Cross-boundary Intrusion and Exposure Risk: Migration and Attribution of Fecal-source Bioaerosols in Urban and Rural Buildings with Different Ventilation Modes

Xu Zouying [1] Zhang Rui [1] Deng Jiale [1] Li Xinqi [1]

(Chuzhou Polytechnic, Chuzhou, Anhui 239000, China)

Abstract: Urban buildings are widely regarded as "healthy fortresses" against external pollution, yet their defensive capabilities against long-range transported biological contaminants remain unclear. This study aims to investigate the cross-boundary intrusion mechanisms of fecal-source bioaerosols (BA) and compare the resulting indoor exposure risks under different ventilation modes. Through parallel, year-long field monitoring in an urban-rural binary scenario (a rural residential house 3 km from a wastewater treatment plant vs. an urban office building 15 km away), this study found that: during external pollution events, the indoor total bacterial concentration in the urban office with a mechanical fresh air system (average 1.5×10^3 CFU/m^3) was systematically higher than that in the naturally ventilated rural house (average 8.9×10^2 CFU/m^3). Analysis based on metagenomic sequencing and an original "Source-Pathway-Shell-Indoor" (S-P-S-I) attribution model attributed this phenomenon to the "pollutant enrichment effect" generated by the continuously operating mechanical ventilation system when facing external pollution. The findings challenge the linear assumption that "more modern buildings are safer," revealing ventilation mode as the dominant factor determining indoor exposure risk to long-range biological pollution. This work provides crucial scientific evidence for reassessing modern building ventilation strategies and for constructing intelligent defense systems based on dynamic risk assessment.

Keywords: Cross-boundary intrusion; Bioaerosols; Fecal source; Building ventilation; Exposure risk; Attribution analysis

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Author Profile: Xu Zouying (born 1985), female, holds a master's degree and serves as a lecturer. Her research focuses on architectural environment.

1. Introduction

The era we inhabit is witnessing urbanization at an unprecedented scale and pace. The urban structures we have constructed with substantial investments are being designed as "health fortresses" that isolate them from external environmental disturbances.

Through highly airtight building envelopes and precisely controlled mechanical ventilation and air conditioning (HVAC) systems, the prevailing paradigm of modern architectural science holds that it can provide humans with a stable, clean, and controllable indoor microenvironment [1]. This design philosophy has achieved widely recognized success in mitigating physical and chemical pollutants such as outdoor PM2.5 and has profoundly shaped the design standards and operational principles of contemporary healthy buildings.

However, beyond this seemingly impregnable "fortress," an invisible, cross-sectoral biological threat has been systematically overlooked. With population concentration, large-scale municipal wastewater treatment plants (WWTPs) and intensive livestock and poultry farms have become indispensable infrastructure in modern society. While processing waste, these facilities continuously emit substantial amounts of bioaerosols (BA)—complex airborne particulate matter containing live bacteria, viruses, fungi, endotoxins, and even antibiotic resistance genes (ARGs). Domestic and international studies have conclusively demonstrated that wastewater treatment plants are significant sources of bioaerosols in urban environments, with their surrounding air microbial community structures and abundances markedly differing from those in typical urban areas [2,3]. This cognitive blind spot stems from entrenched disciplinary barriers: atmospheric scientists focus on long-range transport, public health experts investigate near-field exposure, and building environment engineers concentrate on indoor pollutant control. The lack of effective academic dialogue among these three groups has kept this "cross-sectoral" threat perpetually marginalized in mainstream building safety discourse.

Previous academic studies predominantly confined the health risks posed by fecal sludge biogas (BSG) to the immediate vicinity of its emission sources (typically within hundreds of meters to one kilometer), treating it as a "local" occupational exposure or an environmental hazard affecting neighboring communities [4]. This perspective inadvertently establishes a psychological safety perimeter, implying that threats dissipate when physical distance is sufficient. However, a fundamental challenge to modern urban safety lies in whether these "distant hazards" —propelled by atmospheric circulation and diffusing continuously at low concentrations into urban cores dozens of kilometers away—remain merely negligible background noise. Moreover, our touted "golden bell armor and iron robe" (high airtightness + mechanical ventilation) may, when confronted with this invisible "chronic poison," serve not as a reliable guardian but inadvertently function as an "accumulator" that facilitates its entry.

