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*Research Article*

# Investigation of Indoor Airborne Bacteria in the Severe Cold Region in China: Genera, Levels, and the Influencing Factors of Concentration

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In regions experiencing severe cold, inadequate ventilation during winter months often leads to increased concentrations of indoor pollutants. While there have been several studies on indoor particulate matter and inorganic pollutants in such regions, bioaerosol pollution has not been as extensively investigated. This study examines the indoor bioaerosol situation in a university located in one of the severe cold regions in China, focusing on bacteria as a representative pollutant. It investigated random samples of an office and a dormitory (including washrooms) and spanned heating and nonheating periods. The findings indicated that bacterial abundance in the dormitory and office was approximately equivalent. The predominant airborne bacterial communities identified were Proteobacteria, Bacteroidota, Actinobacteriota, Firmicutes, and Myxococcota. Opening windows effectively reduced bacterial concentrations during both heating and nonheating periods. When windows remained closed, bacterial concentrations exceeded the standard by 9.1% during the nonheating period and by 14.3% during the heating period. Furthermore, temperature and relative humidity influenced bacterial particle size, activity, and consequently, aerosol concentrations. In the office, the highest percentage of bioaerosols was observed in particle sizes <1.1 and 1.1–2.1 $\mu$m, with smaller percentages observed in other particle sizes. Conversely, the percentage of particle sizes 2.1–3.3 $\mu$m in the dormitory was higher. The highest bacterial aerosol concentrations were detected in the morning in both the dormitory and office, during heating and nonheating periods. Bacterial concentrations in the office were lower on weekends than on weekdays, whereas in the dormitory, concentrations were higher on weekends than on weekdays. The above results indicate that indoor bacterial aerosol pollution is serious in winter in severe cold regions, which needs more attention.

**Keywords:** bacterial concentration; indoor air quality; severe cold region; space heating; university dormitory and office

## 1. Introduction

In recent years, indoor air quality (IAQ) has emerged as a paramount concern as living standards have experienced significant improvements [1, 2]. Bioaerosols, which serve as vital indicators of IAQ, consist of particulate matter (both solid and liquid) originating from biological sources such as fungi, bacteria, viruses, and pollen [3]. The transmission of bioaerosols entails emissions, environmental transport, and subsequent exposure to susceptible individuals [4]. Respiratory infectious diseases, including measles, mumps, chickenpox, influenza, SARS, and the common cold, are transmitted via bioaerosols [5]. The recent COVID-19 pandemic has further underscored the significance of IAQ in relation to bioaerosol transmission [6–8]. Primarily transmitted in indoor environments, bioaerosols pose an elevated risk in poorly ventilated spaces [9]. Considering that individuals spend a considerable portion of their time indoors [10, 11], bioaerosol concentrations can accumulate, resulting in potential health hazards [12, 13]. Consequently, understanding

indoor bioaerosol conditions is crucial for safeguarding public health and informing the development of effective IAQ control measures.

Harbin, with a quite long and cold winter, is a typical city located in a severe cold region in China. Harbin endures long, frigid winters, with a heating period extending up to 6 months. There are some significant weather characteristics: the outdoor air temperature is −16.9°C on average, and the average daily highest/lowest outdoor temperatures are −13°C/−25°C in January [14]. The temperature differential between indoor and outdoor environments might exceed 40°C during the heating period. Thus, residents typically refrain from opening windows for ventilation to maintain indoor warmth, which may result in significant IAQ issues. Moreover, Harbin primarily relies on central radiators for heating and experiences relatively mild summers, rendering mechanical ventilation installations unnecessary. In accordance with Chinese energy efficiency standards [15, 16], buildings in Harbin necessitate more airtight doors and windows. As a result, it becomes difficult to exchange indoor air with the outside environment, which leads to serious IAQ problems [14]. Several studies have been conducted on IAQ in the severe cold region, revealing that indoor bacterial concentrations are higher in these areas [17]. This can be attributed to insufficient indoor ventilation and a lack of focus on indoor microorganisms due to economic development constraints [18]. During the winter, the severe cold regions frequently see a substantial decrease in temperature. The significant shift in climate is accompanied by a decrease in the frequency of ventilation, as the low outdoor air temperature makes it less preferable to exchange indoor air with the outside environment. The restricted airflow indoors can foster an environment that is favorable for the rapid growth and spread of bacteria. Additionally, confined areas with limited ventilation can result in the accumulation of moisture, which, when paired with the heat generated by heating systems, creates an optimal environment for bacterial growth. Although indoor air pollution has garnered attention, there remains a dearth of studies on indoor bioaerosols in the severe cold region, rendering the extent of bioaerosol pollution unclear. Exposure to indoor airborne bioaerosols is closely associated with human health. As a potential transmission route for COVID-19, bioaerosol transmission could pose a severe threat to public health, especially in enclosed indoor environments [3]. Indoor bioaerosols are also closely linked with various diseases, such as pneumonia, infectious diseases, cancer, asthma, and allergic diseases [19, 20]. Therefore, it is essential to investigate the bioaerosol situation in a severe cold region.

The composition of indoor microbiota constitutes a complex and dynamic multidimensional dataset [21, 22], influenced by various environmental factors such as latitude, room usage, ventilation systems, and surface materials [23, 24]. The abundance, diversity, and concentration of indoor microbes tend to vary according to the room's type [25]. In educational institutions, offices and dormitories are often densely populated spaces. Chinese university dormitories typically exhibit relatively homogeneous architectural features, with each high-density residence accommodating numerous students. A single room can house four to eight individuals, and long corridors connect approximately 20–50 rooms per floor. Frequent interaction occurs between residents of adjacent rooms [26]. These living spaces share similar building materials, ventilation systems (natural ventilation), furniture, and architectural layouts. However, due to differing thermal preferences among residents, ventilation in dormitories is often insufficient. Chinese university offices similarly experience high occupancy rates, with users frequently engaging in conversation. In many instances, ventilation systems are outdated, inadequately filtered, and poorly maintained [27, 28]. Mechanical ventilation is often absent in northern China [29]. As a result, pollutant concentrations in these spaces can increase, heightening the risk of infection [30]. Students, particularly postgraduates, spend a significant portion of their time in dormitories and offices, where poor air quality can negatively impact motivation and learning potential. Consequently, poor IAQ may significantly affect students' health, an issue that has been largely overlooked.

