than in spring (26.51–28.47 %). Conversely, the indoor proportions of microbes in fine particles were lower in winter (30.27–34.42 %) than in spring (32.58–39.46 %). Furthermore, during both seasons, the indoor proportion of microbes in fine particles was consistently greater than the outdoor proportion, ranging from 1.01 to 1.49 times the outdoor proportion.

3.2.2. Indoor and outdoor size distributions of bioaerosols during pollution events

Fig. 7 shows the indoor and outdoor size distributions of microbes from March 2023 to April 2024 during pollution events. On clean days, the proportion of VBs increased with increasing particle size in both the indoor and outdoor environments. However, the indoor proportions within the 0.65–1.1 μm range were greater (14.46–16.20 %) than the outdoor proportions (12.39 %). During haze events, VBs exhibited an approximately bell-shaped distribution outdoors, peaking at 3.3–4.7 μm (27.18 %). The office and laboratory samples yielded similar size distributions, with proportions fluctuating between 12.23 % and 22.11 %. On foggy days, outdoor VBs showed a distribution with peaks of 1.1–2.1

μm (26.30 %) and 3.3–4.7 μm (20.55 %). In the indoor environment, VBs showed a distribution with fine peaks at 1.1–2.1 μm in both office (20.05 %) and laboratory (18.87 %) but coarse peaks ranging from 4.7 to 7.0 μm (20.49 %) and 3.3–4.7 μm (22.73 %) in office and laboratory, respectively. During dust events, VBs in outdoor and laboratory exhibited distributions with peaks at 3.3–4.7 μm (23.36 % and 21.58 %, respectively). However, VBs in office showed a different size distribution, with a high value of 0.65–2.1 μm (16.72–22.83 %) and the lowest proportion of 11.85 % within the 2.1–3.3 μm range. Moreover, the indoor proportions of VBs in fine particles were significantly greater (32.16–39.55 %) than the outdoor proportions (25.89 %). This indicated that dust events significantly affected the size distribution of VBs, with differences between indoor and outdoor environments.

On clean days, TAMs in outdoor environments exhibited peaks of 2.1–3.3 μm (18.53 %) and > 7.0 μm (19.64 %), respectively, whereas the proportion of TAMs in office was high within the 1.1–4.7 μm range (18.30–18.75 %). Moreover, TAMs exhibited a peak of 3.3–4.7 μm (19.89 %) in laboratory. During haze events, the outdoor size distributions of TAMs were similar to those in office, both peaking at 2.1–4.7 μm (19.31–20.33 % and 18.12–20.23 %, respectively). The laboratory data exhibited peaks of 1.1–2.1 μm (20.13 %) and 3.3–4.7 μm (19.33 %). On foggy days, TAMs showed similar peaks ranging from 3.3 to 4.7 μm (19.71 % and 20.00 %) in the outdoors and laboratory environments, respectively, but in the office, TAMs only showed a high value of 21.03 % within the 0.65–1.1 μm range. During dust events, TAMs exhibited a distribution peaking at 2.1–3.3 μm outdoors (22.80 %) and at 3.3–4.7 μm in laboratory (23.44 %). In office, TAMs had peaks ranging from 1.1 to 2.1 μm (18.74 %) and 3.3–4.7 μm (20.84 %). For both pollution events and clean days, the indoor proportions of TAMs in fine particles were 1.15–1.31 times greater than those outdoors.

Obviously, the particle size distributions of VBs, NVBs and TAMs on clean days were relatively similar both indoors and outdoors. However, during pollution events, the particle size distribution changed greatly, especially in office. During dust and haze events, the indoor proportion of microbes in fine particles was significantly greater than that outdoors.

