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Article

Characterization and Health Risk Assessment of Airborne Fungi in a Semiunderground Municipal Wastewater Treatment Plant

Published as part of Environment & Health special issue "Grand Environmental Challenge: Indoor Air Pollution, Health Effects, and Mitigation".

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Cite This: *Environ. Health* 2025, 3, 227−237

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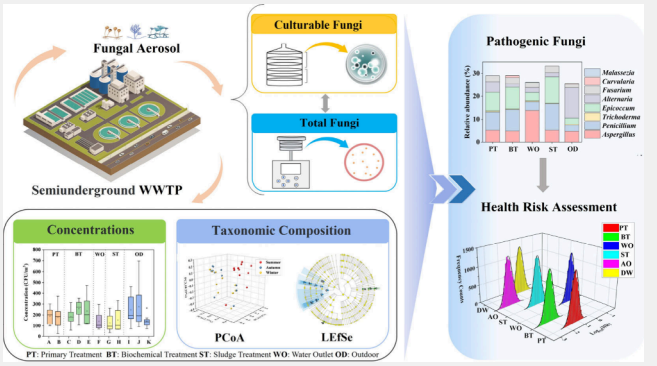
ABSTRACT: Municipal wastewater treatment plants (WWTPs) are recognized as the significant source of fungal aerosols, which pose a significant threat to human health globally. Herein, the occurrences characterization, community structure, and health risk assessment of airborne fungi were investigated from a semiunderground WWTP. The concentrations of culturable fungi emitted into the air from the WWTP ranged from 30.6 to 1431.1 colony forming units (CFU)/m^3, with primary and biochemical treatments constituting the principal sources of emission ($P < 0.05$). Diversity analysis revealed seasonal and facility-dependent fluctuations in culturable fungal communities. Approximately 13.5% of the total airborne fungal genera detected in the WWTP were culturable. Some airborne fungi in the WWTP with relatively low abundance but high cultivability, such as *Cladosporium*, *Trichoderma*, *Neurospora*, *Filobasidium*, and *Hannaella*, tended to be overlooked because of their limited presence in airborne environments. We also developed a health risk assessment method for fungi, utilizing seven indicators to characterize the risk posed by fungal pathogens from multiple perspectives, providing a comprehensive evaluation of potential health impacts. The simulated risk values of the air outlet and biochemical treatment exceeded those of other treatment facilities, with median risks of 2.2×10^2 and 1.4×10^2, respectively. Consequently, management strategies should prioritize enhanced controls for fungal aerosols to mitigate the risk of disease transmission.



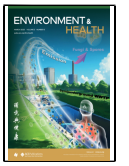
KEYWORDS: *Semiunderground WWTP, Airborne fungi, Culturability, Community structure, Health risk*

INTRODUCTION

Fungal pathogens and infections pose a significant global public health concern, affecting over a billion individuals and causing >1.5 million deaths annually.[1] Approximately 3%−10% of the general population is estimated to be sensitive to fungal allergens.[2,3] Municipal wastewater treatment plants (WWTPs) are recognized as significant emitters of fungal aerosols.[4,5] Recent studies have highlighted the significant role of WWTPs and composting facilities in emitting bioaerosols, which pose potential health risks to workers and nearby communities.[6−9] During wastewater treatment, pathogenic microorganisms are aerosolized and dispersed via mechanical aeration and other processes, impacting air quality in and around these facilities, especially in densely populated urban environments.[6,7] Accumulating evidence suggested that municipal wastewater contains large amounts of potential fungal pathogens that can be aerosolized during wastewater treatment processes.[10,11] Several opportunistic fungi, such as *Aspergillus*, *Fusarium*, *Rhodotorula*, and *Candida*, have been detected in the surrounding air of WWTPs.[4,10] In addition, fungal aerosols, as an important source of microbial volatile organic compounds (MVOCs), can cause adverse environmental effects such as odor problems.[12]

The concentrations and compositions of fungal aerosols have been characterized in the traditional WWTPs.[4,13] However, since 2016, China has witnessed a substantial increase in the total treatment capacity and investment in underground and semiunderground WWTPs, representing 18% and 30% of the total sewage treatment capacity and investment during the same period, respectively.[14] Unlike traditional WWTPs, semiunderground WWTPs are typically sealed by capping that significantly reduces odor diffusion and noise pollution.[15−17] Therefore,

Received: September 24, 2024
Revised: November 22, 2024
Accepted: November 25, 2024
Published: January 7, 2025



https://doi.org/10.1021/envhealth.4c00195
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semiunderground WWTPs are becoming the prevailing choice in China, especially in major cities such as Shanghai and Beijing. While fungal aerosols in traditional WWTPs have been extensively studied, research on underground or semiunderground facilities remains limited. The enclosed nature of these environments may alter air circulation and bioaerosol behavior.[6,7,17] Consequently, comprehensive investigations are required to assess the characteristics, multiplication capacity, and health risks of fungal aerosols in semiunderground or underground WWTPs.

Studying airborne fungi in the environment relies on culture-based and molecular methods.[18] Culturing is a standard method for clinically diagnosing pathogenic fungi and has been widely used for isolating fungi from the air.[19,20] However, > 95% of airborne microorganisms are nonculturable.[17,21] Molecular methods that target the DNA/RNA, can reveal the abundance and diversity of airborne microorganisms.[22,23] Integrating both culture-based and molecular methods can enhance the understanding of the composition and activity of airborne fungi emitted from WWTPs.

Given the growing concern over the health impacts of bioaerosols, it is essential to assess the exposure risks for workers and nearby residents of semiunderground WWTPs. While Quantitative Microbial Risk Assessment (QMRA) is commonly applied to evaluate the health risks of bacterial pathogens in bioaerosols, there remains a significant gap in assessing fungal risks due to the absence of well-defined dose−response relationships.[6,7]

This study aims to comprehensively characterize the concentrations, particle size distribution, and community composition of airborne fungi in a semiunderground WWTP by using culture method, high-throughput sequencing (HTS), and metagenomics sequencing. The activity and reproductive capacity of airborne fungi were evaluated by comparing the relative abundance of culturable and total fungal communities according to previous studies.[17] In addition, Fungi associated with pathogenicity and odorous MVOCs were also investigated, along with their health risks and environmental effects. We also developed a health risk assessment method for fungi, using seven indicators to characterize the risk posed by fungal pathogens from multiple perspectives. The findings of this study will provide pivotal understanding on the health risk assessment and control of airborne fungi in semiunderground WWTPs. This study addresses a significant gap in the literature by investigating the emissions of fungi aerosols from semiunderground WWTPs, a topic that has been under-researched compared to traditional open-air facilities. By focusing on the unique environmental conditions of these systems, this research provides valuable insights into the health risks associated with fungi aerosols.