In light of these aforementioned challenges, it is both meaningful and imperative to examine the IAQ of dormitory and office buildings in the severe cold region during winter, particularly throughout the heating period. To this end, field studies on IAQ were conducted in Harbin, a representative city in China's severe cold region, between September 2022 and November 2022. These studies encompassed heating and nonheating periods, facilitating comparisons of indoor air microbe alterations before and after heating commenced. Generally, bacterial aerosol concentrations are higher in the severe cold region, whereas fungal aerosol contamination is more prevalent in the warmer southern regions [18]. Consequently, to gain a comprehensive understanding of bioaerosol pollution in the severe cold region, this field study selected bacteria as the representative pollutant for bioaerosols. The primary objective of this research was to identify airborne bacterial contamination within university dormitories and offices in China's severe cold regions. Furthermore, the study investigated the impacts of open windows, indoor environments, and occupant habits on the indoor airborne bacterial environment. The findings of this study will serve as foundational data for the future control of indoor bioaerosol contamination.

## 2. Methodology

*2.1. Sampling Sites.* As depicted in Figure 1, Harbin (45°41′ N, 126°37′ E) is a city situated in northern China's severe cold region and belongs to the severe cold climate zone. The climate of Harbin is characterized by an extremely low outdoor air temperature in January. As a quintessential city characterized by an extended and severe cold winter, Harbin adheres to stringent building energy-saving standards. In comparison to other cities in China, Harbin's buildings boast superior thermal insulation and increased air tightness. Predominantly, natural ventilation governs air exchange within Harbin's structures. To preserve stable indoor temperatures during winter, residents tend to minimize window opening usage. Consequently, when doors and windows remain closed, fresh air can only infiltrate rooms through gaps and

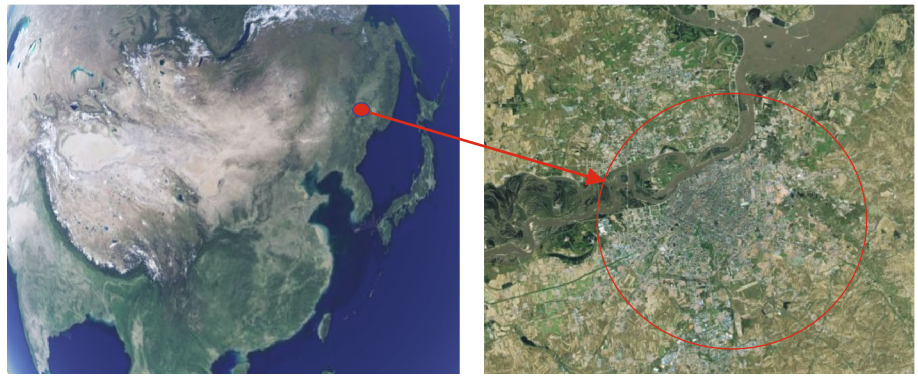

Figure 1: Location of the selected city.

crevices in the building envelope. Given the heightened air tightness of enclosures in the severe cold region, indoor bacterial levels may escalate due to inadequate fresh air supply. Moreover, individuals are more inclined to remain indoors throughout the winter months, further contributing to elevated bacteria concentrations in indoor air.

To examine the variations in bacterial concentration within the indoor environment of China's severe cold region between the heating and nonheating periods, an investigation was carried out in a university's dormitory and office building from September to November. This timeframe encompasses both the heating and nonheating periods. Four sampling sites were selected, comprising work and living areas as well as washrooms. Two of these sites were office and dormitory rooms, while the remaining two were washrooms, facilitating comparisons of bacterial concentrations across different locations. The positions of samplers employed for collecting interior airborne bacterial concentration data are illustrated in Figure S1.

Considering the human breathing zone delineated in the IAQ standard, each sampling point was positioned approximately 1.0 m above ground level [31]. The locations of these sites are depicted in Figure S2. Additionally, fundamental information regarding the selected four sites can be found in Table S1.

*2.2. Sampling Method.* In this investigation, the impaction method was employed for sampling, primarily utilizing a six-stage Andersen sampler. This sampler facilitates the analysis of bacterial particle size at the surveyed sites, as it can categorize bacteria of varying particle sizes, including >7, 4.7–7, 3.3–4.7, 2.1–3.3, 1.1–2.1, and<1.1 $\mu$m. The flow rate of the six-stage Andersen sampler was calibrated to 28.3 L/min. Furthermore, the Luria bertani (LB) agar plate, a widely employed medium for bacterial collection, was utilized in this investigation. Sampling was divided into three periods: morning (9:00–11:00), afternoon (15:00–17:00), and evening (20:00–22:00) to enable comparisons of bacterial concentration fluctuations within the exact location throughout the day. Each sampling lasted 2 h, totaling 6 h/day. The data reported in this study are the mean values of three replicates. Concurrent with measuring bacterial concentration, indoor environmental factors (temperature and relative humidity) were also monitored to analyze their effects on bacterial concentration. Information on the instruments utilized for testing parameters can be found in Table S2.