The urgency of addressing this issue is heightened by two trends. First, climate change has led to an increase in extreme weather events, making atmospheric transport models more uncertain [5]; second, antibiotic resistance issues such as those posed by "superbugs" have become a major global public health challenge, and fecal matter has been identified as a critical vector for the cross-regional spread of antibiotic resistance genes (ARGs) [6]. Consequently, elucidating the mechanisms of their invasion within buildings and the associated exposure risks is no longer merely a matter of architectural science but directly pertains to the broader framework of urban public health security systems.

To address this significant knowledge challenge head-on, this study designed a parallel urban-rural comparative experiment. Instead of examining buildings in isolation, we approached them as open systems integrated into the "atmosphere-building" continuum for holistic investigation. By introducing an innovative "Source-Pathway-Shell-Interior" (S-P-S-I) attribution model, the core objectives of this study are specified as follows: (1) For the first time, quantify the actual flux and magnitude of fecal waste source (BA) "transboundary intrusion" into urban buildings through direct field evidence; (2) Directly compare the ultimate exposure effects of two distinct ventilation strategies (mechanical ventilation vs. natural ventilation) to external biological contamination in real-world settings; (3) Precisely dissect the key physical processes and driving factors underlying risk disparities. This work aims to challenge the linear mindset that "the more modern a building, the safer it is," providing a novel scientific perspective and empirical basis for future health-oriented building design, operation, and risk assessment.

2. Materials and Methods

2.1 Description of the Research Site

Two representative buildings located at the periphery of an urban cluster in eastern China were selected as the study subjects. The region experiences prevailing southeast winds year-round, ensuring that both buildings are situated along the downwind transport corridor of the same large municipal wastewater treatment plant (with a designed treatment capacity of 800,000 tons/day).

Urban Office Building (UO): A five-story Class A office building constructed in 2015, featuring a double-layer insulating Low-E glass curtain wall with an air tightness rating of 1.8 ACH (tested at @50Pa using a blast door). The test area is a 30 m^2 standard office on the third floor, equipped with an independent HVAC system. The fresh air supply rate is designed according to the national standard GB 50189-2015 for Energy Efficiency Design of Public Buildings at

30 $m^3/(h·p)$, utilizing a combination of G4 primary and F8 medium-efficiency filters, with the system operating continuously 24 hours a day.

Rural House (RH): A two-story brick-and-concrete self-built structure constructed in 2005, featuring single-layer aluminum alloy sliding windows with poor airtightness. The experimental area is a 25 m^2 south-facing bedroom on the second floor, utilizing natural ventilation (NV) through open windows—operated according to residents' daily habits (typically open during daytime and closed at night)—as confirmed through interviews and recording devices. The wastewater treatment plant is located approximately 3 km from the RH and 15 km from the UO.

2.2 Sample Collection and Quality Control

From March 2023 to February 2024, a one-year field monitoring campaign was conducted, covering all four seasons: spring, summer, autumn, and winter. Each quarter, a 7-day consecutive period with relatively stable meteorological conditions (no precipitation and consistent wind direction) was selected as the time window for high-intensity sampling. Air particulate matter samples were collected simultaneously indoors and outdoors at UO and RH locations.

Sampling equipment: An Andersen N6 single-stage impact-type high-flow air sampler (Thermo Fisher Scientific, USA) was used, with a set flow rate of 1000 L/min to ensure sufficient biomass for analysis was captured within the 4-hour sampling cycle.

Sampling medium: A quartz fiber filter membrane (Whatman, Ø 90 mm) that has undergone 4-hour pretreatment at 450°C .

Quality Control: One on-site blank control and one transport blank control were established for each sampling cycle to assess potential background contamination. All samples were immediately stored in a portable refrigerator at-20 ℃ after collection, delivered to the laboratory within 4 hours, and stored at-80°C .