MATERIALS AND METHODS

WWTP and Sampling Sites

Sampling sites were situated in a semiunderground WWTP in Shanghai, China, which treats approximately 2.0×10^5 m^3/day of domestic wastewater through the anaerobic−anoxic−aerobic process. All treatment processes in this facility are enclosed or covered, unlike traditional open sewage treatment plants. The plant operates in a fully enclosed indoor environment and is equipped with a ventilation system that includes fresh air and deodorization systems.[17] The sampling period was from July 2021 to February 2022. Sampling was conducted once a month, and each sampling lasted for about 2 days (Table S1). Sampling sites comprised the coarse screen (A), aeration tank (B), anaerobic tank (C), aerobic tank (D), anoxic tank (E), water outlet (WO; F), sludge dewatering house (G), sludge outlet (H), odor vents 1 (I), odor vents 2 (J), and a downwind site (K), as shown in the schematic in Figure S1. All sampling sites were categorized into five regions according to their functions (Table S2). Each sampling site was located at an average human respiratory height of 1.5 m above the ground. All sampling points are located within the same large, well-ventilated room with unobstructed air circulation, ensuring the reliability of our bioaerosol results derived from the same treatment process.

Detection of Culturable Fungi and Total Fungi in the Air of the WWTP

Culturable airborne fungi were collected using six-stage Andersen impactors with aerodynamic cut-size diameters of 7.0, 4.7, 3.3, 2.1, 1.1, and 0.65 μm over a 10 min period at a flow rate of 28.3 L/min. Sabouraud dextrose agar (SDA) plates were used to capture and culture airborne fungi.[24] After sampling, plates were incubated at 25 °C ± 1 °C for 5 days, and colonies were counted to determine the microbial concentrations via positive well correction.[25] We counted colony forming units (CFU) results using two methods: manual counting for verification and a bacterial colony counter for accurate quantification. The results were calculated as the geometric mean of the replicates and expressed as CFU/m^3. Finally, colonies were scraped from the plates, combined, washed with sterile phosphate-buffered saline (PBS) from all six plate of the Andersen sampler for each sampling, and added to two 2 mL centrifuge tubes for subsequent analysis.

Total fungi and spores were collected using a 90 mm diameter quartz filter membrane with a TSP sampler (TH-150C, Tianhong, Wuhan, China) and a sampling pump (TH-150, Tianhong, China), operating continuously at a flow rate of 100 L/min for 24 h. Before sampling, the quartz filter membrane was heated to 500 °C and maintained at that temperature for 5 h to eliminate any potential contaminants, such as DNA, microorganisms, and organic compounds. Tweezers and sampling heads were sterilized with 75% ethanol to ensure the integrity of the samples. To ensure that the microorganisms collected by the quartz filter were suitable for metagenomic sequencing, three samples from the same site were combined into a single composite sample. All samples were stored at −20 °C for subsequent DNA extraction and metagenomic sequencing. Additionally, temperature (T), relative humidity (RH), $PM_{2.5}$, and PM_{10} were measured using a hand-held detector during sampling. At the beginning of sampling, in the process of sampling, and at the end of sampling, respectively, the average value is taken.

Although temperature and humidity varied during sampling, these factors were recorded for each event to account for potential impacts on fungal concentrations. Negative controls were included to monitor for contamination throughout the sampling process, and positive controls were used during DNA extraction to ensure accuracy. Sampling sites were carefully chosen to cover the entire treatment process, and the same calibrated equipment and standardized procedures were used throughout to minimize bias. These measures ensured consistency in data collection and allowed for reliable comparisons across different sites and conditions.

ITS Gene Amplicon and Metagenome Sequencing

During the 8-month sampling period, we collected monthly air samples from 11 sites within the WWTP (Figure S1). Each site was sampled using both culture-based and filter membrane methods to comprehensively assess airborne fungal diversity and abundance (Text S1). Filter membrane samples were used for metagenomic sequencing, while culturable samples were used for amplicon sequencing. A total of 88 culturable fungi samples were collected. These samples were combined into 33 mixed samples according to different sampling points and seasons for ITS sequencing (Figure S3). Additionally, ten filter membrane samples were used for metagenomic sequencing analysis, with one sample taken from each of the ten sites, as the sample from one of the odor vents did not meet the requirements for sequencing. DNA extraction, along with DNA quality control, PCR amplification, and Illumina Miseq sequencing were conducted at Shanghai Majorbio

https://doi.org/10.1021/envhealth.4c00195
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Biopharm Technology. Blank samples and positive controls were included to ensure the accuracy and reliability of the results.

The internal transcribed pacer region 1 (ITS1) of the fungal rDNA gene was amplified using forward primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and reverse primers ITS1R (5′-GCTGCGTTCTTCATCGATGC-3′).[26] PCR cycling conditions were as follows: denaturation at 95 °C for 3 min; amplification for 30 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s; and a final extension at 72 °C for 10 min, then held at 4 °C.

Bioinformatic analysis was performed using QIIME2. Demultiplexed sequences were merged using FLASH (V1.2.11) and filtered using FASTP (0.19.6). After performing quality control using the DADA2 algorithm, 1,944,129 high-quality reads from 8,000 features at a single-nucleotide resolution were generated. Representative sequences were classified using the classify-sklearn classifier and the SILVA database (version 138). To improve the accuracy of the diversity and composition analyses, sequence flattening was performed for each sample according to the minimum number of sample sequences (22572 sequences/sample).[27]

Health Risk Assessment

We emphasized the importance of determining the health risk of fungal pathogens to humans from multiple perspectives rather than basing risk only on abundance. Consequently, seven indicators were defined from different perspectives, namely, average case fatality rate, annual incidence (AI), average treatment duration (TD), complications and sequelae (CS), antifungal resistance (AR), preventability (PR), and evidence-based treatments (ET). These indicators were used to evaluate the selected fungal pathogens based on priority and numerical scores and were divided into high, medium, low, and unknown categories. The specific evaluation standards are shown in Tables S3 and S4. These indicators are defined based on the "WHO List of Fungal Priority Pathogens".[28]

The risk index (RI) of the fungal pathogens to human health was calculated using the formula:

$$\mathrm{RI} = \frac{\mathrm{FR} \times \mathrm{AI} \times \mathrm{TD} \times \mathrm{CS} \times \mathrm{AR}}{\mathrm{PR} \times \mathrm{ET}}$$

The RI was calculated for each sample using the formula:

$$\mathrm{RI}_{\mathrm{sample}} = \sum_{i}^{n} \mathrm{Abundance}_i \times \mathrm{RI}_i$$

As inhalation is the most important mode of exposure for airborne fungi, key factors such as breathing rate (br, m^3/h), exposure time (t, h), fungi concentration in air (C_{air}, CFU/m^3) and aerosol ingestion rate (ag, %) were considered to assess the health risk (HR).