*2.3. DNA Extraction, Polymerase Chain Reaction (PCR) Amplification, and Sequencing Analysis.* In this study, using the air pump, air is passed through a 0.22 $\mu$m filter membrane. A total of 12 total suspended particulate (TSP) samples were collected from the office and dormitory buildings. All experiments were conducted in triplicate, and the results were expressed as the mean values. The filter membrane is placed in a cryovial and sent to the laboratory for the next step of DNA extraction, PCR amplification, and sequencing analysis.

DNA isolation and analysis were performed by employing TB Green Premix Ex Taq II (Tli RNaseH Plus; Dalian, China) as instructed by the manufacturer. 16S rRNA sequences, which are high-variability areas seen in bacteria V3–V4, were amplified using the primers 5′-ACTCCTACGGGAGGCA GCA-3′ and 5′-GGACTACHVGGGTWTCTAAT-3′. The genes were amplified via PCR experiments [32]. The amplified products were sequenced at Yacheng Biotechnology (Harbin) Co. Ltd. using an Illumina MiSeq platform (Illumina, San Diego, CA, United States). The sequencing data were analyzed according to Jiang et al. [33].

*2.4. Statistical Analysis.* All data have been reported as the mean values of triplicates. The Sequence ReadArchive (SRA) is used for storing high-throughput sequencing raw data, including 454, Illumina, SOLiD, IonTorrent, Helicos, and Complete Genomics. The metagenomic datasets were evaluated for the organism abundance (i.e., abundance of microbial groups) by annotating against the SRA database. Functional abundance (i.e., abundance of functional genes) was determined by separately annotating the metagenomic dataset against the subsystems database [34]. The paired-end (PE) reads produced from sequencing on the Illumina MiSeq platform were initially aligned based on their overlapping regions. The quality of the sequences was then assessed and filtered for any low-quality reads. Following the differentiation of the samples, an OTU clustering analysis and species taxonomy analysis were conducted. OTU clustering analysis results can be used to assess various diversity indices for both OTUs and the detection of sequencing depth.

Statistical analysis of community structure can be conducted at each taxonomic level using the available taxonomic information. Origin 2022 (OriginLab, United States) was employed to create the graphs. IBM SPSS Statistics 22.0 was used to analyze the analysis of variance (ANOVA) and Pearson's correlation in data, and $p < 0.05$ was established as the limit for statistical significance.

## 3. Results and Discussion

*3.1. Biological Analyses of the Indoor Environment in Severe Cold Regions.* High-throughput sequencing of air microorganism samples from office and dormitory environments enabled comprehensive statistical analysis of community structure across all taxonomic levels. Utilizing the PCR technique, a specific genomic region was duplicated and amplified a millionfold, thereby facilitating subsequent analyses. Following high-throughput sequencing, Figure S3 illustrates the species composition and relative abundance of airborne bacteria in the office and dormitory settings during winter. Graph segments representing less than 1% were amalgamated to depict other categories. The airborne bacterial community composition and the proportion of each constituent were approximately similar in the dormitory and office environments. Proteobacteria emerged as the predominant airborne bacterial community, with marginally higher levels observed in the dormitory (72%) compared to the office (65.6%). Conversely, Bacteroidota was more abundant in the office (25%) relative to the dormitory (17%). Actinobacteriota levels remained relatively consistent between both settings, with 3.8% and 4.3% in the dormitory and office, respectively. Firmicutes were twice as prevalent in the dormitory (3.5%) compared to the office (1.8%), whereas Myxococcota levels were doubled in the office (2.4%) relative to the dormitory (1.2%). Furthermore, the dormitory housed numerous airborne bacterial species absent in the office, including Entotheonellaeota, Patescibacteria, Hydrogenedentes, and Armatimonadota, among others.

Regarding specific species, several were selected with the highest abundance (at the genus level), either individually or across all samples. Figure S4 presents the taxonomic information for these species at the phylum level, wherein species of the same color denote their affiliation to the same phylum. *Vibrionimonas*, belonging to the Bacteroidota community, exhibited the highest relative abundance at 16.6%. It was followed by various airborne bacteria within the Proteobacteria community, such as *Bradyrhizobium* (12.9%), *Variovorax* (12.1%), and *Methylovirgula* (4.8%), among others.

The extent of variation in species abundance distribution between the dormitory and office environments was assessed using a statistical distance metric. The Bray–Curtis algorithm, a commonly used metric in ecology and environmental science for comparing differences in species composition between two samples, was employed to calculate the distance between the two samples, yielding a distance matrix. The Bray–Curtis distance measure was often more appropriate than other distance measures because it was not affected by large differences in species abundance (or individual counts). The Bray–Curtis distance $D_{BC}$ was calculated using the following formula:

$$D_{BC} = \frac{\sum_{i=1}^{n} |A_i - B_i|}{\sum_{i=1}^{n} (A_i + B_i)} \quad (1)$$

where $D_{BC}$ is the Bray–Curtis distance, $A_i$ is the abundance (or count) of the $i$ species in sample $A$, $B_i$ is the abundance (or count) of the $i$ species in sample $B$, and $n$ is the total number of species.

A smaller coefficient of variation signified a smaller disparity in species diversity between the samples. Based on calculations, the $D_{BC}$ of dormitory and office was 0.186, which is shown in Figure S5. There was minimal difference in species diversity between the dormitory and office environments, with both exhibiting nearly identical airborne bacterial communities.

In prior research, the diversity of bioaerosols in indoor and outdoor environments had been analyzed [35, 36]. However, there needed to be more focus on the examination of bioaerosol diversity between different indoor rooms. The findings in this study indicated that the composition of indoor bacterial aerosols was similar across various rooms, with minimal correlation to the room type. As such, the room type did not appear to significantly influence diversity in this study. Additionally, it was observed that the contribution of bacterial aerosols in cold regions was inconsistent with those found in other climatic zones by PCR quantitatively evaluated. This discrepancy might be attributed to distinct sources of airborne bacteria and specific conditions conducive to their survival, resulting in differences in bacterial aerosol components. A list of bacterial aerosol components for different climatic zones to further elucidate this point is presented in Table 1.