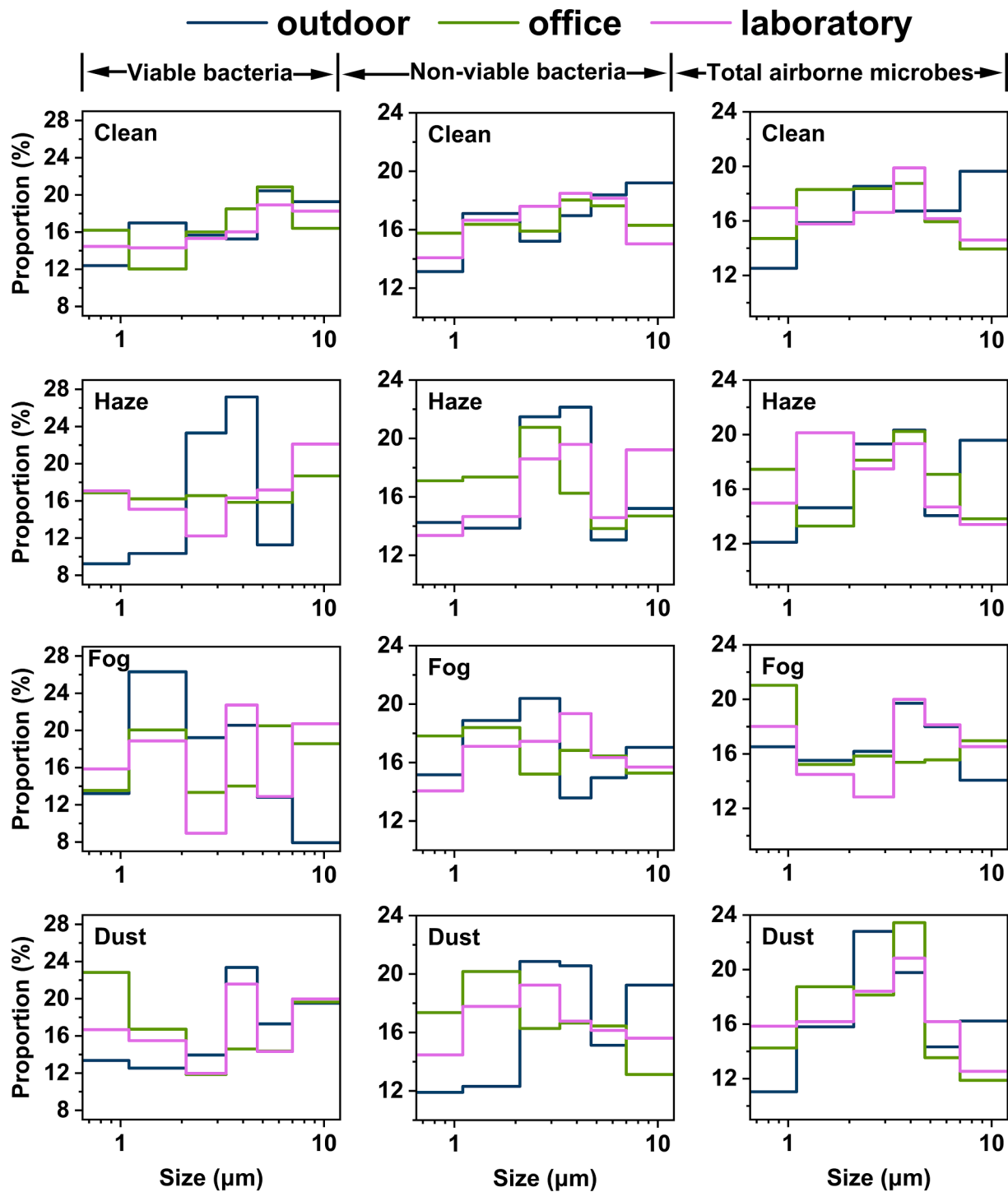


Fig. 7. Proportions of the concentrations of VBs, NVBs and TAMs in bioaerosols for different particle size ranges in the indoor and outdoor environments during pollution events.

3.3. I/O ratio of bioaerosols

The I/O ratio, which represented the ratio of indoor to outdoor bioaerosol concentrations, served as an indicator of bioaerosol emission sources. Notably, I/O ratio > 1 suggests that bioaerosols originate primarily from indoor sources, whereas I/O < 1 indicates outdoor sources. Figs. 8 and 9 show the I/O ratios across different seasons and pollution events. The results showed that the I/O ratio was typically

lower than 1 for both TAMs and NVBs, with averages of 0.76 ± 0.16 and 0.73 ± 0.22 , respectively. Nevertheless, there were several days when the I/O ratio reached or exceeded 1. For example, the average I/O ratio for VBs was 0.85 ± 0.38 , yet 5–50 % of the I/O ratios reached 1 or greater, particularly in spring or on clean days. This suggested that on some occasions, indoor sources contributed significantly to VBs, which might originate from both indoor and outdoor environments.

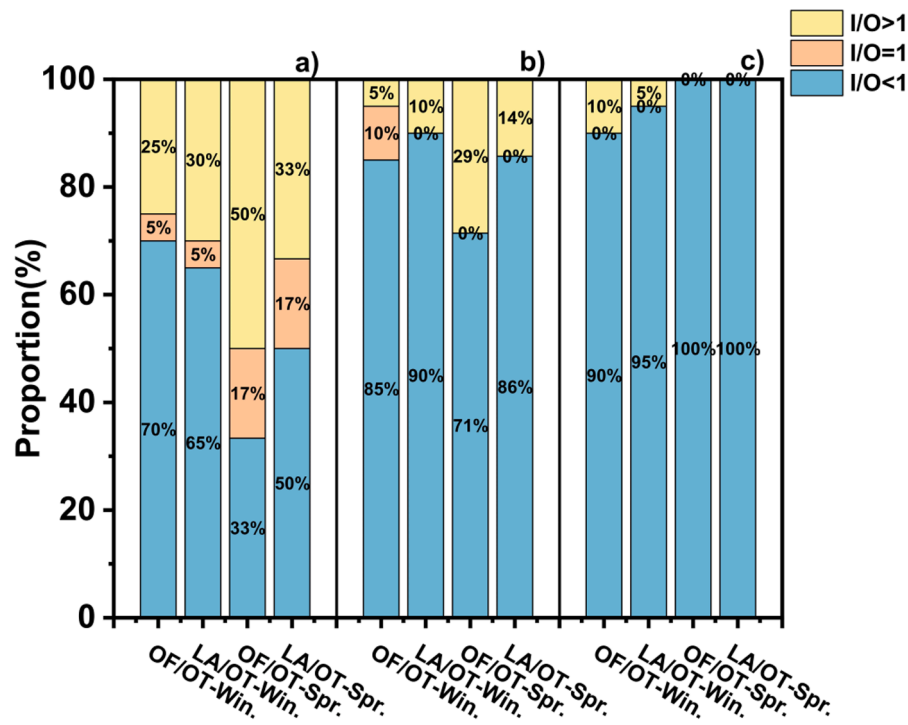


Fig. 8. Distributions of the indoor/outdoor (I/O) ratios for VBs (a), NVBs (b), and TAMs (c) during the different seasons (OT—outdoor; OF—office; LA—laboratory).

3.4. Deposition of bioaerosols in the human respiratory tract

3.4.1. Deposition of microbes in the human respiratory tract in spring and winter

PM_{2.5} were deposited primarily in the respiratory tract [57]. Thus, in this study, we calculated the DD values in the human respiratory tract for adults with the MPPD model and the microbial concentrations in fine particulate matters (0.65–2.1 μm), given that the primary population within universities consists of adults. Owing to the influence of seasonal indoor–outdoor concentration differences, the DD values of microbes were greater in spring $((2.40\text{--}3.36) \times 10^4$ cells/h) than in winter $((1.16\text{--}1.35) \times 10^4$ cells/h). Notably, the indoor DDs of VBs in fine particles in the human respiratory tract exceeded those outdoors during both seasons. The outdoor DDs of TAMs exceeded those indoors during both seasons.