The HR was calculated for each sampling site using the formula:

$$\mathrm{HR} = \mathrm{RI}_{\mathrm{sample}} \times \mathrm{br} \times t \times \mathrm{ag} \times C_{\mathrm{air}}$$

The relevant parameters in this model are presented in Table S5. These parameters were varied over probability distributions generated during the study or reported in literature through Monte Carlo simulation (10,000 iterations). The median and peak (95th percentile) risks were considered to represent the most probable and potential high-risk scenarios.[29] To ensure the reliability of the risk calculations, we implemented rigorous quality assurance and control (QA/QC) measures, including multiple data replicates and the use of Monte Carlo simulations to account for variability and uncertainty. Additionally, we referenced relevant studies that have applied similar risk assessment models in bioaerosol and fungal pathogen research, supporting the validity of our approach.[28,30] These measures ensure that the risk assessment presented is both scientifically robust and grounded in reliable data.

RESULTS AND DISCUSSION

Concentrations and Size Distribution of Culturable Airborne Fungi in and around the Semiunderground WWTP

The concentrations of culturable fungi in the air surrounding the semiunderground WWTP ranged from 30.6 to 1431.1 CFU/m^3, with an average of 666 CFU/m^3 (Figures 1 and S3, Table S6). The concentrations in this study were relatively lower than those observed in traditional open WWTPs (Table 1). For instance, the highest concentration of culturable fungi in a WWTP located in Beijing reached 1.44×10^4 CFU/m^3.[31] The concentrations of fungi observed in nine WWTPs in Poland which reached up to

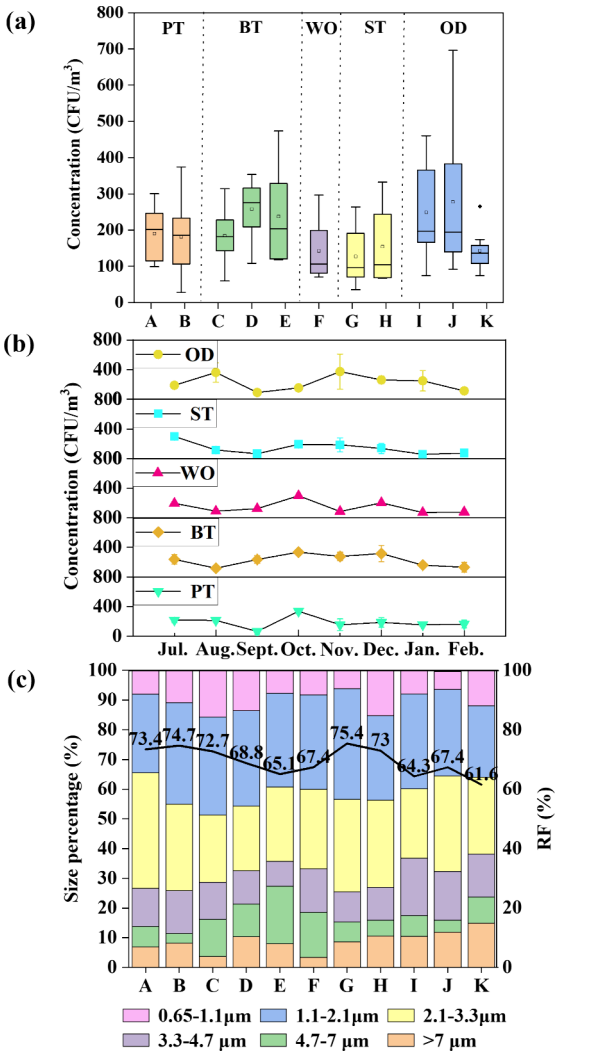

Figure 1. Spatial and temporal distribution of culturable fungi in the air surrounding the semiunderground WWTP. a: Concentrations of culturable fungi at the 11 sampling sites. b: Concentrations of culturable fungi in the five treatment units. c: Size distributions of airborne culturable fungi at the 11 sampling sites of coarse screen (A), aeration tank (B), anaerobic tank (C), aerobic tank (D), anoxic tank (E), water outlet (F), sludge drying house (G), sludge outlet (H), odor vents 1 (I), odor vents 2 (J), and a downwind site (K). These sites were divided into five treatment units according to their functions, which were primary treatment (PT) (sampling sites A and B), biochemical treatment (BT) (sampling sites C, D, and E), WO (sampling sites F), sludge treatment (ST) (sampling sites G and H), and outdoor (OD) (sampling sites I, J, and K).

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Table 1. Variations in Fungal Concentration Levels among Different Types of WWTPs

Sampling location in WWTP	Type of WWTP	Study Location	Sampling method	Fungal concentration	refs
Whole Process	Semiunderground WWTP	China	Andersen impactor sampler	30.6−1431.1 CFU/m^3	This study
Whole Process	Traditional open WWTP	China	Andersen impactor sampler	8775 ± 406 CFU/m^3	34
Aeration Basins	Traditional open WWTP	Iran	Andersen impactor sampler	85−2627 CFU/m^3	46
Aerobic Tank	Traditional open WWTP	China	Andersen impactor sampler	1.44×10^4 CFU/m^3	62
Aeration Basin	Traditional open WWTP	China	Medium-flow sampler	879 CFU/m^3	63

6700 CFU/m^3, were significantly higher than the concentrations found in the present study.[32] The discrepancy can be attributed to the unique closed structure and capping process employed in this semiunderground WWTP, which effectively reduces the release of fungal aerosols into the surrounding air. Additionally, the use of fresh air systems and deodorization systems in WWTP further reduces airborne fungal concentrations.

