



Bioaerosols in the atmospheric environment of oil exploitation sites: spatial and temporal distribution, sources, influencing factors, health risks, and reduction efficiency of masks

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ABSTRACT

Particle-bound microorganisms have attracted increasing attention due to their health effects and pollutant metabolic activity. However, the characteristics of airborne microbes in oil exploitation sites with heavy particulate matter and volatile organic chemicals (VOCs) pollution remain unclear. This study collected bioaerosol samples in a city polluted by oil exploitation in the Beijing-Tianjin-Hebei region of China, and investigated the spatial and temporal distributions, sources, influencing factors, health risks, and filtration efficiency of bioaerosols. The results showed that the average culturable and total bioaerosol levels in autumn were significantly higher than in summer, whereas microbial activity exhibited an inverse seasonal trend. Higher concentrations were found in steel plants, inland oilwell, and chemical industry park, suggesting that industrial activities may increase bioaerosol levels. No significant correlation was observed between microbial activity and concentration. The microbial communities' diversity and abundance varied significantly between seasons, with crude oil degradation bacteria, *Anoxybacillus* and *Curtobacterium* as dominant bacteria, *Cladosporium* and *Alternaria* as dominant fungi. SourceTracker analysis showed that airborne bacteria were primarily derived from leaf surface, while the contribution of leaf surface and soil communities to atmospheric fungi is more prominent. Airborne bacteria were more influenced by VOCs, while environmental factors have greater impact on fungal community structures. The hazard quotient (<1) of inhaling bioaerosols was negligible, but long-term exposure to dominant pathogens, *Bacillus* and *Mycobacterium*, could be harmful. Actual wearing masks could only remove 13.7% of bioaerosols, far lower than laboratory-measured values. In summary, this study elucidates the influence of oil exploitation activities on shaping the airborne microbial community, offering practical insights for risk assessments and control of bioaerosols in oil exploitation sites.

1. Introduction

Particle-bound microorganisms constitute a non-negligible proportion of particulate matter (PM), accounting for 30%-80% of atmospheric PM (Humbal et al., 2018; Xie et al., 2018a). In addition to influencing atmospheric environmental quality and climate dynamics, these microorganisms also exert substantial impacts on human health (Joung

et al., 2017). It is reported that due to the existence of pathogenic organisms, exposure to bioaerosols may induce or aggravate asthma, allergies, and other respiratory diseases (Kim et al., 2018). Potentially infectious diseases such as tuberculosis, pertussis, influenza, and pneumococcal disease are associated with a broad array of airborne transmitted pathogens, such as *Mycobacterium tuberculosis*, *Bordetella pertussis*, SARS-CoV-2 virus, and *Streptococcus pneumoniae* (Dinkele

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et al., 2024; Ristori et al., 2024). Furthermore, elevated mortality rates from respiratory diseases are correlated positively with specific pathogenic genera, particularly *Acinetobacter*, *Corynebacterium*, *Mycobacterium*, and *Staphylococcus* (Hong et al., 2024). Therefore, heightened attention to particle-bound microorganisms is critically warranted due to their potential to spread airborne pathogens and contribute to respiratory diseases.

Bioaerosols can be categorized into natural and anthropogenic sources. Bioaerosols originating from natural sources generally exhibit lower pathogenicity (Feng et al., 2024), whereas anthropogenic and industrial activities have been shown to specifically increase the relative abundance of potentially pathogenic organisms in the air (Jiang et al., 2022). Emissions of particle-bound microorganisms from man-made facilities have been shown to exhibit an increasing trend with economic growth in China. Numerous studies have devoted considerable efforts to evaluate the bioaerosols pollution profiles, particularly pathogen levels, in microbially dense environments including farms (henery, pig farms, and cattle farms), municipal solid waste landfills, and wastewater treatment plants (Feng et al., 2024). For example, Xie et al. reported that main pathogenic bacteria in farm atmospheres include *Bacillus*, *Corynebacterium*, and *Streptococcus*, which are mainly derived from animal excrement and feed or associated farming activities (Xie et al., 2021). Pathogenic bacteria (e.g., *Bacillus*, and *Burkholderia-Paraburkholderia*) and allergenic fungi (e.g., *Aspergillus*, *Cladosporium*, and *Curvularia*) prevailed in the landfill (Liang et al., 2023). In comparison, bioaerosols originating from industrial activities have long been overlooked, as industrial activities do not discharge bioaerosols directly. However, given that bioaerosols are formed via microbial attachment to particulate matter and subsequent agglomeration, industrial emissions of particulate matter and volatile organic chemicals (VOCs) may modify bioaerosol generation from surrounding environmental media (e.g., water, soil, and vegetation), thereby altering their associated health risks. Zhang et al. investigated indoor bioaerosols' community composition, sources, and influencing factors near an electronic waste industrial park and found that higher microbial viability may be caused by pollutants released during e-waste dismantling (Zhang et al., 2023a). Nevertheless, for the industry activities like oil exploitation, characterized by high particulate matter and VOCs emissions, the spatial and temporal distributions, sources, influencing factors, and health risks of bioaerosols were still unknown.

The most common and cost-effective tool for individuals to protect against inhalation of airborne pathogens is mask-wearing. Masks can filter out most particles and droplets, thereby reducing the possibility of pathogens attached to airborne particles entering the respiratory tract (Greenhalgh et al., 2024). Previous studies have compared the protective effects of different masks, evaluated surgical masks' filtration efficiency (FE) towards bacteria aerosol and influenza virus, and revealed their influencing factors such as wearing duration and respiratory volume (Li et al., 2022; Makison Booth et al., 2013; Salimnia et al., 2021). However, these studies are carried out under laboratory conditions with simulated temperature, humidity, aerosol size, and expiratory volume. A lot of factors that may affect mask FEs were not considered in these laboratory experiments. For example, particle detachment from masks can occur when masks are touched with hands. Besides, leakage through gaps between the mask and facial contour, resulting from improper mask wearing or sub-optimal mask design, could significantly diminish FEs (Li et al., 2022). Therefore, it is imperative to assess the protective efficiency of masks from inhaling bioaerosols under real-world wearing conditions.

Hebei province, belonging to the Beijing-Tianjin-Hebei region, is one of the most highly polluted areas in China owing to oil exploitation activities. According to the national environmental quality status released by the Ministry of Ecology and Environment, several cities in Hebei province, such as Tangshan, where higher population density and intensive industrial manufacturing activities are concentrated, are still in the bottom 20 of China's key cities in terms of air quality (Li et al., 2025). Caofeidian District, a key industrial zone in Tangshan, is mainly dominated by oil exploitation and steel industries and is polluted

by VOCs and particulate matter, posing acute and chronic occupational disease risks to workers (Wang et al., 2023; Zhang et al., 2023b).

To reveal the effects of oil exploitation activities on airborne microorganisms, the culturable bioaerosol levels and microbial diversity and abundance in the oil exploitation area were investigated using a combination of culture-based techniques and amplicon sequencing analysis. Besides, bioinformatic analytical method, such as SourceTracker, was used to trace the sources of bioaerosols. Additionally, the risk assessment of inhaling bioaerosols for adults and children was performed using the model recommended by the United States Environmental Protection Agency. Finally, the reduction effects of inhaling bioaerosols by wearing surgical masks were also evaluated. This study aims to provide data support and theoretical guidance for government departments to formulate reasonable and effective bioaerosol pollution prevention and control measures.