As shown in Fig. 10, the indoor and outdoor DDs of TAMs in fine particles in the human respiratory tract varied with population, with values ranging from $(1.20\text{--}3.36) \times 10^4$ cells/h and $(1.16\text{--}3.22) \times 10^4$ cells/h for females and males, respectively. In addition, the indoor and outdoor DDs of VBs in fine particles in the human respiratory tract varied with values ranging from $(2.69\text{--}3.81) \times 10^3$ cells/h and $(2.59\text{--}3.66) \times 10^3$ cells/h for females and males, respectively. These findings indicated that the indoor and outdoor DDs of TAMs and VBs in the human respiratory tract were greater for females than for males, potentially due to the differences in breathing patterns, lung anatomy, airway geometry, and physical activity.

3.4.2. Deposition of bioaerosols into the human respiratory tract during pollution events

Fig. 10 shows the DDs of microbes in fine particles in the human respiratory tract during pollution events, with DDs ranging from $(2.24\text{--}53.02) \times 10^3$ cells/h. Generally, except for dust and haze events, the DDs of TAMs in fine particles in the indoor and outdoor environments were largely consistent. With adult females as an example, the DDs of TAMs in the indoor and outdoor environments ranged from $(1.64\text{--}1.87) \times 10^4$ cells/h on clean days and from $(1.35\text{--}1.70) \times 10^4$ cells/h and $(1.04\text{--}1.40) \times 10^4$ cells/h on foggy and haze days,

respectively. During dust events, the outdoor DDs of TAMs ranged from $(3.29\text{--}5.30) \times 10^4$ cells/h for females, which were significantly greater than those in the office $(1.67\text{--}2.59) \times 10^4$ cells/h and laboratory $(1.81\text{--}2.80) \times 10^4$ cells/h environments.

We found that the indoor DDs of VBs in fine particles in the human respiratory tract were greater than those outdoors during haze and dust events, while they were slightly lower than those outdoors on clean and foggy days. For example, the DDs of VBs for females ranged from $(2.27\text{--}2.44) \times 10^3$ cells/h and $(2.23\text{--}2.52) \times 10^3$ cells/h on clean and foggy days, respectively, which were slightly lower than the outdoor values $((2.96\text{--}4.38) \times 10^3$ cells/h).

Overall, the indoor and outdoor deposition characteristics of different microbes differed. During dust and haze events, the DDs of TAMs were higher outdoors than indoors, while VBs exhibited the opposite trend to that of TAMs.

4. Discussion

4.1. Influence of outdoor environment on indoor bioaerosol concentration and bacterial viability

4.1.1. Influence of outdoor air intake on indoor microbial concentrations

In most cases, the I/O ratio was < 1. The indoor and outdoor microbial concentrations were strongly correlated, with R^2 values of 0.935 and 0.881 for offices and laboratories, respectively. These findings indicated that the outdoor environment was the main source of indoor bioaerosols. Chen et al. [24] and Zhang et al. [58] reported the same results in investigations of indoor and outdoor microbial characteristics. Microbes from outdoor air could infiltrate indoor spaces by building openings, such as gaps in doors and windows, and ventilation systems, thereby influencing indoor microbial concentrations [59,60].

Owing to the geographical features of coastal cities, the sources of microbes primarily encompassed natural origins, such as those from oceans, lakes, rivers, plants, and soil [8], along with anthropogenic origins, including those stemming from landfills, sewage treatment, agricultural undertakings, and industrial activities [7]. Studies have demonstrated that natural sources played a dominant role in microbial