ANOVA analysis revealed that the concentrations of culturable fungi significantly differed in the air among the different treatment facilities ($P < 0.05$, Table S7). Notably, the concentrations of culturable fungi from the primary treatment (PT) (sampling sites A and B) and biochemical treatment (BT) (sampling sites C, D, and E) ranged from 56.5 to 992.9 CFU/m^3, which were relatively higher than those at other sampling sites (Figure 1a). Thus, these two treatments may be the primary sources of airborne fungi in semiunderground WWTPs. Previous studies have consistently shown that high concentrations of bioaerosols in WWTPs tend to be concentrated around treatment steps involving aeration or mechanical agitation.[33] Conversely, the sludge treatment (ST) sampling sites (G and H) in this semiunderground WWTP exhibited the lowest concentration of culturable fungi, with an average of 116.6 CFU/m^3 (Figure 1). Notably, ST areas have often been reported to have high bioaerosol concentrations.[34] Nevertheless, in the WWTP evaluated here, the encapsulation and capping of the ST process may limit the release of fungi escaping from the sludge into the air. Additionally, the presence of an integrated ventilation system in the ST area helps minimize airborne fungal levels.

In all outdoor sampling sites, the culturable fungal concentrations at odor vent1 (sampling site I) and vent2 (sampling site J) were significantly higher than those at the downwind site (sampling site K). The odor vents exhibited a higher concentration of culturable fungi, as these carry fungal bioaerosols when discharging gas from the WWTP. In particular, the odor vents serve as the outlet for the entire indoor treatment process, allowing a substantial number of fungi to be emitted along with the gas. These airborne fungi tended to settle near the ground of the odor vents, leading to the high concentration of fungi found in this area. However, the concentrations of culturable fungi at the downwind site were diluted by the effect of the wind.

The concentration profiles of culturable fungi over time are presented in Figure 1b. ANOVA 1 revealed a significant reduction in the concentrations of culturable fungi during summer and winter compared with that in autumn ($P < 0.05$, Table S7). Additionally, emissions of culturable fungi from distinct treatment processes in the semiunderground WWTP displayed notable temporal variations. Specifically, the concentrations of culturable fungi in the ST and WO treatment facilities exhibited significant temporal fluctuations, with coefficient of variation (CV) values of 58.51% and 56.84%, respectively (Table S8). Conversely, the concentrations of culturable fungi in the BT treatment facility showed the least variation over time, with a CV of 36.54% (Table S8). The temporal and seasonal fluctuations in fungal concentrations are primarily caused by changes in temperature and relative humidity.[35,36] Herein, a significant positive correlation was present between the concentrations of culturable fungi and temperature ($r = 0.41$, $P < 0.05$, Table S9), suggesting that temperature is a predominant factor influencing airborne fungi in this semiunderground WWTP.

The size distributions of culturable airborne fungi in and around the semiunderground WWTP are shown in Figure 1c. Notably, the vast majority of airborne fungi are present in the form of spores. The highest concentration of culturable airborne fungi was observed in stages 4 and 5, with an aerodynamic size range of 2.1−3.3 and 1.1−2.1 μm, respectively (Figure 1c). These stages accounted for 21.8%−39.0% and 24.1%−37.2% of the total concentrations, respectively (Figure 1c). Fungal particles in this size range can reach the alveoli and could cause infections in immunocompromised individuals.[4,37] However, the proportion of culturable airborne fungi with particle size >4.7 μm in WWTP was only 11.4%−27.2% (Figure 1c). The particle size distribution characteristics of the culturable airborne fungi were similar to those observed in landfills, conventional wastewater treatment plants, and composting facilities.[3,38,39]

The respiratory fraction (RF), referring to particles with a diameter <3.3 μm, is noteworthy as these particles can enter the human lower respiratory tract and are recognized as a significant contributor to inflammation.[23] The RF of culturable airborne fungi in and around this WWTP is shown in Figure 1c. Compared with that at other treatment facilities, the highest RF was found at the ST sampling sites (G and H), accounting for 73%−75.4%, followed by that at the PT sampling sites (A and B), accounting for 72.7%−74.7%. The RF of culturable airborne fungi at the outdoor (OD) sampling sites (I, J, and K) was the lowest of those tested, with an average of 61.6%−67.4%. Fungal aerosols released from the WWTP, characterized by a high proportion of RF, can pose a serious health risk to both workers at the facility and nearby residents. Therefore, the particle size distribution of culturable fungal aerosols in the WWTP should be a matter of concern regarding the discharge of fungal aerosols from the WWTPs.

Taxonomic Composition of Culturable Airborne Fungi in and around the Semiunderground WWTP

Herein, Illumina Miseq HTS technology was used to probe the taxonomic composition of culturable airborne fungal aerosols in and around the semiunderground WWTP. A total of 296 amplicon sequence variants (ASVs) were detected in 33 samples and classified into 110 fungal genera (Figure S6).

Principal coordinate analysis (PCoA) was used to illustrate the variation in culturable airborne fungal populations (Figure 2). No apparent clustering was observed in the samples collected from different treatment facilities (Figure 2a), indicating that the culturable airborne fungi in and around the semiunderground WWTP were homogeneous. The results in Figure 2b

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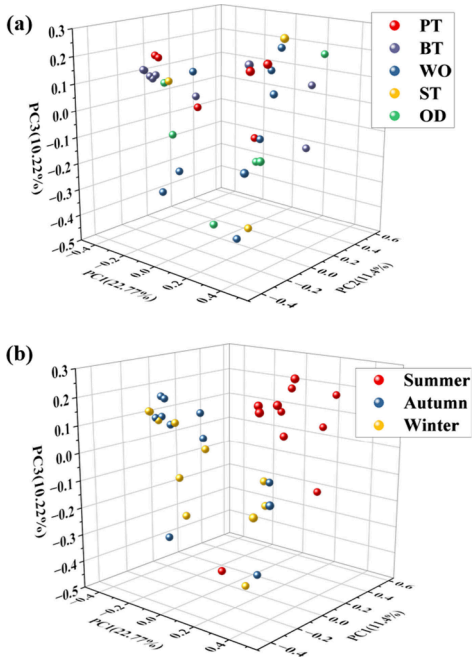

Figure 2. PCoA of the culturable fungal populations in the air surrounding the WWTP. a: Comparison of the abundance and community composition of culturable among five different treatment units. b: Comparison of the abundance and community composition of culturable fungi among different seasons.

demonstrate significant individual aggregation among samples representing summer fungal populations. Conversely, a partial overlap and aggregation were present among the samples representing autumn and winter populations, with a notable separation between the summer and autumn/winter culturable fungal populations. This indicated that the culturable airborne fungal populations collected in the summer differed from those obtained in the autumn and winter.