2. Materials and methods

2.1. Study sites and sample collection

Sampling sites were situated in Caofeidian District, Tangshan City, Hebei Province, China (39°01'50"–39°18'05"N, 118°11'10"–118°37'55"E), which is a major economic growth center. It is also an important port and industrial zone in the Beijing-Tianjin-Hebei region. Geographically, Caofeidian District is located on the southern coast of the Bohai Sea with a total area of 2114 km² and a resident population of approximately 367,700 and belongs to a temperate semi-humid continental climate with an average annual temperature of 11°C and annual precipitation of 636 mm. In this area, oil exploitation activities are intensive with scattered distribution of many oil pumping machines, and it has a crude oil reserve of 451.39 million tons. A total of 26 site-season unit air samples were collected from six regions during autumn (October 8–15, 2022) and summer (June 21–28, 2023). The sampling sites are shown in Fig. S1 and they were located in densely populated towns (Town1, Town2), Caofei lake park (Lake1, Lake2), a steel plant (SP), inland oilwell areas (IOA1, IOA2), seaside oilwell areas (SOA1, SOA2, SOA3, SOA4), and chemical industry park (CIP1, CIP2). For each site, three independent samples were collected at three random places.

Airborne culturable bacteria, fungi, and total microorganisms were sampled on nutrient agar plates (with 5 mg/L nystatin), rose Bengal agar plates (with chloramphenicol), and sterilized polycarbonate membranes (pore size: 0.2 µm, diameter: 80 mm), respectively, using a FA-1 six-stage cascade impactor (Applied Technical Institute of Liaoyang, China). The detailed method was provided in Test S1 of the Supporting Information (SI). Besides, leaf, water, and soil samples were also collected at each sampling point to trace the source of bioaerosols. As the main strategies to protect residents from inhaling particles, surgical masks worn by volunteers for 3 h in each region were also collected to evaluate the FE of bioaerosols. The analysis method of the mask samples are described in Text S3. Gas samples were simultaneously collected using vacuum stainless Summa canisters (2.7 L, Silonite™, Entech Instruments Inc., Simi Valley, CA, USA) and used to detect VOCs concentration and composition by a gas chromatography-mass spectrometer (GC–MS) (7890A GC-5975C MS, Agilent Technologies, Santa Clara, CA, USA) coupled with a pre-concentrator (Entech 7200, Entech Instruments Inc. Simi Valley, CA, USA). Temperature and relative humidity were measured using a digital thermohygrometer (KDWS-01, Beijing Cadillac Instrument Equipment Co., Ltd, China). Wind speed was measured using a hot-bulb anemometer (QDF-6, Beijing Cadillac Instrument Equipment Co., Ltd, China). PM₁₀, PM_{2.5}, NO₂, SO₂, O₃, and CO were obtained from the Hebei Provincial Department of Ecological Environment (<https://hbebpb.hebei.gov.cn/>) and are presented in Table S1.

2.2. Airborne microbial activity and concentrations analysis

One-eighth of a polycarbonate membrane was used to determine the bioaerosol activity using the fluorescein diacetate (FDA) hydrolysis

method (Qi et al., 2015), and the detailed steps are described in Text S2. To reveal the concentration of total airborne bacteria and fungi, genomic DNA was isolated from one-third of a polycarbonate membrane using the Ezup Column Soil DNA Purification Kit (Sangon Biotech, Shanghai, China). qPCR amplification was conducted with primers for total bacteria (16S rDNA F: 5'-GGTAGTCYAYGCMSTAAACG-3' and 16S rDNA R: 5'-GACARCCATGCASCACCTG-3') and fungi (ITS1F: 5'-CTTGGTCATTTAGAGG-3') and fungi (ITS2R: 5'-GCTGCGTTCTTCATCGATGC-3'), respectively (Adams et al., 2013; Pravinkumar et al., 2010). In the meantime, the total bacteria and fungi in the central area (16 cm²) of surgical masks were quantified by qPCR amplification on the CFX96 Touch System (BioRad). To eliminate the interference of microorganisms exhaled by the volunteers, the inner layer of the mask was discarded. The standard curves were constructed according to the method described by (Liang et al., 2020). Detailed procedures for DNA extraction and qPCR amplification can be found in Text S3.

AAGTAA - 3' and ITS2: 5' - GCTGCGTTCTTCATCGATGC - 3'), respectively (Adams et al., 2013; Pravinkumar et al., 2010). In the meantime, the total bacteria and fungi in the central area (16 cm²) of surgical masks were quantified by qPCR amplification on the CFX96 Touch System (BioRad). To eliminate the interference of microorganisms exhaled by the volunteers, the inner layer of the mask was discarded. The standard curves were constructed according to the method described by (Liang et al., 2020). Detailed procedures for DNA extraction and qPCR amplification can be found in Text S3.

2.3. Airborne bacterial and fungal communities' structure and source analysis

The genomic DNA isolated from the membrane and surgical mask was also used for Illumina sequencing with the primers (338F: 5'-ACTCC-TACGGGAGGCAGCAG-3', 806R: 5'-GGACTACHVGGGTWTCTAAT-3') targeting V3-V4 region of the bacterial 16S rRNA gene (Xu et al., 2016), and the primers (ITS1F: 5'-CTTGGTCATTTAGAGGAAGTAA-3', ITS2R: 5'-GCTGCGTTCTTCATCGATGC-3') targeting the Internal Transcribed Spacer (ITS) region of fungi (Adams et al., 2013). Illumina sequencing was performed on an Illumina MiSeq platform (Illumina, CA, U.S.) following the standard protocol by Majorbio Bio-Pharm Technology Co. Ltd., Shanghai, China, and data processing methods were described in Text S4. The sequencing data were deposited in the NCBI under the accession number of PRJNA1422116.

Besides, to track the source of bioaerosols, the bacterial and fungal communities' structures from the leaves, water, and soil samples were also analyzed by Illumina sequencing. Then, SourceTracker, a Bayesian approach, was employed to track the source of sinks according to the community structure of collected samples (Knights et al., 2011). Herein, Air samples were considered as a sink, while leaves, water, and soil samples were considered as three primary known sources. The pretreatment method of leaves and water samples was described in Text S3.

2.4. The reduction efficiency of bioaerosols by surgical masks

The reduction efficiency (RE, %) of bioaerosols by wearing the masks was evaluated using the following Eq:

$$RE = \frac{C_{SMB} + C_{SMF}}{C_{AB} + C_{AF}} \times 100\%$$

where C_{SMB} and C_{SMF} are the total bacterial and fungal concentrations in the surgical mask (copies/μL), respectively; C_{AB} and C_{AF} are the total airborne bacterial and fungal concentrations (copies/μL); the detailed calculation method can be found in Text S3.

2.5. The health risk of inhaling bioaerosols

To assess the inhalation risk of atmospheric bioaerosols, the average daily dose (ADD) and hazard quotient (HQ) were calculated using a model recommended by the United States Environmental Protection Agency (US EPA) (Yang et al., 2019). This assessment model was approved by US EPA and has been widely used in previous studies (Han et al., 2018; Luo et al., 2024). Health risks could be ignored when HQs are lower than 1; however, they cannot be overlooked when the HQs are higher than 1. The detailed calculation method, equations, and parameters were described in Text S5 and Table S2.