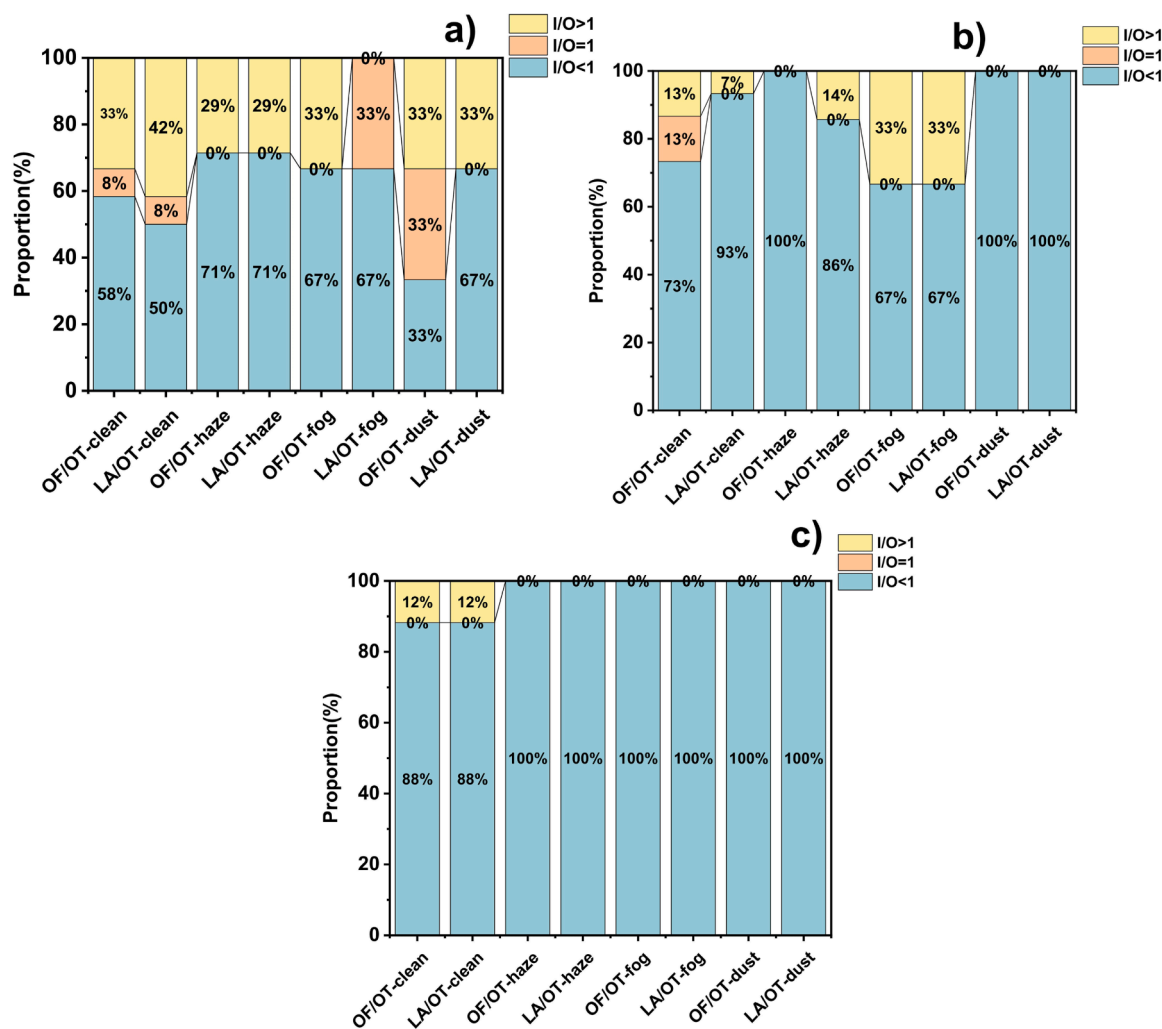


Fig. 9. Distributions of the indoor/outdoor (I/O) ratios for VBs (a), NVBs (b), and TAMs (c) during pollution events (OT—outdoor; OF—office; LA—laboratory).

emissions, significantly surpassing anthropogenic sources [61]. Throughout the sampling period, both the ventilation and air-conditioning systems within the laboratory and office were deactivated, and the pipeline system was scarcely utilized. Thus, the principal route of microbe entry was through outdoor air exchange, instead of originating from within the indoor environment. Meteorologically, the outdoor RH (53.95 %) was higher than that in offices (21.88 %) and laboratories (25.81 %), being more favorable for microbial growth and reproduction. High humidity contributed to preserving cellular water content and extended viability duration [62]. Additionally, the higher outdoor wind speed facilitated the release of microbes from the ground, plants, and water bodies compared with that indoors. This further intensified the microbial input from the outdoors to the indoor environment.

However, the I/O ratio for VBs was greater than or equal to 1 in some cases. Specifically, during pollution events when doors and windows were closed, we observed that the VBs I/O ratio was greater than or equal to 1 when the $PM_{2.5}$ I/O ratio was ≥ 1 . This indicated that the indoor environment might serve as a significant microbial source through the release of microbes from human activities under low ventilation conditions and the carrier role of $PM_{2.5}$ in transporting microbial agents [63,64,32].

The concentrations in office were generally higher than those in laboratory, largely because of human activities and the composition of indoor air. Human activities in offices, such as the use of electronic devices [65], breathing, walking, and floor cleaning, cause the release of

more PM. The chemical composition of office air mainly comprises ozone, inhalable particles, volatile organic compounds (VOCs) such as polychlorinated biphenyls [66,67] emitted by old appliances such as computers, printers, and semivolatile organic compounds from wall cracks. These substances might serve as nutrient sources for microbes, thus promoting their growth and reproduction [68]. However, in laboratories, strong disinfectants, cleaners, and volatile chemicals such as ether, methanol, and acetonitrile were commonly used, which inhibited or killed microbes, thereby significantly reducing their concentrations in air and on surfaces [69,70]. The observational data also revealed that PM_{10} concentration ranged from 60.80 to 179.05 $\mu g/m^3$ in office, whereas it ranged from 75.97 to 156.13 $\mu g/m^3$ in laboratory, creating a more favorable surface environment for microbial survival.

Our results indicated that indoor microbes primarily originated from outdoor air, and the influence of outdoor air on indoor microbial concentrations was significant. However, it was difficult to precisely quantify the relative contributions from diverse sources due to the lack of a microbial community. Future research exploring the regulatory mechanisms of outdoor air on indoor microbial sources would be needed.

4.1.2. Influence of seasonal variations on the distribution of microbial concentrations

Bacteria accounted for 2.5 % to 72.0 % of TAMs both indoors and outdoors, with a mean proportion of 32.14 ± 18.38 %, which was consistent with previous findings [52–54]. This phenomenon occurred