The Shannon and Simpson indices for culturable airborne fungi across different seasons and facilities are shown in Figures S5a and b. The mean Shannon indices of culturable airborne fungi from the PT, BT, WO, ST, and OD sites were 1.32, 1.39, 1.45, 1.54, and 1.31, respectively (Figure S6a), while the mean Simpson indices were 0.45, 0.40, 0.32, 0.36, and 0.45 (Figure S6c), respectively. These results indicate a minimal difference in the diversity of culturable fungal communities among the different treatment processes. The mean Shannon indices of culturable airborne fungi in summer, autumn, and winter were 0.95, 1.69, and 1.53, respectively (Figure S6b), while the mean Simpson indices were 0.58, 0.28, and 0.36 (Figure S6d), respectively. These findings suggest that the community diversity of culturable airborne fungi was highest in the autumn, slightly lower in the winter, and lowest in the summer.

Figure S7 illustrates the high proportional distribution of Ascomycota and Basidiomycota across all samples collected from the semiunderground WWTP. Ascomycota and Basidiomycota have been reported as the dominant fungal phyla in aerosol particles in different environments,[3,40,41] and these phyla are frequently observed in soil, aquatic, and airborne habitats.[42] The composition of culturable airborne fungi in different seasons and treatment facilities at the genus level is shown in Figure S8. The genera with high abundance of culturable airborne fungi in and around the semiunderground WWTP were *Aspergillus*, *Penicillium*, and *Cladosporium* in order (Figure S8a). *Cladosporium* and *Aspergillus* have also been reported as the most abundant genera in the air of other composting facilities,[43] landfills,[44] and wastewater treatment plants.[4] Certain species within the genus *Penicillium* have been recognized as potential allergens and can contribute to extrinsic bronchial asthma.[45]

The taxonomic composition of culturable airborne fungi varied across seasons (Figures S7b–d). Specifically, the relative abundance of *Aspergillus* was highest in summer (69.92%) but decreased in autumn and winter (14.22% and 6.08%, respectively). The relative abundance of *Cladosporium* was 28.30% and 22.11% in autumn and winter, respectively, but only 1.72% in summer. The taxonomic differences in culturable airborne fungi were also observed among different treatment facilities. For instance, the relative abundance of *Aspergillus* was high at the PT sites (39.0%–72.7%) but low at the OD (0.4%–21.8%) sampling sites. Additionally, the relative abundance of *Penicillium* was higher at the BT sites than in other treatment facilities. Previous studies on bioaerosols in WWTPs have also reported significant differences in microbial populations across seasons and facilities.[31,46]

The LEfSe analysis revealed that certain fungi were more prevalent in specific seasons: *Filobasidiales* and *Cryptococcus* at the genus level, Tremellaceae at the family level, Tremellales and Filobasidiales at the order level, and Tremellomycetes at the class level were more common in winter compared to summer

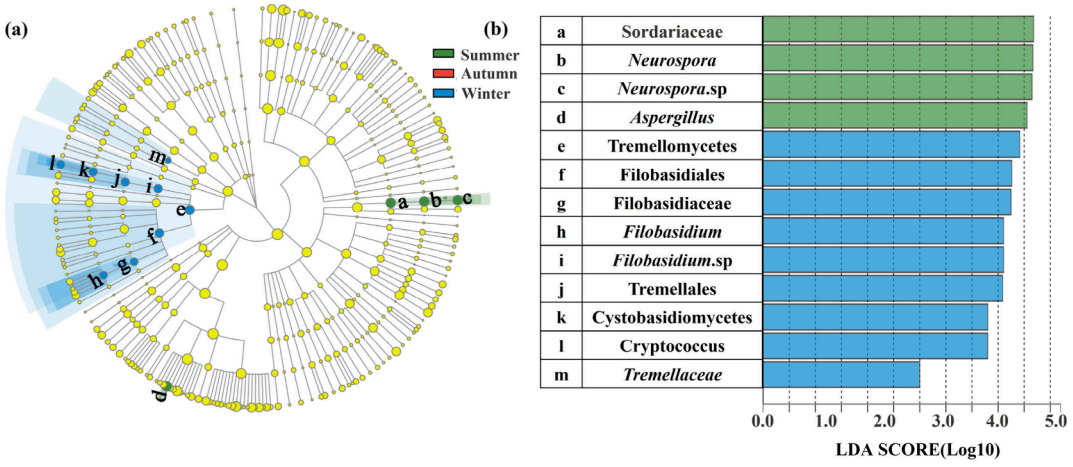

Figure 3. Discriminative culturable fungi associated with seasons. a: LEfSe Hierarchical tree of multiple species. b: LDA discriminates histogram.