2.6. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 22 (Version 22.0). A p -value < 0.05 indicates a statistically significant difference. The relationships between bioaerosols and meteorological factors as well as VOCs were assessed using Spearman correlation analysis and the Variance Inflation Factor method was used to exclude VOCs with large autocorrelation. Principal co-ordinates analysis (PCoA) was used to reveal the discrepancy of community structure between sampling sites.

3. Results

3.1. Culturable bioaerosols concentration, particle size, and activity

3.1.1. Concentration and particle size of culturable bioaerosols

As shown in Fig. 1a and b, the average concentration of culturable bioaerosols in summer was 906 CFU/m³, which was significantly lower than that in autumn (2011 CFU/m³). Specifically, in summer, the concentrations of airborne bacteria and fungi in Town1 ($n = 3$) and Lake1 ($n = 3$), respectively, were significantly higher than those of other sites. These might be related to increased release of bacteria and fungi from human activities and vegetation, respectively, since Town1 was located in a densely populated town, while Lake1 was located adjacent to a lake. It has to be mentioned that the seaside oil well (SOA1, SOA2, and SOA3) has the lowest concentrations (170-635 CFU/m³). In autumn, the airborne microorganisms' concentrations in the inland oil well (IOA1 and IOA2) and the chemical industry park (CIP1 and CIP12) were significantly higher compared to other locations, suggesting that industrial activities in these sites may exacerbate pollution and increase the concentration of airborne microorganisms.

The particle sizes of bioaerosols determine their deposition location within the respiratory system (Morgado-Gamero et al., 2021). As shown in Fig. 1c-f, in autumn, the proportion of coarse particles (>2.1 μm) reached 76.7% (bacteria) and 69.9% (fungi), both of which were lower than that in summer (81.2% and 88.9% for airborne bacteria and fungi, respectively). Specifically, the airborne microorganisms (except for fungi in autumn) were mainly distributed in the size range of >7.0 μm, suggesting that these microorganisms mainly existed in coarse particles. Given that particles with an aerodynamic diameter less than 4.7 μm can penetrate deep into the respiratory tract, causing irreversible damage to the respiratory system (Liang et al., 2020; Xing et al., 2023), the proportion of bioaerosols within this size range was evaluated. The results showed that the proportions of inhalable bacterial and fungal aerosols in autumn were 60.9% and 73.5%, higher than those in summer (53.0% and 52.2%). In autumn, the size distribution of airborne fungi was bimodal, accounting for 27.0% and 26.5% for 2.1-3.3 μm and 1.1-2.1 μm, respectively. This indicates that individuals in this region face a higher risk of inhaling fungal aerosols than bacterial aerosols.

3.1.2. Concentration and activity of total microbial aerosols

Given that culturable bioaerosols account for only 1% of the total bioaerosols (Ni et al., 2024), and non-culturable microorganisms contribute to a considerable proportion, we evaluated the concentration of total airborne microbes. As shown in Fig. S2, consistent with the trend observed for culturable microorganisms, the average concentrations of total airborne bacteria (1.13×10^7 copies/m³) and fungi (2.07×10^6 copies/m³) in autumn were significantly higher than those in summer (7.83×10^6 copies/m³ and 1.64×10^6 copies/m³). Specifically, significantly higher total bioaerosol concentrations were detected in the steel plant (SP), inland oilwell (IOA1 and IOA2), and chemical industry park (CIP1 and CIP2) compared to other locations, suggesting that industrial activity may increase total bioaerosol concentrations.

Further exploring the activity of bioaerosols, we found that, different from the concentration trends of culturable and total microorganisms, the average airborne microbial activity in autumn (32.15×10^4 ng/m³)

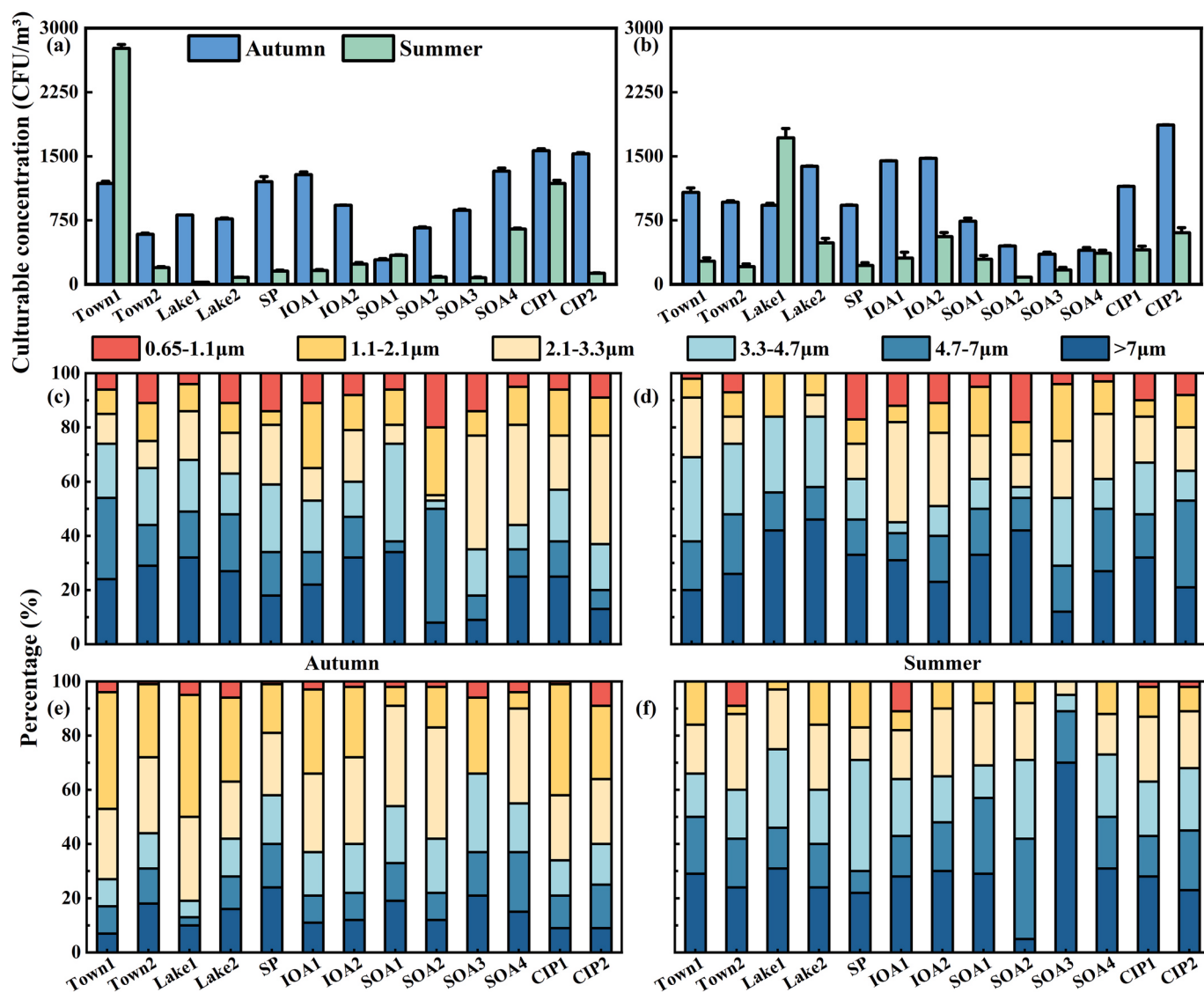


Fig. 1. Concentration of culturable airborne bacteria (a) and fungi (b) in autumn and summer, and the size distribution of airborne bacteria (c, d) and fungi (e, f) in autumn and summer. (n = 3 for each site and season).

fluorescein sodium) was lower than that in summer ($47.67 \times 10^4 \text{ ng/m}^3$ fluorescein sodium) (Fig. S3). This phenomenon may be attributed to human activities, since higher bioaerosol activity was found in densely populated town areas (Town1, Town2), and commercial centers near inland oil well areas (IOA2). Overall, further exploring microbial communities of bioaerosols are helpful to figure out the reason for the seasonal variation of concentration and activity.