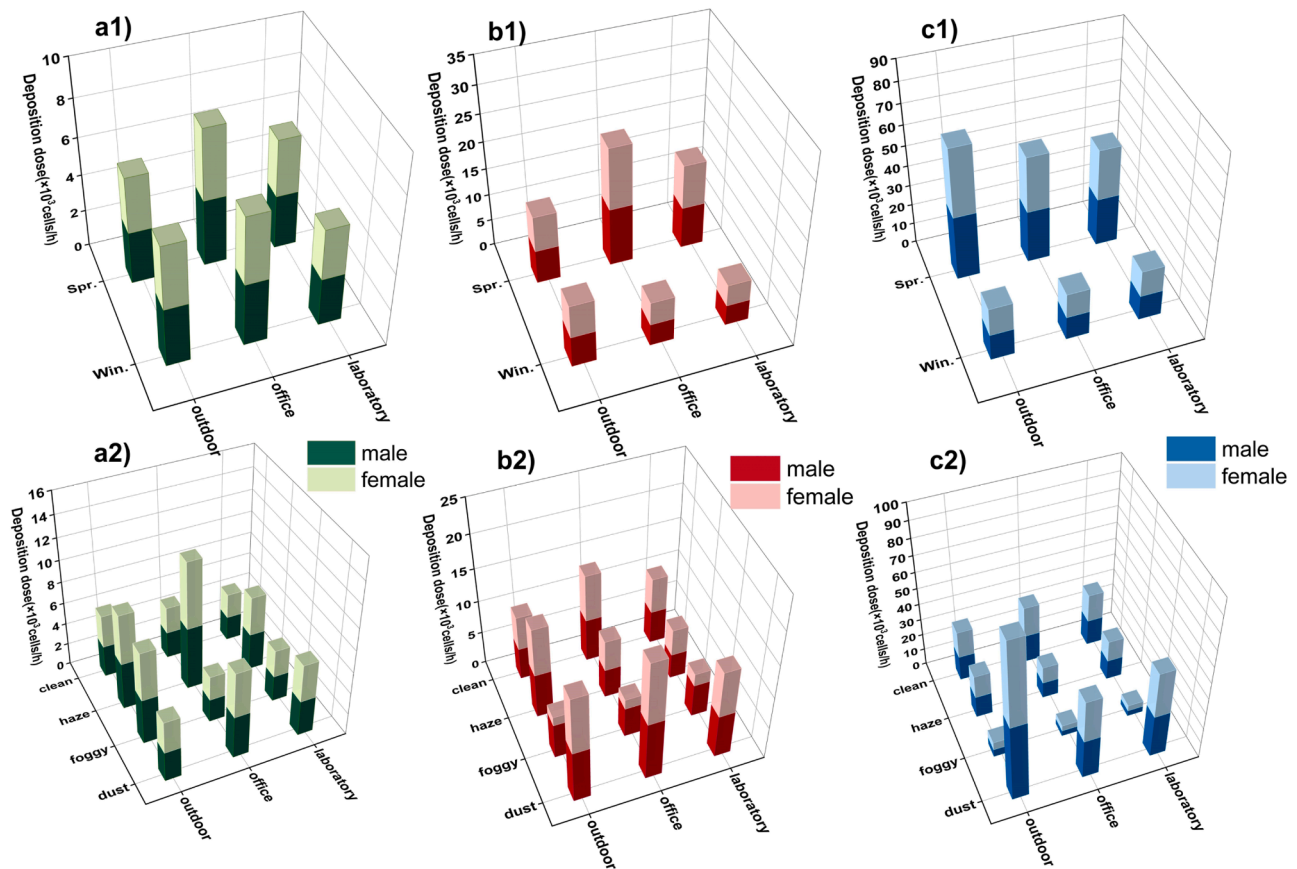


Fig. 10. Comparison of the indoor and outdoor DDs of VBs (a), NVBs (b) and TAMs (c) in fine particles in the human respiratory tract in spring and winter (1) and during pollution events (2).

because TAMs measured via DAPI staining combined with fluorescence microscopy included bacteria (VBs and NVBs) rchaea and unicellular eukaryotes (fungal spores and heterotrophic and autotrophic protists) [54].

In both spring and winter, the outdoor microbial concentrations were higher than indoors. Regardless of the environment (indoors or outdoors), the concentrations of NVBs and TAMs were higher in spring than in winter, whereas VBs showed the opposite trend. To clarify the primary factors influencing the indoor and outdoor distribution characteristics of bioaerosols, Spearman correlation analysis and redundancy analysis (RDA) (Fig. 11) were performed. Meteorological conditions, including T, RH, and PM concentration, significantly affected both the indoor and outdoor microbial concentrations, which was consistent with the findings of other studies [24,58]. Variance analysis also revealed significant differences in outdoor meteorological parameters, including T, visibility, CO_2 , O_3 and CO between spring and winter.

The elevated concentrations of NVBs and TAMs in spring relative to those in winter might be attributed to the favorable temperature for microbial growth as temperature positively influenced the TAM and NVB concentrations. Higher temperatures in spring (9.48°C) facilitated the long-distance spread of microbes [49,71]. Temperatures above 10°C boosted cell metabolism and reduced cold-induced cell damage (2.6°C in winter). As carriers for microbes, the PM_{10} concentration in spring increased to $161.56 \mu\text{g}/\text{m}^3$ compared with $120.10 \mu\text{g}/\text{m}^3$ in winter, due to particle resuspension caused by atmospheric turbulence under increasing temperatures [72] and high wind speed of 4.37 m/s in spring.

The seasonal differences in microbial concentrations were also influenced by increased biological sources such as pollen and plant growth in spring [73]. In this study, The RH (62.56 %) in winter was greater than that in spring (42.44 %). The survival patterns of microbes

were affected by combined effects of temperature and RH. Under low temperature and high RH, microbes could endure for an extended period with constrained reproductive capacity. Conversely, under high-temperature and low RH, the mortality of microbes was expedited [74,75]. As a result, the concentration of VBs in winter was higher than that in spring.

Therefore, it is crucial to control RH within a proper range to decrease indoor VBs pollution levels, since the human health will be also affected when RH is too low. For example, the ideal RH for preventing the spread of aerosolized respiratory viruses at room temperature is between 40 % and 60 % [76].

4.1.3. Influence of pollution events on the distribution of microbial concentrations

The NVB and TAM concentrations were highest during dust events, reaching 1.25 to 3.94 times those on clean days, whereas the VB concentrations during haze events significantly increased to 1.65 to 2.07 times those on clean days. This indicated that the influence of pollution events on microbes depended on microbial species and pollution types. Spearman correlation showed that NVBs and TAMs were primarily influenced primarily by particle transport and wind speed, whereas VBs were more affected by humidity and PM (Table S8–9). During the different pollution events, the outdoor microbial concentration was greater than the indoors.