The bacterial aerosol components are probably related to the climatic conditions of ambient air (e.g., temperature, humidity, and solar radiation intensity) and building element factors. Human occupants and their activities had a minor effect on bacterial aerosol components. In some specific occasions (such as botanical gardens), the bacterial aerosol components might be affected by the elements of the spaces. Kozdrój, Fraczek, and Ropek [39] mentioned that distinct plant- or soil-derived bacteria were identified in the air of botanical gardens. Meanwhile, these locations would provide a unique indoor environment (such as warm and moist), which might lead to different airborne bacterial diversity. In this study, we investigated the bacterial aerosol components in offices and dormitories of a university. During the winter in severe cold regions, offices and dormitories were equipped with heating systems to regulate the indoor environment. The indoor temperature in the severely cold area could reach 24°C, while the indoor humidity is dry, about 32% [42]. Proteobacteria and Bacteroidota thrived in this warm and dry environment [43]. And in the university's dormitories and offices, the building element factors were approximately the same, with wooden and iron tables, chairs, and beds, as well as some household appliances.

Table 1: Bacterial aerosol components in different climatic zones.

| Place | Climatic zone | Bacteria | Ref. |
|---|---|---|---|
| Harbin | Medium temperate continental monsoon climate (severe cold regions) | Proteobacteria<br>Bacteroidota<br>Actinobacteriota<br>Firmicutes | This study |
| Urbana-Champaign | Temperate climate | Proteobacteria<br>Firmicutes<br>Bacteroidota<br>Actinobacteriota | [37] |
| Ankara | Temperate continental climate | *Micrococcus*<br>*Bacillus*<br>*Auricularis*<br>*Haemolyticus* | [38] |
| Kraków | Temperate maritime climate | *Arthrobacter*<br>*Bacillus*<br>*Curtobacterium*<br>*Exiguobacterium* | [39] |
| Berkeley | Mediterranean sea climate | *Cloacibacterium*<br>*Limnohabitans*<br>*Pseudomonas*<br>*Acinetobacter* | [40] |
| Barcelona | Mediterranean climate | *Methylobacterium*<br>Chitinophagaceae<br>*Bradyrhizobium*<br>*Paracoccus* | [41] |

Due to the similar climatic conditions of ambient air and building element factors between classrooms and offices, the bacterial aerosol components exhibited minimal variation.

*3.2. Effect of the Opening State of Windows on Bacterial Concentration.* Human behavior was widely recognized to considerably influence indoor environments and energy consumption, with window operation constituting a particularly significant factor. Consequently, examining the effects of open or closed windows on variations in indoor bacterial aerosol concentrations was of notable interest. For this study, a dormitory was randomly selected as the test site, excluding offices due to user feedback and their distinct usage characteristics. Figure S6 presents a schematic representation of the window in the actual testing room. The room features walls measuring 3.3 m × 3 m, windows measuring 1.5 m × 1.8 m, a window-to-wall ratio of 0.27, and openable windows measuring 0.6 m × 1.1 m. During the test, doors remained predominantly closed, ensuring no strong convection was generated within the room when windows were open. The only way to exchange air between indoor and outdoor areas was through windows.

Figure 2 illustrates the variations in bacterial concentrations and particle size distributions in the dormitory during nonheating periods following window closure. Regarding bacterial particle size distribution during these periods, bacterial aerosols with particle sizes <1.1 and 2.1–3.3 $\mu$m constituted the majority of the distribution. With windows closed, the proportion of particle sizes between 2.1 and 3.3 $\mu$m was 53% in the morning (9:00–11:00), declining to 26% in the afternoon (15:00–17:00), and ultimately reaching 15% in the evening (20:00–22:00). Conversely, particle sizes >7, 4.7–7, and 3.3–4.7 $\mu$m exhibited the smallest percentages in the morning, increasing to their highest in the evening. The proportions of particle sizes <1.1 $\mu$m and 1.1–2.1 $\mu$m were minimal in the morning, peaking in the afternoon, and decreasing in the evening. Upon closing the window, the percentage of particle sizes within the 2.1–3.3 $\mu$m range was reduced compared to when windows are open, while the percentages of 3.3–4.7, 4.7–7, and >7 $\mu$m increased in the morning. The proportion of bacterial aerosols <1.1 and >7 $\mu$m rose during the afternoon. At night, the percentage of bacterial aerosols <3.3 $\mu$m elevated, while the proportion of particles >3.3 $\mu$m diminished. Overall, the percentage of bacterial aerosols with particle sizes >3.3 $\mu$m decreased when windows were opened.

As to indoor bacteria concentration, Figure 2 reveals that the peak during nonheating periods in the morning decreased at noon and reached a minimum at night. In the morning, bacterial aerosol concentrations attained 948 CFU/m$^3$, whereas 224 and 118 CFU/m$^3$ were observed in the afternoon and evening, respectively. When the window was closed, a similar trend was evident. The bacterial aerosol concentration in the morning amounted to 1637 CFU/m$^3$, compared to 289 and 153 CFU/m$^3$ in the afternoon and evening, respectively. Compared to open windows, indoor bacterial concentrations were higher when windows remained closed, with increases of 72.7%, 28.9%, and 30% observed in the morning, afternoon, and evening, respectively.