3.2. Bioaerosols' community structure and source

3.2.1. Community composition of airborne bacteria and fungi

To explore seasonal variations in bioaerosol microbial communities, we compared the Shannon and Chao1 indices of bioaerosol between summer and autumn. The results showed that the diversity and richness of bacterial communities were higher than those of fungal communities (Table S3). Besides, fungal diversity and richness were higher in autumn than in summer, whereas an opposite trend was observed for bacteria. Specifically, for bacteria, the diversity and richness in the seaside oil well area (SOA1, SOA2, and SOA4), inland oil well (IOA1 and IOA2), and town (Town1 and Town2) were higher than in other sites, with the lowest values recorded in the chemical industry park (CIP1 and CIP2). For fungi, the highest Shannon and Chao indices were observed in the

chemical industrial park (CIP1 and CIP2), while the lowest were found in the seaside oil well area (SOA1, SOA2, and SOA3). This suggested that oil exploitation activities may increase the diversity and richness of bacterial aerosols, while decreasing those of fungal aerosols. Notably, although the industry activities in the chemical industrial park impaired the alpha diversity of bacterial aerosol, they enhanced fungal diversity.

Among 1080 bacterial genera and 291 fungal genera detected, 44.7% (bacteria) and 20.3% (fungi) were co-shared between summer and autumn. Besides, more genera were detected in autumn (861 bacteria and 212 fungi) than in summer (Fig. 2a and b). PCoA analysis showed clear separation of bacterial aerosol communities between summer and autumn, whereas fungal communities were clustered together, indicating distinct seasonal variations in bacterial community structure (Fig. 2c and d).

In autumn, Bacillota, Pseudomonadota, and Actinobacteriota dominated the bacterial community, accounting for 23.2%, 34.1%, and 30.8% of the total bacteria, respectively (Fig. S4). The dominant position of Actinobacteriota, especially in the chemical industry park (CIP1 and CIP2) and steel plant (SP), may be due to their metabolic flexibility and capacity to endure severe environments. While in summer, Bacillota (60.3%) had absolute abundance, followed by Pseudomonadota (29.9%). At the genus level, *Anoxybacillus* (16.3%) and *Curtobacterium*

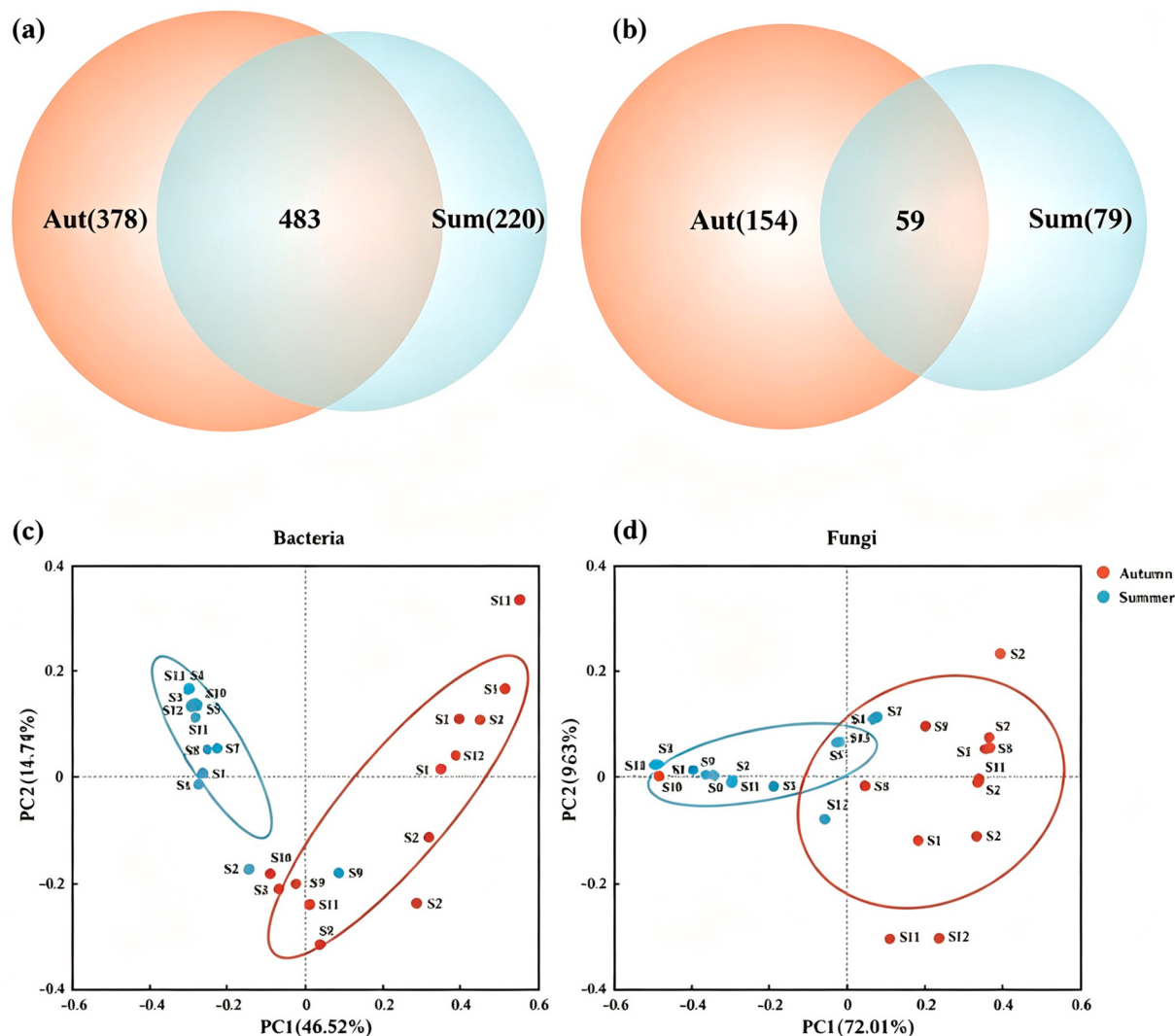


Fig. 2. Venn diagram showing the number of shared and unique bacterial (a) and fungal (b) communities at the genus level between autumn and summer. Principal coordinate analysis (PCoA) plot of airborne bacterial (c) and fungal (d) community structure. PC1 and PC2 are the first and second components.