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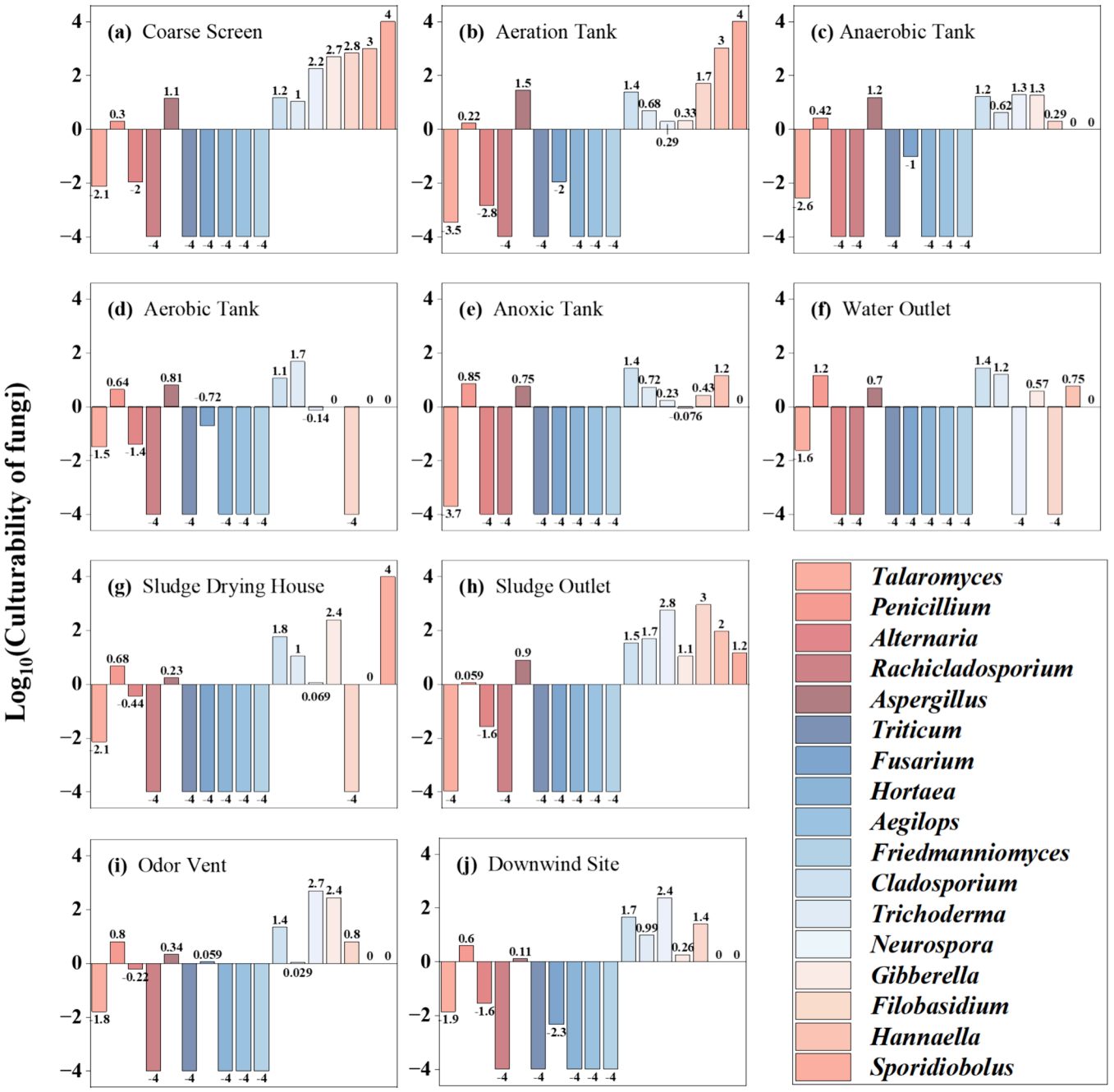

Figure 4. Culturability of fungi in the air surrounding the WWTP. Fungal culturability was assessed based on the ratio of the relative abundance of culturable fungi to the relative abundance of total fungi. This ratio was then reflected in the score. A score of 4 indicates detection only in culturable fungi, − 4 indicates detection only in total fungi, and 0 indicates no detection. The horizontal coordinate ordination was determined by the relative abundance in the total fungal community. (a) to (j) represent the following sampling sites: (a) coarse screen, (b) aeration tank, (c) anaerobic tank, (d) aerobic tank, (e) anoxic tank, (f) water outlet, (g) sludge drying house, (h) sludge outlet, (i) odor vent, and (j) downwind site.

and autumn (Figure 3). *Cryptococcus* is a key mammalian fungal pathogen and may cause cryptococcosis, a life-threatening infection.[47,48] *Neurospora* and *Aspergillus* were more prevalent in the summer but not in autumn or winter. *Neusporidium* was particularly prevalent during the hot season. Certain species of *Aspergillus* can develop in the lung cavities of immunocompromised patients or those with chronic lung diseases, forming a pulmonary fungal ball.[49] Several species of *Aspergillus* are listed by the WHO as fungal pathogens, with immunocompromised individuals being more susceptible to infections. Notably, most of the fungi which were more prevalent in specific seasons in this study were potential pathogens at the genus level, such as *Aspergillus* and *Neurospora*. The list of prevalent fungi can provide a reference for understanding the risk of fungal aerosols in semiunderground WWTP and for taking effective control measures.

The Relationship between Total and Culturable Fungi in and around the Semiunderground WWTP

The community structure of total airborne fungi in and around the semiunderground WWTP was investigated via metagenomic sequencing. Following species annotation, we identified 6,045,136 fungal sequences, representing 10 phyla, 585 genera,

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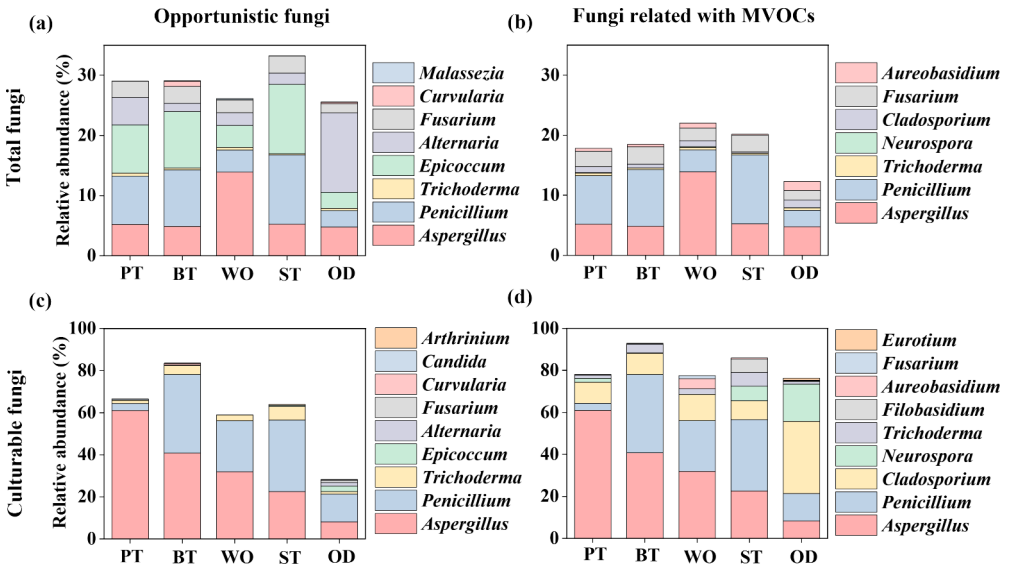