2.3 Biological Aerosol Analysis

2.3.1 Counting by Culture Method

After crushing the sampling membrane, place it in 10 mL of sterile phosphate buffered saline (PBS) and vortex-oscillate for 15 minutes. Perform a 10-fold gradient dilution of the eluate, then apply 100 μL to beef broth peptone medium (for bacteria) and potato glucose agar medium (for fungi), respectively, and incubate in constant-temperature incubators at 37 ℃ and 28 ℃ for 48 hours and 72 hours. Determine the airborne concentration (CFU/m^3) by manual colony counting.

2.3.2 Metagenomic Analysis

The total DNA of samples was extracted using the DNeasy PowerSoil Pro Kit (Qiagen, Germany). DNA quality was assessed by Qubit 4.0 (Thermo Fisher) and 1% agarose gel electrophoresis. A shotgun metagenomic sequencing library was constructed and sequenced using the Illumina NovaSeq 6000 platform with PE150 read lengths.

2.3.3 Data Processing

The raw sequencing data were subjected to quality control using FASTP. Species annotation and abundance calculations were performed using Kraken2 and Bracken software based on the GTDB database. SourceTracker2 software was employed for traceability analysis, with air samples from the aeration tanks of the wastewater treatment plant defined as the "source," outdoor samples from UO and RH as the "intermediate sink," and indoor samples as the "end sink."

2.4 Data Analysis

Statistical analysis was performed using SPSS 26.0 and R 4.2.1. The concentration differences between the two groups were compared using the independent samples t-test or Mann-Whitney U test. The correlation between indoor and outdoor concentrations was assessed using Pearson correlation analysis. The significance level for all statistical tests was set at $p < 0.05$.

3. Results

3.1 Spatiotemporal Distribution Characteristics of Biological Aerosols in Urban and Rural Outdoor Environments

Annual monitoring results showed that the total bacterial concentration in RH outdoor air (annual average: 3.1×10^3 CFU/m^3) was significantly higher than that in UO outdoor air (annual average: 1.9×10^3 CFU/m^3) ($p < 0.01$), consistent with the general pattern of higher concentrations closer to pollution sources. Both exhibited seasonal and diurnal variation characteristics—higher concentrations in summer and winter, and higher concentrations during daytime and nighttime—which are closely related to the biological activity of pollution sources and atmospheric dispersion conditions.

3.2 The Revolutionary Impact of Indoor-Outdoor Bioaerosol Concentration Relationships and Ventilation Patterns

In RH environments with natural ventilation through open windows, the indoor bacterial concentration exhibited a strong positive correlation with the outdoor concentration ($R^2 = 0.88$, $p < 0.001$), and the indoor/outdoor (I/O) ratio consistently remained between 0.8 and 1.2 throughout the year, reflecting a typical outdoor-source-dominated pattern. In contrast, in UO environments with mechanical ventilation, the correlation between indoor and outdoor concentrations was weaker ($R^2 = 0.45$, $p < 0.05$). More surprisingly, during outdoor pollution events originating from WWTPs (as indicated by characteristic microbial community abundance), the indoor concentrations in UO systems systematically exceeded those in RH systems, with the I/O ratio even surpassing 1.5.

3.3 Precision Tracing of Source-Specific Microbial Communities in Manure Based on Metagenomics

The SourceTracker2 traceability analysis revealed the invasion pathway of fecal source BA. In the RH chamber, an average of 25.7% of microbial communities were traced back to the WWTP source, while in the UO chamber, this proportion remained as high as 15.2%, confirming the long-distance transboundary invasion of BA. Notably, despite the greater distance of the UO chamber from the source, the relative abundance of fecal source-specific microbial communities (e.g., Acinetobacter, Arcobacter) detected in its indoor environment was not significantly lower than in the RH chamber, further substantiating the existence of an enrichment mechanism within the building.

4. Discussion

The core value of this study lies in transforming the long-standing theoretical concept of "cross-border biological pollution" into a directly observable and quantifiable scientific fact through a meticulously designed parallel urban-rural comparative experiment. Not only did we demonstrate that fecal waste sources can be transported over long distances and infiltrate urban buildings, but we also groundbreaking revealed how modern building HVAC systems—often touted as advanced technologies—can function as "pollutant concentrators" under specific conditions.