(25.9%) were the dominant bacterial genera in autumn (Fig. 3a). While in summer, *Anoxybacillus* (49.1%) became the dominant genus (Fig. 3b). Additionally, *Pseudarthrobacter* accounted for 27.7% of the bacterial community in the town (Town2), while *Acinetobacter* and *Methylobacterium* were most abundant in the inland oilwell area (IOA2) and seaside oilwell (SOA2), respectively. For the fungal community, Ascomycota exhibited absolute dominance at the phylum level (Fig. S5). Similar to bacteria, the structure of fungal community at the genus level varied seasonally (Fig. 3c and d). Specifically, *Cladosporium* was the most abundant genus in autumn (54.5%), followed by *Alternaria* (18.3%), especially in the air of Lake (Lake1) and the seaside oil well area (SOA3). In contrast, *Alternaria* dominated the summer fungal community (65.5%), with *Cladosporium* ranking second (31.7%).

3.2.2. Sources of bioaerosols

The source of bioaerosols in oil exploitation sites was further analyzed by SourceTracker. Firstly, we analyzed the community composition in the leaf surface and found that phyllosphere (leaf surface) microbiota were dominated by *Pantoea* and *Pseudomonas*, whereas soil and water were, respectively, enriched with *Sphingomonas* and *Tychonema* CCAP.1459-11B as well as *Chloroplast* and *Vibrio* (Fig. S6–S8). Source apportionment based on these community profiles revealed that airborne bacteria at most

sampling sites (excluding Lake1, Lake2, and SOA3) primarily originated from the leaf surface (Fig. 4), indicating that vegetation is a key source of bioaerosol. Lake1 and SOA3 showed a higher proportional contribution of soil-derived bioaerosols than leaf, owing to high wind speed (Table S1). For Lake2 (lake), up to 40.0% of bioaerosols stem from water. This is related to the large lake coverage since many microorganisms in the lake would enter the atmosphere under strong wind forcing. Besides, a substantial fraction of unidentified sources was observed, particularly at Lake1 (lake), SOA1, and SOA2 (seaside oilwell). We suspect that they may derive from anthropogenic/zoogenic activities or marine inputs since the wind direction in SOA1 and SOA2 is southwest.

For fungi, *Cryptococcus* and *Alternaria* have absolute advantage in the leaf surface, while in soil and water, the predominant fungi were both unclassified_k_fungi (Fig. S6 and S8). Compared to bacteria, the contribution of leaf surface and soil fungal communities to the atmospheric fungi is more prominent (Fig. 4). The soil contribution rate was higher in the lake (Lake1), steel plant (SP), inland oilwell (IOA1), and seaside oilwell (SOA1 and SOA2), while the remaining sites (except for Lake2) were predominantly phyllosphere-derived. It has to be mentioned that the proportion of unknown sources of fungi was lower than that of bacteria, suggesting that bacterial communities are more affected by other sources, such as anthropogenic activities, than fungi.

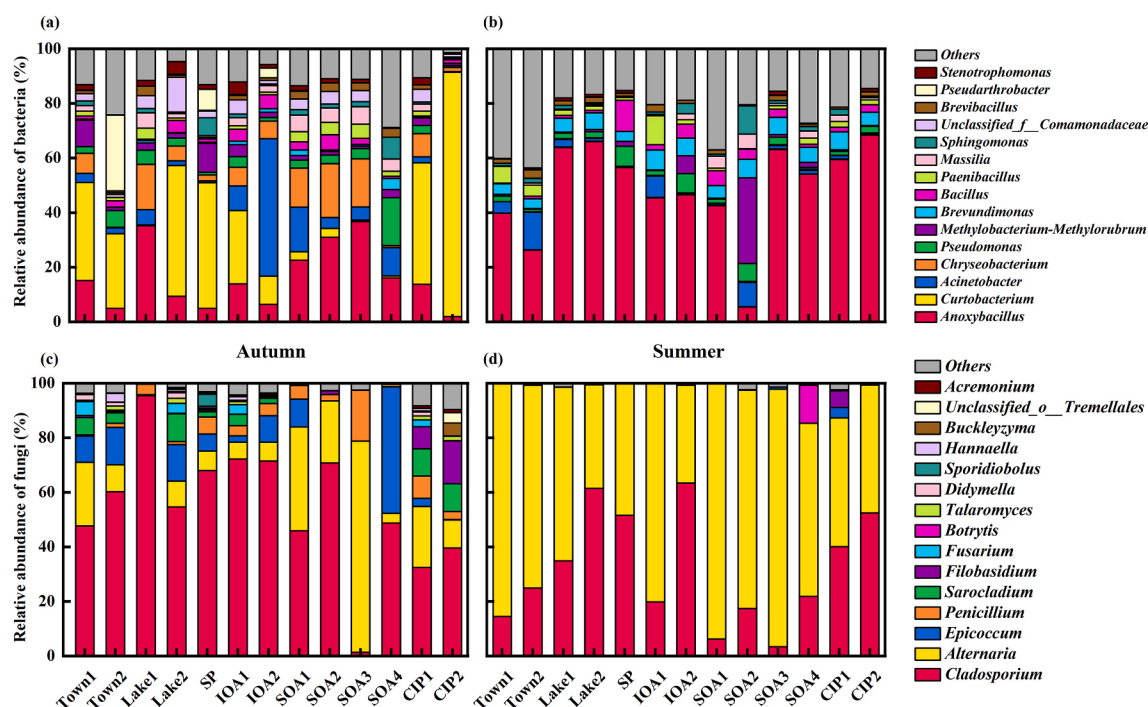


Fig. 3. Relative abundance of the top 15 airborne bacteria (a, b) and fungi (c, d) at the genus level in autumn and summer. Merge the remaining genera into others.

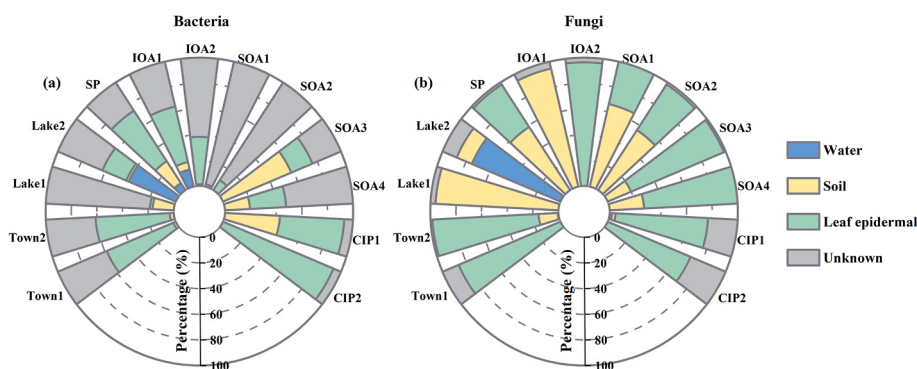


Fig. 4. Rose diagram showing the sources of airborne bacteria (a) and fungi (b).

3.3. Inhalation health risks of bioaerosols

As bioaerosols are an integral part of myriad toxic components in ambient particulate matter, this study clarified the health risk associated with human exposure to culturable airborne microbes. As shown in Fig. 5a and b, the HQ value is higher in children than in adults, but remains below 1, indicating negligible non-carcinogenic risks. The risks varied by season, with a higher health risk found in autumn than in summer. Specifically, the town, steel plant, and chemical industry have a higher potential risk than other sites, suggesting that anthropogenic activities and industrial emissions can enhance the health risk of bioaerosol exposure.