The efficacy of window openings in reducing indoor bioaerosol concentrations was contingent upon the concentration of outdoor bioaerosol. This study demonstrated that opening windows effectively decreased indoor bacterial aerosol concentrations; however, outdoor pollutants continued to contribute to elevated indoor bacterial aerosol levels. By comparing the concentrations during the heating period, it was discovered that bacterial aerosol concentrations reached 1200 CFU/m$^3$ when windows were opened during this time. In contrast, the concentration was only 800 CFU/m$^3$ in the nonheating period. As illustrated in Figure 2, open windows influenced the size distribution of bacterial aerosols, with an increase in the proportion of small-scale (<3.3 $\mu$m) bacterial aerosols. This observation suggested that aerosols within this fraction enter the room from the outside through the windows. Particulate matter, such as PM2.5, could transport bacteria indoors, elevating the proportion of bacterial aerosols at sizes <3.3 $\mu$m.

Occupant density has also been identified as contributing to indoor bacterial aerosol levels. In this study, it was observed that bacterial aerosol concentrations were significantly higher during the morning hours. This phenomenon was attributed to indoor occupant density fluctuations resulting from student schedule variations, with the

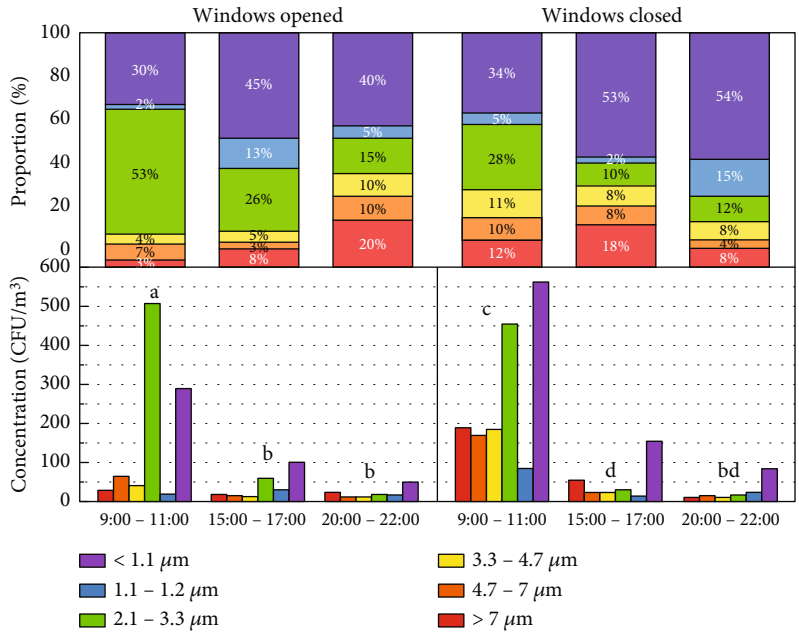


FIGURE 2: Effect of opening windows on indoor bacteria concentration and particle size distribution in dormitories during nonheating periods. Different lowercase letters denote statistically significant differences between the indoor bacteria concentration at different times ($p < 0.05$).

occupant density increasing as students return to their dormitories after evening classes. Consequently, bacteria exhaled by individuals and introduced from external sources contribute to a rise in bacterial aerosol concentrations. Following an overnight accumulation of these bacterial aerosols, their proportion reached a maximum during the morning measurement period.

As shown in Figure 3, upon opening the windows, bacterial aerosols with particle sizes of <1.1 and 2.1–3.3 $\mu$m persisted in constituting the predominant size distribution during the heating period. The proportion of particles with sizes <1.1 $\mu$m exhibited a decrease followed by an increase over time. Conversely, an inverse relationship was observed for particles within the 2.1–3.3 $\mu$m range. No discernible trend was detected in the temporal variation of other particle sizes. Notably, the percentage of bacterial aerosols exceeding 3.3 $\mu$m increased when the windows remained closed. Regarding bacterial aerosol concentrations, the highest levels during the heating period were consistently observed in the morning, which aligned with the observations during the nonheating period. Furthermore, no indoor bacterial aerosol concentrations surpass the established standards when windows remain open. The concentrations were 1213 CFU/m$^3$ in the morning, 194 CFU/m$^3$ in the afternoon, and 141 CFU/m$^3$ in the evening. However, when windows were closed, these concentrations rose to 1714 CFU/m$^3$ in the morning, 347 CFU/m$^3$ in the afternoon, and 224 CFU/m$^3$ in the evening, representing respective increases of 41.3%, 78.8%, and 58.3%. Notably, compared to the nonheating period, the afternoon and evening concentrations substantially increased when windows were closed during the heating period.

As illustrated in Figure 3, elevated overall concentrations were observed during the heating period compared to the nonheating period, attributable to the heightened particulate matter levels during the former. This increased bacterial aerosol concentrations as the particles remained suspended in the air for extended durations. With windows open, bacterial aerosol concentrations experienced a rise of 28% in the morning, 13.2% in the afternoon, and 20% in the evening during the heating period relative to the nonheating period. Conversely, when windows were closed, the heating period saw bacterial aerosol concentrations increase by 4.7% in the morning, 20.4% in the afternoon, and 46.2% in the evening compared to the nonheating period.

In this study, bacterial aerosol concentrations did not surpass the established threshold during the day when windows were open, irrespective of the heating or nonheating periods. However, when windows were closed, the bacterial aerosol concentration exceeded the standard in the morning. Relative to the standard, the nonheating period surpassed 9.1%, while the heating period exceeded it by 14.3%. Consequently, it was recommended to utilize mechanical ventilation in the morning to mitigate elevated bacterial aerosol concentrations that could potentially harm human health.