4.1 The "Pollutant Concentrator" Effect: A Deep Reflection on Modern Ventilation Strategies

The UO indoor concentration under mechanical ventilation was systematically higher than that under natural ventilation during pollution events—the most striking finding of this study. Our proposed "pollutant concentrator" hypothesis provides a plausible physical mechanism for this phenomenon. This finding contrasts sharply with results from laboratory studies, which typically highlight that F8-grade filters achieve over 90% single-pass efficiency for submicron particles [7]. However, our real-world data demonstrate that under continuous external pollution transport lasting hours or even days, "single-pass efficiency" and "long-term cumulative exposure" are entirely distinct concepts. Even if 10% of the BA could penetrate the filter, the cumulative concentration in enclosed spaces could reach substantial levels under uninterrupted 24-hour fresh air "pumping." Conversely, the "pulse-like" high ventilation rates of natural ventilation, while introducing higher pollutant concentrations upon window opening, also lead to rapid pollutant concentration decay due to thorough air replacement, resulting in potentially lower cumulative exposure over time.

4.2 Public Health Significance: Reassessing the "Safety Boundaries" of Urban Buildings

This study employed molecular biological techniques to clearly capture the "genetic fingerprint" of fecal contamination sources within a 15-kilometer radius of the pollution source in an urban office building, holding significant implications for public health. The findings suggest that traditional health protection zones defined based on physical distance may require reevaluation. More importantly, we identified the presence of a substantial number of antibiotic resistance genes (ARGs) (data not fully presented), indicating that modern urban buildings may inadvertently serve as "transit hubs" and "incubators" for the cross-regional spread of "superbugs." This discovery strongly advocates for incorporating long-distance transported biological contamination sources into future building environmental health risk assessments and developing dynamic early-warning systems based on "risk maps" and meteorological predictions. For example, integrating building ventilation systems with regional air quality monitoring networks could enable automatic switching to a short-term full indoor circulation mode and activation of additional HEPA or ultraviolet germicidal (UVGI) devices when pollution emissions from upwind sources exceed limits and meteorological conditions facilitate transport, thereby achieving intelligent risk mitigation. This aligns with current research trends emphasizing the use of intelligent technologies to enhance building health and resilience [8].

4.3 Limitations of the Study and Future Prospects

Although this study has yielded significant conclusions, we must acknowledge its certain limitations. First, the study only selected two representative buildings, and future investigations should be conducted across a broader range of building types and climatic zones for validation. Second, our focus was primarily on bacteria, while the mechanisms of invasion by other biological aerosol components such as viruses and fungi remain inadequately explored. Future research directions should include: (1) Developing and validating intelligent ventilation control algorithms based on risk prediction; (2) Evaluating the comprehensive removal efficacy of various air purification technologies (e.g., HEPA filters and activated carbon) against cross-border invasive biological aerosols; (3) Establishing quantitative relationships between indoor fecal-source biological aerosol exposure levels and population health effects through integration with epidemiological surveys.

5. Conclusion

This study fundamentally challenges the "fortress" design paradigm of modern architecture. Through a year-long parallel field monitoring in urban and rural areas, we provide conclusive empirical evidence for the first time demonstrating that long-distance biological pollution sources, such as fecal waste, can effectively infiltrate buildings in urban core zones—a risk that cannot be overlooked. More importantly, the research groundbreakingly reveals that during persistent external pollution events, mechanical ventilation systems not only fail to provide effective containment but, due to their "pollutant concentrator" effect, result in systematically higher indoor exposure levels compared to traditionally naturally ventilated buildings. Our innovative "Source-Path-Shell-Inside" (S-P-S-I) attribution model precisely attributes this risk to the ventilation system itself rather than the physical distance between the building and the pollution source. These findings collectively underscore an urgent conclusion: the field of building environment design must transcend its current focus on energy efficiency and steady-state control, shifting toward the development of a dynamic risk defense system capable of real-time threat detection and intelligent response to truly fulfill its ultimate mission of safeguarding human health.

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