As noted above, high PM concentrations (PM_{10} , $269.50 \mu\text{g}/\text{m}^3$), a suitable temperature increase (12.8°C), high wind speeds (6.38 m/s) and abundant biological sources during long-distance dust transport could facilitate microbial growth, reproduction, resuspension and input from the ground surface during dust events [77]. Although dust provided abundant carriers, high ozone concentration ($74.25 \mu\text{g}/\text{m}^3$) accelerated microbial inactivation by destroying microbial cell

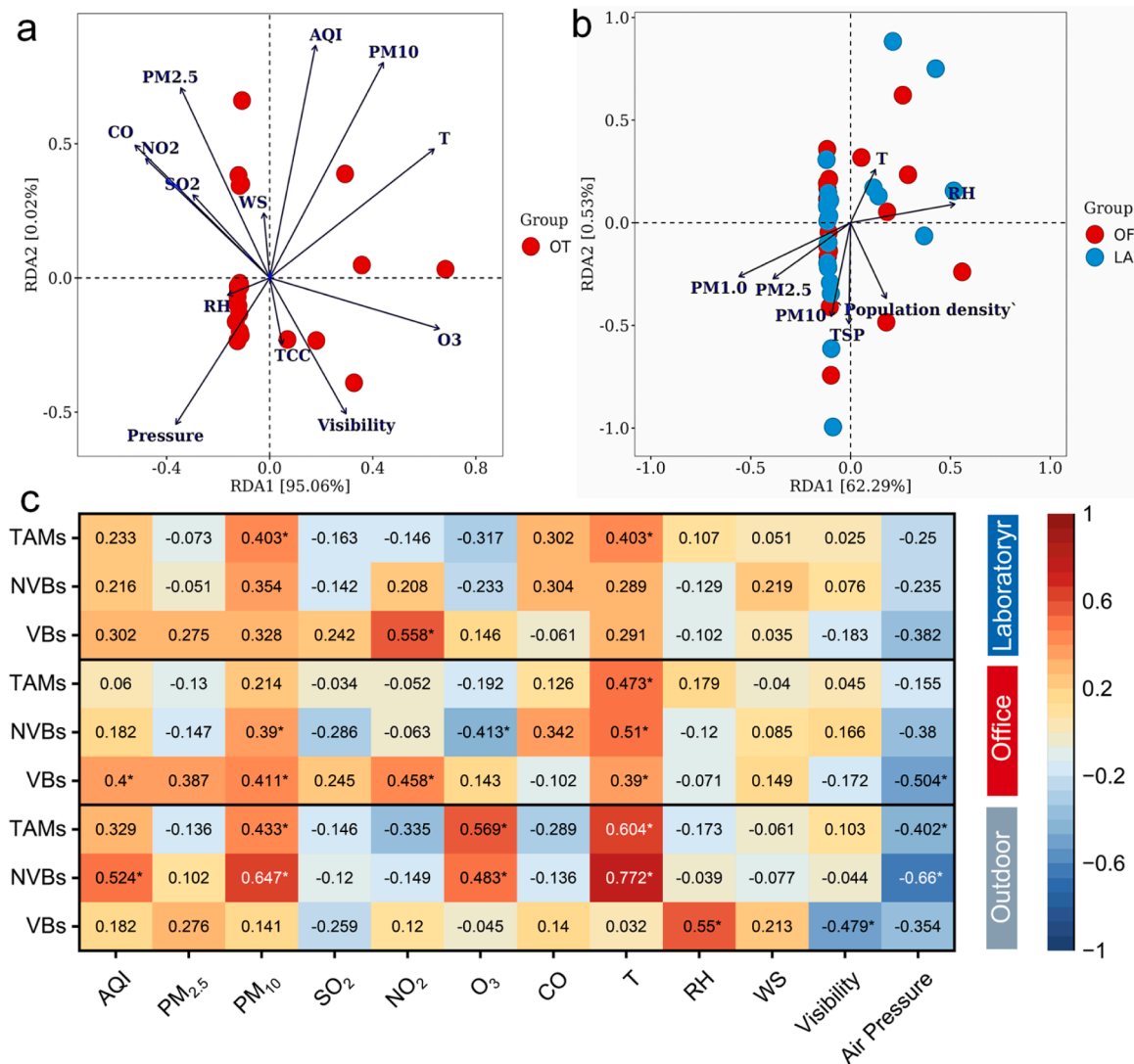


Fig. 11. RDA correlation between the indoor (b) and outdoor (a) microbial concentrations and environmental parameters (OT—outdoor; OF—office; LA—laboratory) and Spearman correlation (c) between indoor-outdoor bioaerosol concentrations and outdoor environmental parameters.

membranes, nucleic acids, and proteins through strong oxidation and enhancing atmospheric oxidation capacity [78]. Meanwhile, low RH (19.50–34.00 %) caused microbial dehydration, increasing the susceptibility of microbes to oxidative damage and further inhibiting their survival and reproduction [79]. It indicated that dust events increased bioaerosol concentration by the increase of carrier, but bacterial viability was restricted by oxidative stress and dry air.

The increase in the VB concentration during haze events was influenced primarily by meteorological conditions, pollutant accumulation, and ultraviolet (UV) radiation. During haze events, high RH levels (61.86 %) and moderate temperatures provided favorable conditions for bacterial survival [80]. High humidity reduced the concentration of oxidative substances in the air (O₃ and ·OH), decreasing oxidative damage to microbes [81]. Adverse atmospheric diffusion conditions caused pollutants and microbes to accumulate near the surface, and PM concentration sharply increased, which might also serve as a nutrient source, providing organic matter for microbes [82]. Haze also partially blocked solar rays, reducing UV radiation and O₃ concentrations from 59.12 µg/m³ on clean days to 31.14 µg/m³, thus helping to maintain high microbial concentrations and activities.

On foggy days, the outdoor RH (84 %) was also relatively high, which not only reduced microbial cell water loss but also formed a water film barrier, reducing oxidative damage and extending microbial

viability duration. On the other hand, the low temperature (3.93 °C) and low PM concentrations (37.67–57.67 µg/m³) were not conducive to microbial reproduction and growth. The particles deposited more easily due to particle hygroscopic growth under high RH conditions [83], leading to a decrease in the carriers of airborne microbes. The combined effect caused the low microbial concentration outdoors on foggy days.

During pollution events, indoor concentrations of TAMs, VBs and NVBs were significantly lower than outdoors. However, indoor microbial concentrations also increased in response to increasing outdoor microbial levels. During pollution events, indoor concentrations of TAMs and VBs increased 1.20 to 2.12 times compared with those on clean days. The indoor RH (24.33–26.00 %) and temperature (17.73–22.16 °C) remained relatively stable. However, the indoor PM concentrations increased significantly during pollution events, since the purifier was not in use during the sampling period of this study. Specifically, compared with those on clean days, the PM_{2.5} levels increased from 55.27 to 78.49 µg/m³ to 127.67–145.24 µg/m³ during haze events, and the PM₁₀ levels increased from 65.32 to 90.25 µg/m³ to 158.65–185.43 µg/m³ during dust events. Therefore, we considered that increased indoor PM concentrations could be one key reason for the increase in microbial levels during pollution events.