In many cases of this study, bacterial aerosol concentrations exceeded the established standard (1500 CFU/m$^3$) for a severe cold region. It was important to note that IAQ standards for bacterial and fungal limits vary across different climatic zones and between countries and regions. There was a lack of uniform international standards for airborne bacterial exposure levels and acceptable limits, with various countries, regions, and organizations adopting different criteria [44]. Table 2 presents the microbiological concentration limits of selected countries and organizations. Recognizing that these guidelines are specific to individual sites was crucial, and discrepancies may exist even within the same country, region, or organization.

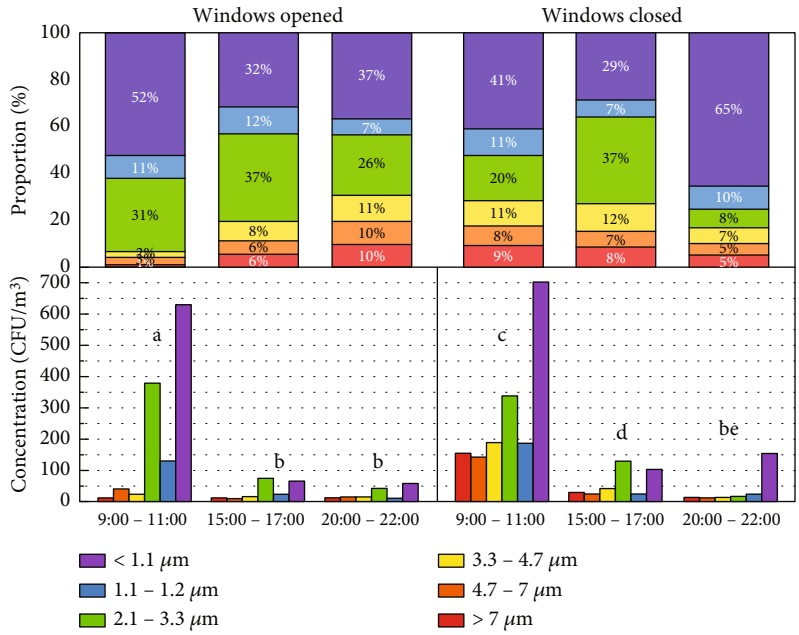


Figure 3: Effect of opening windows on indoor bacteria concentration and particle size distribution in dormitories during heating periods. Different lowercase letters denote statistically significant differences between the indoor bacteria concentration at different times ($p < 0.05$).

Table 2: Guideline of airborne microbiological concentrations in different countries or organizations.

| Country, region, organization | Concentration of fungi (CFU/m$^3$) | Concentration of bacterial aerosols (CFU/m$^3$) | Ref. |
|---|---|---|---|
| Hong Kong, China | No visible mold or odor | 1000 | [45] |
| Taiwan, China | 1000 | 1500 | [46] |
| China | | 1500 | [47] |
| Canada | 500 | | [48] |
| Korea | | 800 | [49] |
| Brazil | 750 | | [50] |
| Germany | 10,000 | 4500 | [51] |
| Portugal | | 500 | [52] |
| Switzerland | 1000 | 1000 | [53] |
| WHO, 2009 | No quantitative, health-based guideline values or thresholds can be recommended for acceptable levels of contamination by microorganisms | | [54] |
| WELL | No visible mold or water damage | | [55] |

*3.3. Bacterial Aerosol Concentrations Under Different Environmental Factors.* Room environmental factors influenced bacterial concentration. In this study, an empty room was chosen as the sampling site to minimize human interference, and the investigation spanned both heating and non-heating periods. Temperature and relative humidity were documented as critical environmental parameters. Figure 4 presents the bacterial concentration under varying temperatures and humidity levels. The test room's temperature ranged from 13.9°C to 27.4°C, while relative humidity varied between 21.1% and 56.3%. At low relative humidity, bacterial concentration increased with rising temperature. As relative humidity escalated, the bacterial concentration peaked between 23.4°C and 26.9°C. The highest bacterial concentration, at 879 CFU/m$^3$, occurred when the temperature was 27.4°C and relative humidity was 41.1%. However, with constant temperature, bacterial concentration initially increased and subsequently decreased as relative humidity rose, particularly within the 36.7%–46.3% humidity range. This trend was observable throughout the entire temperature testing spectrum.

The dynamics of bacterial aerosol concentration in the indoor air were governed by a complex interplay of physical and biological factors, with gravitational sedimentation and bacterial activity being key determinants [56]. The study in question shed light on how temperature, a critical environmental variable, significantly influences bacterial activity, which in turn affects aerosol concentrations.

As illustrated in Figure 4, an intriguing pattern emerged within a specific temperature range of 24.2°C–26.5°C, where the concentration of bacterial aerosols peaked. This optimal temperature window suggested a "most suitable growth

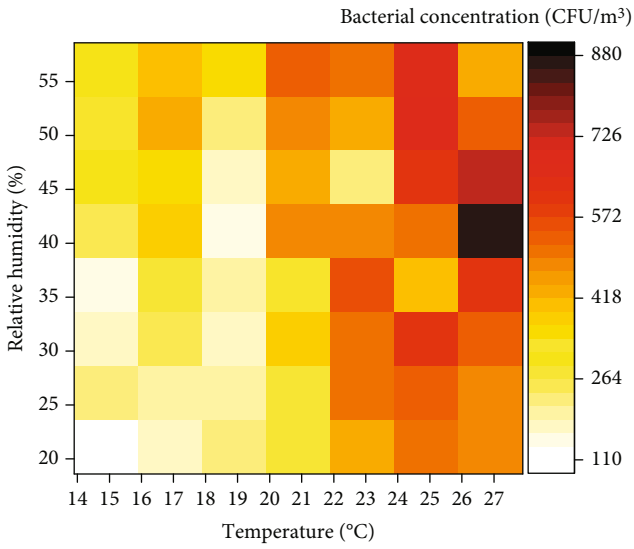


FIGURE 4: Heat map of the relationship between environmental factors (temperature and relative humidity) and bacterial concentrations in the field study.

zone" for bacterial activity, where conditions were ideal for bacteria to thrive and become airborne, maximizing their presence in the aerosol form. Outside this range, bacterial activity waned, possibly due to metabolic slowdown or increased susceptibility to environmental stressors [57, 58], leading to reduced aerosolization and, ultimately, inactivation postincubation.