Figure 5. Relative abundance of pathogenic fungal taxa and genera associated with microbial volatile organic compounds (MVOCs). a: Relative abundance of pathogenic fungi in total fungal taxa. b: Relative abundance of fungal genera associated with MVOCs in total fungal taxa. c: Relative abundance of pathogenic fungi in culturable fungal taxa. d: Relative abundances of fungi associated with MVOCs in culturable fungal taxa. PT, primary treatment; BT, biochemical treatment; WO, water outlet; ST, sludge treatment; OD, outdoor.

and 1,333 species. The total fungal community structures at the class and genus levels are illustrated in Figure S9. At the class level, the predominant fungi in the air of the semiunderground WWTP were Eurotiomycetes and Dothideomycetes (Figure S9a), whereas at the genus level, the most dominant genera included *Talaromyces*, *Penicillium*, and *Rachicladosporium* (Figure S9b). Conversely, different taxonomic compositions were observed in culturable airborne fungal samples, with *Aspergillus*, *Penicillium*, and *Cladosporium* being the most dominant fungal genera (Figure S8a). Culture methods and culture-independent HTS are both established approaches for studying bioaerosol composition.[50] Notably, the results obtained through postculture and direct sequencing without culture significantly differed. These discrepancies can be attributed to variations in the culturability of different fungi, with a significant portion being inactivated or being viable but nonculturable (VBNC).

Additionally, we counted the total and culturable fungi at the genus level and discovered that approximately 13.5% of airborne fungal genera in the sewage treatment plant could be cultured (Figure S10). Most airborne fungi spread through spores, which exhibit greater resilience to external factors such as ultraviolet light.[21] These fungal spores can potentially revive and proliferate under favorable conditions.[51] Therefore, we used the ratio of the relative abundance of culturable fungi to total fungi to assess the culturability of fungi. The degree of culturability can reflect the activity and replicability of fungi. The culturable capacity of fungi in the air of a sewage treatment plant is shown in Figure 4, where a score of 4 indicates detection only in culturable fungal samples, possibly because of sequencing losses during metagenomic sequencing, while a score of −4 signifies detection only in the total fungus sample, indicating that the fungus is dead or in a VBNC state. A score of 0 indicates no detection. Ten genera with the highest abundances of culturable and total fungi were selected for analysis, and the order of different genera of fungi was sorted according to the abundance of total fungi. Several genera with a high relative abundance of total fungi, such as *Talaromyces* and *Alternaria*, exhibited low culturability, with ratios ranging from 0.03×10^{-4}–1.9×10^{-4} and -0.36×10^{-3}–1.6×10^{-3}, respectively (Figure 4). Additionally, certain fungi with relatively low abundance but high cultivability, such as *Cladosporium*, *Trichoderma*, *Neurospora*, *Filobasidium*, and *Hannaella*, tend to be overlooked because of their limited presence in airborne environments (Figure 4). However, their robust cultivation capacity enables them to thrive once they have settled in suitable environments. Notably, certain fungi identified are known to be pathogenic or sensitizing, potentially causing underestimated health risks. For instance, several species of *Aspergillus* are well-documented pathogens that can cause a range of health issues from allergic reactions to more severe infections. Other identified fungi such as *Alternaria* and *Cladosporium* are known allergens that can exacerbate respiratory conditions. Significant differences in the activity and reproductive capacity of fungi within the same genus were not observed at various sampling points, except for the WO site, where the activity and reproductive capacity of *Neurospora* and *Filobasidium* was notably lower compared with that at other sampling sites. This decrease in activity and reproductive capacity may be attributed to reduced fungal activity caused by disinfection processes at the WO.

Environmental Factors Affected the Airborne Fungi in and around the Semiunderground WWTP

Bioaerosols, including fungi, are affected by environmental conditions such as relative humidity and temperature. In addition, $PM_{2.5}$ and PM_{10}, as carriers of airborne fungi and fungal spores, also affect the concentration of airborne fungi.[52] Herein, the relative abundance of 12 out of 30 culturable airborne fungi with the highest abundance was significantly correlated with PM_{10} or $PM_{2.5}$ ($P < 0.01$) (Figure S11), as fungi spores can be carried by $PM_{2.5}$ and PM_{10} particles, affecting their concentration in the air. A study conducted on atmospheric microbes in 31 major Chinese cities revealed that PM is among the most significant factors affecting fungal communities.[53] PM, particularly $PM_{2.5}$ and PM_{10}, influences fungal communities by providing attachment surfaces, acting as carriers for fungal spores, altering environmental conditions like humidity and

https://doi.org/10.1021/envhealth.4c00195
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temperature, and affecting fungal metabolism and community composition.[53] Temperature was negatively correlated with most culturable airborne fungi, such as *Micrococcus*, *Dietzia*, and *Brevibacillus* (Figure S11). However, the relative abundance of *Aspergillus* and *Neurospora*, which were the discriminative fungal genera between seasons, showed a significant positive correlation with temperature. Relative humidity was positively correlated with various culturable airborne fungal populations and was significantly positively correlated with *Trichoderma* and *Hannaella*. In a dry environment, the metabolism and physiological functions of fungi are inhibited, but an increase in humidity can cause cell clumping and improve cell survival rates.[54,55] Previous studies have shown that fungal aerosol concentrations increase during rainy days.[56]

Health Risks Assessment of Airborne Fungi in and around the Semiunderground WWTP

To gain insight into the potential health effects of fungal aerosols, we investigated the culturable and total fungi associated with disease in the air surrounding the semiunderground WWTP. The pathogenic fungi at the genus level in different facilities and seasons are shown in Figures 5 and S11, respectively. The dominant opportunistic pathogenic genera in the air surrounding the WWTP were *Aspergillus* and *Penicillium*, which accounted for 4.8%−19.9% and 2.7%−11.5% of the total fungal sequences, respectively (Figure 5a). *Aspergillus fumigatus* can cause aspergillus disease in humans, mainly affecting the lungs but also spreading to other areas such as the brain.[57] *Penicillium* is considered to be an important pathogen of exogenous bronchial asthma.[45] Notably, the proportion of these two opportunistic fungal pathogens within the culturable fungal taxa was considerably larger than that of other opportunistic fungal taxa (Figure 5c). These observations indicate the high activity and reproductive capability of these fungal genera in the air of the WWTP. This is significant because they can have potential health impacts at high concentrations. The proportions of pathogenic fungi, and particularly culturable species, were notably higher in the treatment facilities (PT, BT, WO, and ST) compared with those at the OD site. We hypothesize that this discrepancy is due to the aerosolization of pathogenic fungi from sewage into the air during the sewage treatment process. Subsequently, these fungi may undergo dilution with nonpathogenic fungi in the atmosphere during the outward diffusion process. Furthermore, the proportions of pathogenic fungi varied by season, with the highest proportion in summer (83.1%), followed by that in autumn (51.4%) and winter (36.5%) (Figure S12). The seasonal variation of the airborne fungal population in WWTPs is often caused by changes in temperature and humidity.[3]