Further analysis of the pathogenic communities, we found that the dominant pathogenic bacteria in this area were *Bacillus* and *Mycobacterium* (Fig. 5c and d). Specifically, in autumn, airborne *Bacillus* and *Mycobacterium*, respectively, accounted for 16.5% and 31.6% of total pathogenic microorganisms. In summer, however, *Bacillus* and *Rhizobiales* become the dominant pathogenic microorganisms, which account for 50.3% and 10.5% of total pathogenic microorganisms, respectively. The infection risk was higher in the chemical industry park (CIP1 and CIP2) than in other sites, with *Mycobacteria* accounting for 46.4–89.8%.

By contrast, populations in the inland oil well area (IOA2) and seaside oilwell (SOA2) face health risk caused by the dominance of *Acinetobacter* and *Rhizobia*, respectively. Overall, the wide existence of *Mycobacterium* and *Bacillus* in both autumn and summer suggests potential exposure risks for people, especially vulnerable groups like children, the elderly, and pregnant individuals, still require attention.

3.4. The reduction effects of inhaling bioaerosol by wearing masks

Since wearing a surgical mask is the most common way to protect residents from inhaling dust and PM, we evaluated the REs of bioaerosols by wearing mask in the actual wearing process. As shown in Fig. 6, the average REs of surgical masks was 13.7%. In specific, REs of surgical masks in the steel plant (18.6%) and the inland oilwell areas (15.2%) were higher than those in other sites. We speculate that more intensive industrial production activities in this area increase volunteers' exposure to emitted particulate matter, thereby improving the capture efficiency of surgical masks. Overall, mask wearing remains an effective measure to reduce inhalation of bioaerosols.

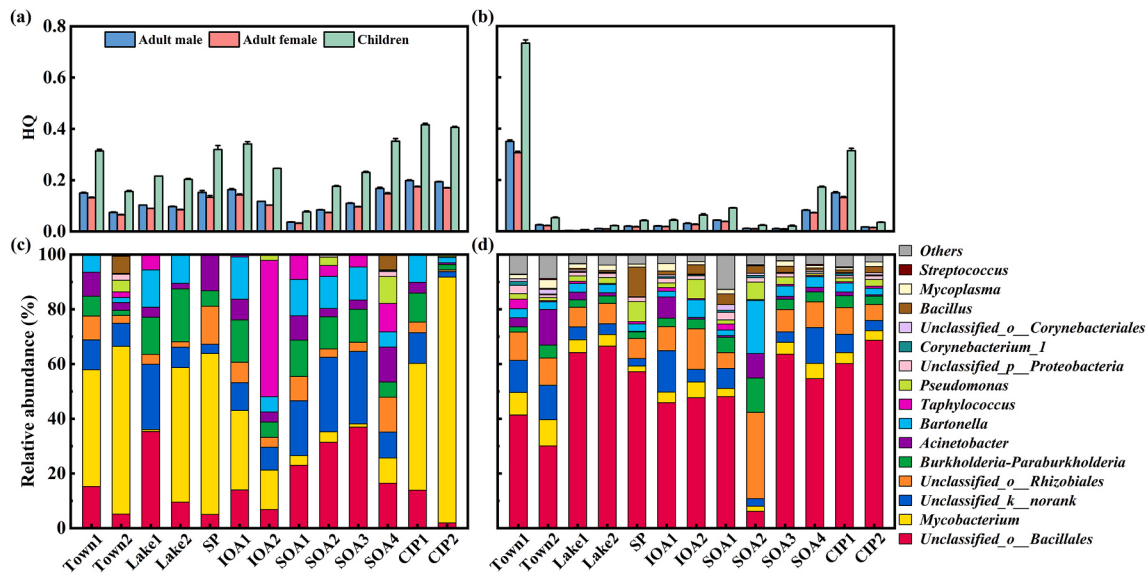


Fig. 5. HQ of inhaling culturable airborne microbes in autumn (a) and summer (b); Relative abundance of the top 15 airborne pathogenic bacteria in autumn (c) and summer (d). Merge the remaining genera into others.

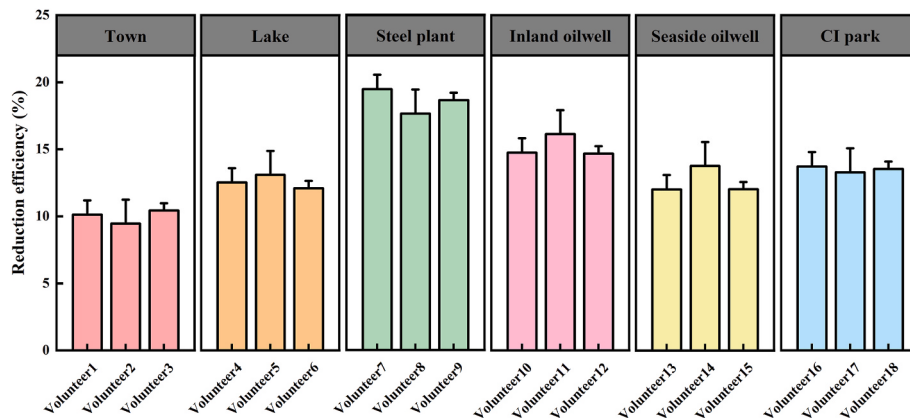


Fig. 6. The bioaerosols' reduction efficiency of masks worn by volunteers.

4. Discussion

4.1. The seasonal variations of bioaerosol concentrations

The concentration of culturable bioaerosols in autumn is significantly higher than in summer. This may be associated with the relatively high average wind speed (4.6 m/s on average) (Table S1), as wind is a critical driver for the emission of viable biological particles from water, soil, and vegetation into the atmosphere (Monahan et al., 1983). A previous study also showed that total and culturable bacterial aerosols as well as total viral aerosols increased with rising onshore wind speeds (Dueker et al., 2017). In summer, we found that the concentration of culturable bioaerosol in the seaside oil well is relatively lower than other continental sites, presumably attributed to the prevailing onshore winds (southwest or south) (Table S1). This suggested that marine source bioaerosol was lower than that derived from continental sources. Previous studies have shown that concentrations of bioaerosols in marine environments are lower than those derived from continental sources (Cao et al., 2024; Shi et al., 2022). Besides, the relatively higher O₃ concentration at the seaside oil well may have contributed to the reduced concentration, as O₃ can destroy microbial DNA and disrupt cells, thereby reducing the concentration of bacteria in the air (Yang et al., 2024). In autumn, the airborne microorganisms' concentrations

in polluted sites were higher than other sites, suggesting that industrial activities in these sites may exacerbate the pollution of bioaerosols. Previous studies have found that culturable bioaerosol concentrations are higher on hazy days than on non-hazy days (He et al., 2017; Xie et al., 2018b).