Studies have revealed that air purifiers can effectively remove inhalable bacteria [58], with the removal efficiencies for PM_{1.0}, PM_{2.5},

and PM₁₀ reaching 72.1 – 73.3 % [84]. Therefore, during pollution events, it was advisable to close doors and windows to minimize outdoor PM infiltration and apply air purifiers to lower indoor PM concentrations, thereby reducing indoor microbial pollution. However, the effects of air purifiers were not evaluated in this study due to the limit of the actual indoor environment.

This study investigated the factors influencing bioaerosol concentration. However, without community structure and functional gene analyses, it is difficult to fully clarify the influencing mechanisms, necessitating further in-depth research.

4.2. Influence of outdoor environment on indoor size distribution of bioaerosols

4.2.1. Differences in size distribution between indoor and outdoor environments

The PM concentration, environmental factors, and human activities can affect indoor and outdoor size distributions. As mentioned above, VBs, NVBs and TAMs mainly occurred in coarse particles, which conformed with earlier findings [24,52–54]. However, during different seasons and pollution events, the indoor proportion of microbes in fine particles exceeded that outdoors. This might be due to the higher indoor concentration of fine particles (PM_{2.5}) than outdoors, with PM_{2.5} concentrations ranging from 44.53 to 145.24 µg/m³ and 31.33–114.14 µg/m³, respectively.

Firstly, the low indoor airflow velocity and Reynolds number facilitate the longer suspension of fine particles than coarse one [85]. Second, during the infiltration of outdoor bioaerosols, coarse particles may be trapped outside the building, as evidenced by a lower indoor PM₁₀ concentration (103.79 µg/m³) than that outdoors (120.03 µg/m³). With doors and windows closed, reduced air exchange and limited ventilation lead to the accumulation of fine particles indoors [86]. Third, indoor activities such as walking and cleaning, along with pollution sources such as aging electrical equipment (e.g., printers and computers) and building materials, could influence indoor fine particle concentrations [85]. Owing to heat dissipation or aging of furniture, electrical devices, for example, computers and monitors, and decorative materials, can release fine particles, such as plastics, metals and VOCs [87,88]. The heating and cooling devices used in the experiments, such as hot plates and condensers, also emit particulates during operation [89,90].

In conclusion, the difference in size distribution between indoor and outdoor microbes was affected by fine particles in the air, which was related to pollution sources and meteorological parameters. In indoor environments, fine particles were more caused by the release of indoor materials and human activities.

4.2.2. Influence of the season on the size distribution of bioaerosols

To identify the main factors influencing the microbial particle size distribution, we performed a correlation analysis between the microbial concentrations in different particle size ranges and the meteorological parameters for both indoor and outdoor environments (Table S8–S9). Overall, PM₁₀ exhibited a strong positive correlation with the outdoor concentrations of TAMs, VBs, and NVBs within the coarse particle size range. Moreover, RH and population density were strongly positively correlated with the indoor concentrations of TAMs, VBs, and NVBs within the fine particle size range.

As mentioned in Section 3.2.1, the TAM concentrations peaked within the 3.3–4.7 µm range in winter and spring, but the proportion of microbial concentrations in fine particles was greater in winter than in spring. Owing to increased emissions of fine PM from coal and other heating sources in winter, PM_{2.5} concentrations were higher in winter (70.81 µg/m³) than in spring (31.33 µg/m³), and the RH in winter was higher than that in spring, which may be conducive to survival of microbes in aerosols [75]. Therefore, the proportion of microbes in 0.65–2.1 µm particles increased in winter. Fine particles in this size range could easily penetrate the lower respiratory tract and deposit in

the alveoli [91], potentially causing respiratory infections or exacerbating asthma and chronic obstructive pulmonary disease (COPD). As temperatures rise in spring, atmospheric instability and turbulence increase, thereby promoting the upward and long-distance transport of coarse particles [72]. Under the influence of northerly and northwesterly winds in spring in Qingdao, the proportion of coarse particles increased significantly. Thus, the concentration of microbes in spring reached a peak within the 2.1–4.7 µm range, and they were largely distributed within the > 7.0 µm range.

Indoor particle size distributions of NVBs and TAMs were very similar to those outdoors, indicating that indoor size distributions were influenced mainly by outdoor microbes. However, the indoor size distribution of VBs exhibited opposite seasonal patterns. The indoor VB concentration was higher in spring than in winter. We found that the indoor temperature and airflow velocity showed no seasonal variation. Thus, this seasonal VB difference might be partly due to indoor RH levels and human activities. The indoor RH value of 23.00–24.86 % in spring was greater than that in winter, and the indoor population density in spring (0.016–0.081 ppsm) was greater than that in winter (0.019–0.064 ppsm). Human activities were likely to cause the release of more microbes.

In summary, seasonal variations in microbial particle size distribution were closely related to RH, PM_{2.5} concentrations, and human activities. These findings highlighted the importance of indoor air quality management during different seasons, especially in environments with high population density. Effective ventilation can limit the transmission of indoor microbes [92], therefore, opening the doors and windows and controlling human activities (for example, eating in the dining areas instead of offices) are likely to serve as one of the effective measures for reducing indoor pollution.

4.2.3. Influence of pollution events on the size distribution of bioaerosols

Although indoor microbes primarily originated from outdoors, their particle size distribution varied significantly during different pollution events. During pollution events, the indoor proportions of TAMs and VBs in fine particles were significantly higher than those outdoors (1.19 to 1.53 times). We believed that the difference in the size distribution of microbes in bioaerosols was caused mainly by the relative ratio of fine particles to total one. During pollution events, the indoor PM_{2.5}/PM₁₀ ratio ranged from 0.32 to 0.82, which was 1.30 to 2.28 times higher than the outdoors.