Relative humidity introduced another layer of complexity to the behavior of bacterial aerosols. At lower levels of humidity, the moisture available for bacterial activity was limited, which could impair bacterial function and result in diminished aerosol concentrations [59, 60]. However, as relative humidity increased and approached an optimal threshold, the survival and activity of bacteria in aerosols were enhanced, leading to an uptick in their numbers. Yet, beyond this optimal point, the increased size of bacterial particles due to water absorption could lead to a higher susceptibility to gravitational forces. This resulted in a higher rate of sedimentation, where bacterial aerosols were pulled back to the ground, thereby reducing their concentration in the air [61–63].

The study's findings underscored the intricate balance between the biological imperatives of bacterial activity and the physical forces of gravity. It was evident that both factors exert significant control over the distribution and concentration of bacterial aerosols in the indoor environment. Understanding these relationships was crucial for predicting the spread of airborne bacteria, which had implications for public health, epidemiology, and our understanding of the atmospheric microbiome. Moreover, this study highlighted the need for further research into the nuances of how environmental factors interact with bacterial physiology to influence aerosolization. Such knowledge could be aided in developing models that predicted the behavior of bacterial aerosols under varying conditions, offering insights into potential health risks and the role of bacteria in an indoor environment.

*3.4. The Bacterial Concentration in Dormitory and Office Buildings During the Nonheating and Heating Periods.* Individual schedules could significantly influence IAQ, and the indoor environment may differ based on the room's type. This study examined bacterial aerosol concentrations in university dormitories (inclusive of washrooms) and offices (including washrooms) on both weekdays and weekends. Figure S7 illustrates the alterations in the particle size distribution of bacterial aerosols on weekdays and weekends in the office before and after the heating period. For the office and office washroom, bacterial aerosols with a particle size of <1.1 $\mu$m were more prevalent throughout the day on weekends compared to weekdays. The proportion of <1.1 $\mu$m particle size bacterial aerosols in the office progressively increased during the day, while it decreased in the office washroom in the afternoon. Over the weekend, the proportion of bacterial aerosols with a particle size between 1.1 and 2.1 $\mu$m diminished in both the office and office washroom, while no discernible pattern was observed for other particle size variations. Compared to the nonheating period, there was a substantial increase in the proportion of bacterial aerosols with a particle size of 1.1–2.1 $\mu$m following heating, except for the office on weekends. Moreover, the proportion of <1.1 $\mu$m particle size bacterial aerosols in the office during the heating period rose.

As shown in Figure S7, in the office, the highest proportion remained that of <1.1 $\mu$m bacterial aerosols; however, the percentage of bacterial aerosols >3.3 $\mu$m (3.3–4.7, 4.7–7, and >7 $\mu$m) had experienced an increase. Compared to the office, the proportion of bacterial aerosols within the 2.1–3.3 $\mu$m range was notably higher in the dormitory and dormitory washroom. The percentage of bacterial aerosols at 2.1–3.3 $\mu$m on weekends tended to be higher than that on weekdays. The particle size of bacterial aerosols was determined by the bacterial components, which primarily originated from outdoor sources and humans [40, 64]. Bacteria could attach to clothing and subsequently be shed, enabling humans to serve as carriers for the built environment [65, 66]. As individuals enter indoor environments from outdoors, they are introduced to external bacteria. In office settings, people frequently go in and out, and bacteria carried on their clothing can infiltrate the indoor environment. In contrast, the primary source of bacterial aerosols in dormitories was human respiration, with less contribution from outdoor sources. Variations in particle size distribution among offices, dormitories, and washrooms could be attributed to these differing sources. Bacterial aerosols in washrooms also included volatile components from human excrement. Dormitory washrooms, which accommodated a more comprehensive range of activities such as bathing, exhibit distinct particle size distributions compared to office washrooms. On the other hand, office washrooms were typically utilized solely for excretion and handwashing.

In examining bacterial aerosol concentrations within an office environment, the study specifically analyzed the data before and after a heating period. As illustrated in Figure S8, prior to the heating period, bacterial aerosol

concentrations in both the office and office washroom exhibited the highest levels in the morning, with a subsequent decrease in the afternoon and the lowest levels in the evening. Furthermore, the office washroom experienced more frequent usage on weekdays than weekends, when its usage was nearly equivalent to that of the office. Following the heating period, an increase in bacterial aerosol concentrations was observed in both the office and office washrooms during the evening on weekends. When comparing the concentrations in the office to those in the office washroom, it could be deduced that bacterial aerosol concentrations in the office restrooms should be higher. In relation to the standard of 1500 CFU/$m^3$, the morning concentrations in the office washroom on weekdays (1867 CFU/$m^3$) prior to heating and in the office (1513 CFU/$m^3$) and office washroom (1549 CFU/$m^3$) on weekends exceeded the standard by 24.5%, 1%, and 1.3%, respectively. After the heating period, the concentration of bacterial aerosols in the office and office washroom generally increased across all periods, including morning, afternoon, and evening. For instance, the morning concentration in the office after heating reached 2078 and 1731 CFU/$m^3$ on weekdays and weekends, respectively, representing increases of 41.2% and 14.4% compared to preheating levels. Additionally, the concentration in the office washroom after the heating period increased by 17.4% and 5% on weekdays (2191 CFU/$m^3$) and weekends (1595 CFU/$m^3$), respectively, relative to the levels before the heating period. Consequently, after the heating period, the bacterial aerosol concentrations measured in the office and office washroom in the morning surpassed the 1500 CFU/$m^3$ standard.