The particle size distribution of culturable bacterial and fungal aerosols showed that more than 76.7% of bioaerosols were coarse particles (>2.1 μm). This may be due to the following two reasons: first, coarse particles may indirectly enhance airborne microbial abundance and diversity by protecting them from severe atmospheric conditions (e.g., UV irradiation); second, these particles may serve as energy and carbon sources for airborne microorganisms (Clements et al., 2014). A previous study also reported that the airborne microbes were primarily in the coarse particle size (>2.1 μm) with a proportion of 71.5% (Dong et al., 2016). Besides, the proportions of inhalable bacterial and fungal aerosols accounted for more than 52%. This proportion was comparable to the studies in other environments, such as landfills (Li et al., 2021), wastewater treatment plants (Wang et al., 2022) and underground garages (Xing et al., 2023). This indicates that people in this region face a high risk for inhaling bioaerosols.

Consistent with the findings of previous research (Zhong et al., 2016), airborne microbial activity is generally higher in summer compared to autumn. This may be attributed to the higher temperatures

in the summer, which are conducive to maintaining the microbial physiological activity, growth, and production. Previous studies also reported that temperature was the key driver of the seasonal differences of bioaerosols in the coastal areas of Nanjing and Qingdao (Guo et al., 2021; Zhang et al., 2024). Notably, there was no correlation between air microbial activity and culturable or total microbial concentrations ($p > 0.05$) (Table S4). This is because the factors affecting microbial activity are very complex, which include climatic conditions, bioaerosol sources, and geographical locations (Zhong et al., 2016), and their combined influence results in the variations in microbial activity.

4.2. Bioaerosol communities and their correlation with environmental factors

Bacillota, Pseudomonadota, and Actinobacteriota were the dominant bacterial communities in autumn. Wang et al. found that pollution levels of oil can affect the relative abundance of Bacillota (Wang et al., 2021). This indicates that Bacillota may have a high decomposition and utilization rate of petroleum hydrocarbons since the air in this region is rich in petroleum hydrocarbons. Besides, considering Actinobacteriota can create secondary metabolites (eg., enzymes), produce siderophores and extracellular polymeric substances that facilitate the trapping and degradation of various pollutants (Makarani and Kaushal, 2025), the dominance of Actinobacteriota suggests that they have the capacity to metabolize pollutants and endure severe environments. This is consistent with previous study that *Acinetobacter baumannii* was enriched in the bioenhanced treatment of crude oil-contaminated soil (Zhang et al., 2021). At the genus level, *Anoxybacillus* (49.1%) and *Pseudarthrobacter* (27.7%) were the dominant genera in summer (Fig. 3b). This is possibly due to the following two reasons: 1) as a thermophilic bacterium, it thrives under high temperature; 2) *Anoxybacillus* is an oil-degrading and biosurfactant-producing strain, capable of utilizing oil as a carbon source for growth (Schultz et al., 2022).

The correlation between bacterial communities and VOCs in Fig. 7a showed that most airborne bacteria exhibited significant correlations with 2,3-dimethylbutane, chloroethane, trichloroethylene (TCE), and 2-

methoxy-2-methylpropane. The correlation between dominant bacteria and 2,3-dimethylbutane as well as 2-methoxy-2-methylpropane was similar, but opposite to that of TCE and chloroethane, indicating contrasting effects of these VOCs on microbial growth and metabolism. This suggested that microorganisms have developed multiple physiological tolerance and metabolic strategies under stressful conditions to degrade complex environmental pollutants (Wu et al., 2022). Specifically, *Anoxybacillus*, *Rhodococcus*, *Staphylococcus*, *Escherichia-Shigella*, and *Brevundimonas* were positively related to 2,3-dimethylbutane and 2-methoxy-2-methylpropane, but negatively related to TCE. The positive relationship between TCE and *Curtobacterium*, *Chryseobacterium*, *Methylobacterium-Methylorubrum*, *Comamonadaceae*, and *Stenotrophomonas* suggests that these microorganisms play a leading function in TCE degradation. A previous study demonstrated that 2,3-dimethylbutane in the light fraction of gasoline can be metabolized by microorganisms (Solano-Serena et al., 2000). *Methylobacter* has been proven to be closely related to the TCE-dechlorinating degradation (Kong et al., 2014). For the correlation of bacterial communities and meteorological factors, we found that most meteorological factors (except for wind speed) were significantly correlated with bacterial communities (Table S5). Among these variables, O_3 exhibited a stronger influence on the distribution of bacterial communities than other factors. Specifically, *Anoxybacillus*, *Brevundimonas*, *Escherichia-Shigella*, *Staphylococcus*, and *Rhodococcus* were positively related to meteorological factors, while negatively related to total bacterial concentrations. *Curtobacterium*, *Chryseobacterium*, and *Comamonadaceae* were negatively correlated with meteorological factors, while positively related to total bacterial concentrations ($p < 0.05$) (Fig. S9a).

For fungi, Ascomycota was the dominant phylum. This is consistent with a previous study reporting that Ascomycota accounted for 90.6% of fungal phyla in oil-contaminated soil (Ramdass and Rampersad, 2021). At the genus level, *Cladosporium* and *Alternaria* were the most abundant genera in autumn and summer, respectively, especially in the air of Lake (Lake1) and the seaside oil well area (SOA3). This may be attributed to the relatively higher temperature and humidity in summer, which are favorable for the growth of *Alternaria* with dry airborne spores (Wang et al., 2020). Besides, high wind speeds at these locations also