On foggy days, the PM_{2.5}/PM₁₀ ratio reached up to 0.67, and fine particles were rich in hygroscopic components such as sulfate and nitrate, which increased the surface area in the high-humidity environment (84.33 % RH), allowing more microbes to adhere. At this time, the particle size peaks of VBs were at 1.1–2.1 µm, and those of TAMs were at 0.65–1.1 µm. During haze events, the PM_{2.5}/PM₁₀ ratio was 0.63, and the peak microbial concentrations were concentrated in the 2.1–4.7 µm range. Additionally, haze particles might have carried pathogenic microbes, further increasing the risk of infections. On dust events, the PM_{2.5}/PM₁₀ ratio was lower (0.14), showing coarse particles (>2.1 µm) dominated, and the proportion of microbes adhering to mineral particles (e.g., silicon and calcium) was high.

Furthermore, changes in human activities during pollution events further influence the size distribution. The increased indoor human density (from 0.010 to 0.064 ppsm on clean days to 0.016–0.111 ppsm during pollution events) released more microbes into the air [93], further increasing the proportion of microbes in fine particles indoors.

4.3. Health risks and mitigation strategies

Our survey indicated that 86.86 % of university faculty and students stayed indoors for more than 10 h every day. An increase in the number of indoor microbes could contribute to the increased indoor exposure risk for occupants [94]. The deposition of fine particles containing microbes in the respiratory tract varied significantly among the different

populations, with higher deposition in females than in males. This finding agrees with that of Wei et al. [95] and might be attributable to some factors, such as breathing patterns, lung anatomy, airway geometry, and level of physical activity. Studies had shown that the airways of females might be narrower than those of males, with a lower functional residual capacity (2680 mL), which increased the airflow velocity and leads to greater deposition of aerosol particles [96]. Additionally, the unique immune responses and physiological characteristics (such as hormonal fluctuations) of females could render microbes more prone to lingering in the respiratory tract, thereby increasing deposition, especially during menstruation and pregnancy [97].

The health risk values for inhalation of bioaerosols in the indoor and outdoor environments, as determined with the EPA model, are provided in Tables S7. The HQ for VBs, NVBs, and TAMs ranged from 4.27×10^{-3} to 1.38×10^{-2} , from 7.70×10^{-2} to 2.86×10^{-1} , and from 2.00×10^{-1} to 7.55×10^{-1} , respectively. Although both the indoor and outdoor HQ for microbes were less than 1 for the different populations, the indoor (especially in the office environment) risk for VBs during pollution events were significantly higher than those on clean days and outdoors. We performed headquarter calculations for extremely high values during dust and haze events and found that the results were far above the average. Compared with that on clean days, the potential health risk of microbes during dust events reached 0.752 (HQ). Although the result remained below 1, the variability in individual organisms and chemical compounds was not considered here. Some people are more vulnerable to infection, resulting in high health risks during pollution events.

To reduce the risk of exposure to indoor microbes, we recommend enhancing indoor air filtration systems, opening the doors and windows, and controlling the sources of fine particles and humidity during high-pollution events (e.g., haze or dust events). Furthermore, during pollution events, adults should wear masks to reduce the deposition risk of bioaerosols.

5. Conclusion

In this study, we analyzed the concentrations of TAMs, VBs, and NVBs in bioaerosols in outdoor environments, offices, and laboratories in coastal cities. The outdoor concentrations of microbes were significantly greater than those indoors, and the indoor and outdoor microbial concentrations exhibited a notable linear relationship. The average I/O ratios of VBs, NVBs and TAMs were all lower than 1, which indicated indoor microbes largely originated from outdoor air. Outdoor microbial sources significantly influenced the indoor concentration and size distribution of microbes.

Microbes were affected by temperature, RH, PM concentration and human activity, with significant variations observed across seasons and pollution events. These factors variations caused the indoor and outdoor concentrations of microbes to vary with season and pollution events. The indoor and outdoor VBs concentrations and bacterial viability were high in winter and low in spring, whereas those of the concentrations of TAMs and NVBs were the opposite. The indoor and outdoor VBs, NVBs and TAMs concentrations during pollution events were 1.24–3.94 times higher than those on clean days, especially during haze and dust events. The indoor proportion of microbes in fine particles was consistently greater than the outdoor proportion, without dependence on season or type of pollution event. Indoor BV was higher than outdoor one in haze and dust events, while outdoor BV was higher than indoors on foggy days. The main factors influencing indoor microbes and BV include indoor and outdoor air exchange, RH and population density. In addition, the $PM_{2.5}/PM_{10}$ ratio was one of the main factors influencing the microbial particle size distribution.

Both the indoor and outdoor DDs of microbes were high in spring and low in winter, and the DDs of VBs in fine PM in the human respiratory tract were higher indoors than outdoors during haze and dust events. The potential health risks of microbes were significantly greater during dust and haze events than on clean days. Moreover, our results revealed

that the deposition characteristics of females were consistently greater than those of males. To mitigate the risk of indoor microbial exposure, strategies such as closing doors and windows during pollution events, using air purifiers, controlling indoor humidity levels, and minimizing the release of indoor fine PM are recommended. Meanwhile, people should strengthen individual protection measures and implement behavioral interventions, such as wearing masks. In this study, we documented differences in microbial concentrations and distributions between indoor and outdoor environments, providing a reference for further research on the dynamics of airborne microbes and their impacts on health.

CRediT authorship contribution statement

Ru Zhao: Writing – original draft, Investigation, Formal analysis, Data curation. **Liang Guo:** Writing – review & editing, Supervision, Resources. **Jianhua Qi:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Taicheng An:** Writing – review & editing, Visualization, Resources.

Declaration of competing interest

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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.buildenv.2025.113056](https://doi.org/10.1016/j.buildenv.2025.113056).

Data availability

Data will be made available on request.

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