As depicted in Figure S9, the concentration of bacterial aerosols in the dormitory was found to be lower than that in the office setting. Interestingly, only the weekend dormitory bacterial aerosol concentration surpassed the established standard of 1500 CFU/$m^3$. Prior to the heating period, bacterial aerosol concentrations in the dormitory exceeded the standard by 9.1%, while after the heating period, the excess increased to 14.2%. The office experienced a more pronounced increase in bacterial aerosol concentrations during the afternoon and evening. The elevated bacterial aerosol concentration in the office during the morning could be attributed to occupants' schedules; however, this differs from the situation in the dormitory. Individuals entering the room from outside may introduce bacterial aerosols into the office. As people exited for lunch and dinner and returned to the office, the concentration of bacterial aerosols maintained a certain level throughout the afternoon and evening. Conversely, in the dormitory, individuals typically did not return during the day but continued attending classes.

## 4. Limitations

This study presents three limitations. Firstly, it solely focuses on bacterial aerosols, neglecting the potential impact of fungal aerosol contamination on human life. Bacteria and fungi exhibit varying adaptability to environmental conditions (e.g., temperature and relative humidity), resulting in different concentrations in a given area. Nevertheless, the findings of this study remain valuable for mitigating bioaerosol pollution. Secondly, because this study focuses on a severe cold region, the results may not be applicable to warmer regions. Lastly, the experiments conducted in this study were repeated a minimum of three times, but increased replication is necessary in future investigations to acquire more reliable data. Therefore, further exploration is needed to explain bioaerosols' crucial role in indoor environmental quality and human health.

Despite these limitations, this study offers foundational data for examining bioaerosol pollution in severe cold regions. The findings presented in this paper contribute boundary conditions for numerical simulation research in related fields and provide recommendations for mitigating bioaerosol pollution.

## 5. Conclusions

The presence of bioaerosols is known to significantly impact IAQ and may potentially lead to health issues if they contain allergens or pathogens. In this study, a 60-day field test examined indoor bacterial aerosol concentrations in a severe cold region. Several conclusions can be drawn from the findings.

- The bacterial composition in university dormitories and offices within severe cold regions is fundamentally similar. Proteobacteria, Bacteroidota, Actinobacteriota, Firmicutes, and Myxococcota constitute the predominant airborne bacterial community.
- In severe cold regions, during both heating and nonheating periods, bioaerosol concentrations are highest in the mornings in university dormitories, influenced by occupants' schedules. When windows remain closed, bioaerosol concentrations in the morning exceed the standard. The nonheating period surpasses the standard by 9.1%, while the heating period exceeds it by 14.3%.
- The study identified an optimal range of environmental factors for bacterial aerosol concentrations. These factors impact bacterial aerosol concentrations by affecting their activity and particle size. Room temperature varied between 13.9°C and 27.4°C, and relative humidity ranged from 21.1% to 56.3% during the test period. The highest bacterial concentration value was 879 CFU/$m^3$, with a temperature of 27.4°C and relative humidity of 41.1%.
- Their functions determine the particle size distribution of bacterial aerosols in dormitories, offices, and washrooms. Bacterial aerosol concentrations in offices are higher than in dormitories, with more instances exceeding the 1500 CFU/$m^3$ standard. Therefore, increased attention should be paid to IAQ in offices in severe cold regions.

Overall, not only because of the air pollutants emitted from coal and straw combustion but also because of

maintaining an indoor thermal environment in winter, the occupants tended to close the windows in severe cold regions. This has led to excessive indoor bioaerosol concentrations in winter in severe cold regions. This study offers insights into bioaerosol pollution in severe cold regions. Future studies would expand the sample range to include more types of buildings and areas to improve the generalizability of the results. The findings will serve as a foundation for enhancing IAQ in these areas.

## Data Availability Statement

The data used to support the findings of this study may be released upon application to the Harbin Institute of Technology, which can be contacted at chaoshen@hit.edu.cn.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Author Contributions

Xiaojian Duan: drafting, writing up, investigation, and data analysis.

Chao Shen: supervision, writing up, investigation, and resources.

Guozheng Chen: investigation.

Xi Deng: writing up.

Phil Jones: supervision and proofreading.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section. Supporting Information includes detailed procedures for sampling sites, biological analyses of the indoor environment in severe cold regions, the effect of the opening state of windows on bacterial concentration, and the bacterial concentration in dormitory and office buildings during the nonheating and heating periods. Figure S1: locations of samplers for collecting interior microbial concentration data. Figure S2: appearances of four sampling sites. Figure S3: microbial (a) community barplot and (b) community heat map analysis. Figure S4: dominant species barplot. Figure S5: heat map analysis for distance matrix. Figure S6: schematic diagram of the window. Figure S7: changes in the particle size distribution of bacterial aerosol on weekdays and weekends in the (a) office and (b) dormitory before and after the heating period. The inner ring is in the morning (9:00–11:00), the middle ring is in the afternoon (15:00–17:00), and the outer ring is in the evening (20:00–22:00). Figure S8: changes in bacterial aerosol concentrations in the office on weekdays and weekends before and after the heating period. Different lowercase letters denote statistically significant differences between the indoor bacteria concentrations of different periods ($p < 0.05$ ). Figure S9: changes in bacterial aerosol concentrations in the dormitory on weekdays and weekends before and after the heating period. Different lowercase letters denote statistically significant differences between the indoor bacteria concentrations of different periods ($p < 0.05$). Table S1: the basic information of the selected four sites. Table S2: the instruments for tested parameters. *(Supporting Information)*

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