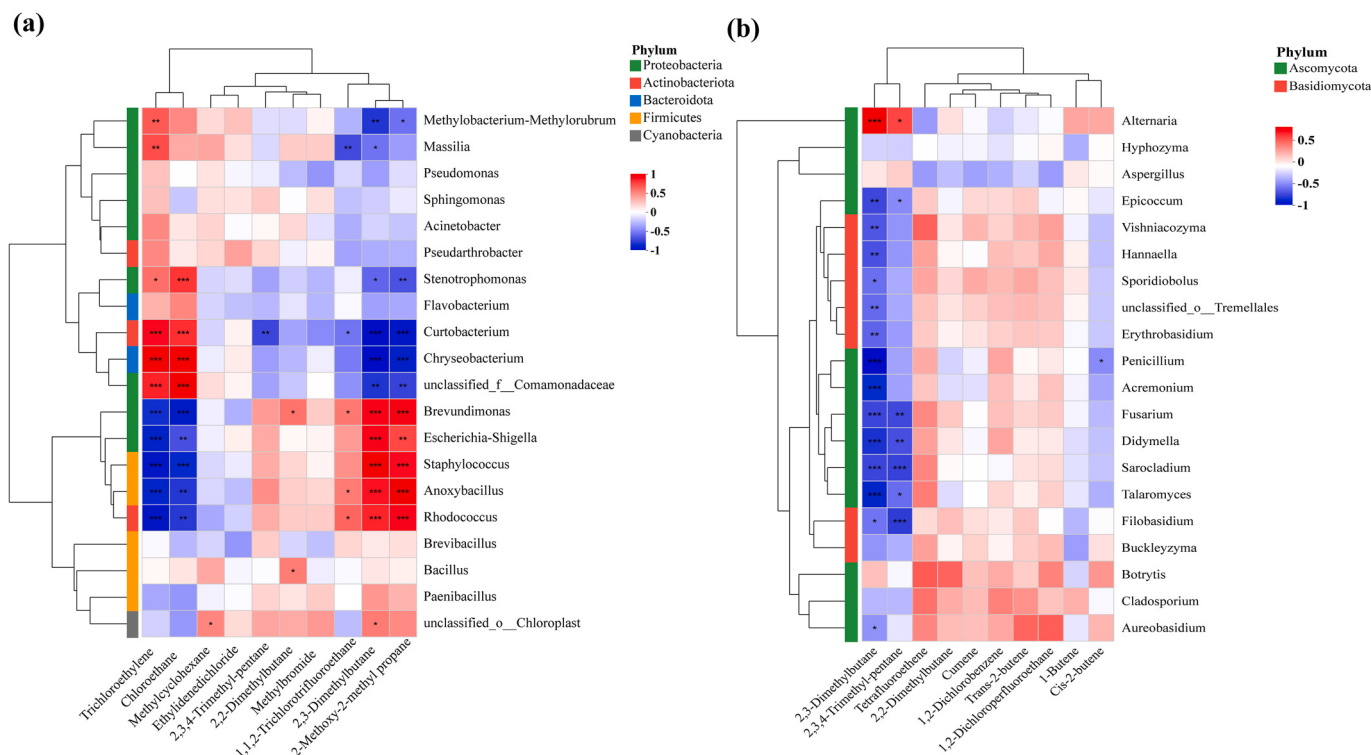


Fig. 7. Spearman correlations between VOCs and the top 20 airborne bacteria and fungi at the genus level. One, two, and three asterisks were used when $0.01 < p^* \leq 0.05$ (relatively strong correlation), $0.001 < p^{**} \leq 0.01$ (strong correlation), and $p^{***} < 0.001$ (significantly strong correlation), respectively.

facilitate the dispersal of *Cladosporium* (primarily from vegetation) and *Alternaria* (from soil and halophytes) into the atmosphere (Awad, 2005; Khalmuratova et al., 2020).

The correlation between fungal communities and meteorological factors in Fig. S9b showed that most fungi (except for *Alternaria*) were negatively correlated with meteorological factors (except for wind speed), while positively correlated with total fungal concentrations ($p < 0.05$). Overall, environmental factors have a greater impact on fungal community structures than bacterial communities, suggesting that fungal communities are more sensitive to environmental conditions than bacteria. This is consistent with a previous study conducted in an industrial park, which showed that pathogenic fungi were more strongly affected by environmental factors than bacteria (Zhang et al., 2023a). In comparison, less significant correlations were observed between VOCs and fungi, with only two VOCs (2,3-dimethylbutane and 2,3,4-trimethyl-pentane) significantly negatively correlated with most airborne fungi (except for *Alternaria*) (Fig. 7b). This suggests that VOCs may inhibit the growth and accumulation of airborne fungi.

4.3. Health risks of bioaerosol exposure and the protective effects of surgical masks

Bacillus and *Mycobacterium* were the dominant pathogenic bacteria in this area.

This phenomenon may be related to high dust levels, as previous studies have shown that dust is an important carrier for the spread of pathogens, such as *Bacillus* and *Mycobacterium* (Maki et al., 2019; Maki et al., 2022). Their dominance deserves our close attention as certain species exhibit pathogenicity to humans. For example, *Bacillus anthracis* can induce respiratory infections, while *Bacillus cereus* is associated with diarrheal food-borne illness (Ehling-Schulz et al., 2019). *Mycobacterium tuberculosis*, *Mycobacterium leprae*, and *Mycobacterium ulcerans* are responsible for tuberculosis, leprosy, and Buruli ulcer, respectively (Lagier et al., 2015). Besides, the dominance of *Acinetobacter* and *Rhizobia* in the oilwell area suggested potential exposure risks for people. Specifically, *Acinetobacter baumannii* infections manifest most frequently as ventilator-associated pneumonia, followed by bloodstream, skin, and soft tissue infections (Yao et al., 2023), whereas bacteremia is the most common manifestation of infections of *Rhizobium radiobacter* in saline containing environments (Pereira Lynette et al., 2009).

Surgical masks can be used to reduce the risk of inhaling these pathogenic bioaerosols. We found that the average bioaerosol REs of surgical masks in real-world wearing conditions were significantly lower than values in some studies (28 - 98%) conducted under laboratory conditions (Bin Jeong et al., 2021; Li et al., 2022). This may be attributed to the detachment of trapped particle matters, particularly coarse particles, from the masks during the movement of volunteers during the actual use scenario, resulting in lower REs. However, there was no statistically significant correlation between bioaerosol concentration and REs ($r = 0.2127$, $p = 0.6857$ for total bioaerosols' concentration; $r = -0.7993$, $p = 0.0564$ for culturable bioaerosols' concentration), indicating that REs of surgical masks are not simply related to bioaerosol concentrations, the degree of fit between masks and the wearer and the intensity of production activities also contribute to the overall protective effect of masks (Makison Booth et al., 2013). In the future, it may be necessary to develop improved evaluation methods of masks to more realistically reflect the role of masks in protecting human health.

5. Conclusions

The work investigated the pollution profile and inhalation health risks of bioaerosols in a typical oil exploitation area in Hebei Province, China. The concentration of bioaerosols varied with seasons, with significantly higher concentrations found in the steel plant (SP), inland oilwell (IOA1 and IOA2), and chemical industry park (CIP1 and CIP2) than in other places, suggesting that industrial activity contributes to elevated

bioaerosol concentrations. Bioaerosols were predominantly distributed in the size range of $>7.0 \mu\text{m}$ with the contribution of coarse particles ($>2.1 \mu\text{m}$) reaching 76.7% and 81.2% (bacteria) as well as 69.9% and 88.9% (fungi) in autumn and summer, respectively. The diversity and richness of bacterial communities were higher than those of fungal communities. *Anoxybacillus* and *Curtobacterium*, which could use crude oil and hydrocarbons as carbon sources, were the dominant bacteria, while *Cladosporium* and *Alternaria* were the dominant fungi. The sources of airborne bacteria were more complex than those of fungi. Among VOCs, 2,3-dimethylbutane, chloroethane, TCE, and 2-methoxy-2-methylpropane showed more significant correlations with bacteria, while fungal communities were more influenced by environmental conditions. The health risks caused by inhalation of bioaerosols are negligible ($HQ < 1$), but attention should be paid to the risks posed by *Bacillus* and *Mycobacterium* to the human body. The use of masks can reduce the inhalation of bioaerosols, but their interception efficiencies were substantially lower than reported laboratory-measured values.

However, this study has the following two limitations: Firstly, sampling was conducted only in autumn and summer, excluding transitional seasons when microbial dynamics may differ due to changes in temperature, humidity, and industrial activities. Secondly, the reduction efficiencies of masks for bioaerosols were not compared between different seasons; they were also not detected in the lab under controlled conditions, which is not favorable for comparing the protective effect and influencing factors of the masks. Nevertheless, the findings of this study provide critical insights for improving local air quality and mitigating human exposure risks to bioaerosols.

CRediT authorship contribution statement

Hongjie Yang: Writing – original draft, Formal analysis. **Shen He:** Writing – original draft, Investigation. **Zongting Yao:** Data curation. **Hao Li:** Visualization, Investigation. **Qihao Feng:** Investigation. **Simin Huang:** Writing – review & editing, Methodology. **Zhen Chen:** Visualization, Investigation. **Jiasheng Ni:** Investigation, Data curation. **Zhishu Liang:** Writing – review & editing, Supervision, Methodology.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2026.129020>.

Data availability

The data that has been used is confidential.

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