The health risks associated with different pathogenic fungi vary greatly. Therefore, judging risks by the total abundance of pathogenic fungi alone may not be reliable. According to the Critical Fungal Pathogen List (FPPL) published by the World Health Organization, *A. fumigatus*, *Fusarium*, and *Talaromyces marneffei* were selected as typical fungal pathogens for risk assessment in and around the semiunderground WWTPs. We evaluated the overall health risk for three typical fungal pathogens using the seven calculated metrics (FR, AI, TD, CS, AR, PR, and ET) as determined above (Tables S3 and S4). The health risks of the three fungal pathogens are shown in Figure 6, with *A. fumigatum*, *Fusarium*, and *Talaromyces* in order of risk magnitude. This is consistent with WHO priorities for fungal pathogens.

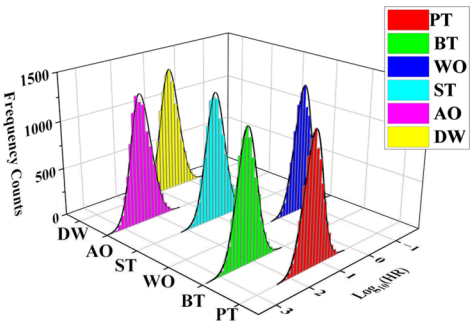

Figure 6. Health risks of fungal aerosols in different treatment units in the WWTP. The horizontal coordinate represents different treatment facilities, and the specific information is consistent with Figure 1. The vertical coordinate represents the health risk value. The z-coordinate represents the frequency counts of each health risk zone, with the higher the value, the higher the likelihood. PT, primary treatment; BT, biochemical treatment; WO, water outlet; ST, sludge treatment; AO, air outlet; DW, downwind.

In this study, culturable fungal concentrations and sequencing results were used to estimate the actual concentrations of fungal pathogens in the air of semiunderground WWTP. The actual health risk is much higher because VBNC state fungal pathogens are not considered. The simulated health risk is shown in Figure 6, which displays the distribution of risk values and frequencies obtained by Monte Carlo simulation, showing a clear normal distribution. Compared with that of other treatment facilities, the risk at the air outlet (AO) sampling sites (I and J) and BT sampling sites (C, D, and E) was higher, with median risks of 2.2×10^2 and 1.4×10^2, respectively. The median health risks at PT sampling sites (A and B) and ST sampling sites (G and H) were 2.2×10^1 and 1.1×10^1, respectively. The WO (sampling site F) had the lowest health risk, with a median risk of only 1.7×10^{-1}. The WO is equipped with chlorine disinfection and ultraviolet disinfection facilities, which considerably reduces the abundance of pathogenic fungi and thus the risk of infection. The risk values for the downwind sampling point ranged from 4.5×10^{-1} to 2.8×10^2, with a median of 4.4, which was significantly lower than that of the AO sites. This may be due to environmental factors such as wind dilution and ultraviolet radiation. These results suggest that culturable airborne pathogenic fungi in and around the semiunderground WWTP may also pose health risks to workers and nearby residents. Managers should therefore focus on controlling the discharge of pathogenic fungi, especially at the AO as a major source of discharge.

To effectively control fungal emissions in high-risk areas, several mitigation strategies should be implemented. Enhancing ventilation systems can significantly improve air circulation and reduce the concentration of bioaerosols. Additionally, employing bioaerosol filtration technologies, such as high-efficiency particulate air filters, can capture airborne fungal spores. Regular maintenance and cleaning of ventilation and filtration systems are also crucial to ensure their effectiveness.

In addition to the health risks, the potential environmental impact of fungal aerosols should also be considered. Fungal degradation of complex organic matter is accompanied by the release of MVOCs.[58] The MVOCs released by fungi include many malodorous gas compounds, which may also have adverse effects on workers and nearby residents. From an environmental perspective, the problem of malodorous gases in WWTP has been a long-standing and challenging issue. Therefore,

https://doi.org/10.1021/envhealth.4c00195
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identifying fungi associated with MVOC production is of considerable significance and value for subsequent research.

The most predominant fungal genera related to MVOC generation in this study were *Aspergillus*, *Penicillium*, *Cladosporium*, *Neurospora*, and *Trichoderma*, which accounted for an average of 13%−22% and 76%−93% in total fungal and culturable fungal taxa, respectively (Figure 5b and d). *Aspergillus*, for example, is associated with the release of 2-methyl-1-alcohol,[59] while *Penicillium* metabolism releases dimethyl disulfide, one of eight malodorous pollutants.[60] Certain species of *Cladosporium* can release MVOCs such as decane, decaldehydes, and methyl benzoate.[59,61] The proportion of MVOCs in the air at the BT, WO and ST sites was higher than that in other areas, supporting the fact that these areas will probably have unpleasant odors. The proportion of MVOCs in the air of the WWTP was the highest in summer, followed by that in autumn and winter. Rising summer temperatures may exacerbate the spread of malodorous gases, thereby increasing their negative environmental impact.

CONCLUSIONS

The characteristics of culturable and total fungal emissions and potential impacts in a semiunderground municipal wastewater treatment plant were investigated. The following conclusions can be drawn from the results of this study:

1) The concentrations and communities of airborne culturable fungi in and around the semiunderground WWTP exhibited variations that were facility- and season-associated.
2) The culturable rate of airborne fungi in the WWTP is approximately 13.5%. Some fungi, such as *Cladosporium*, *Trichoderma*, *Neurospora*, *Filobasidium*, *and Hannaella*, exhibit relatively low total abundance but high cultivability. These fungi are frequently disregarded due to their limited presence in the air environment.
3) Fungi genera that were potentially pathogenic or could produce MVOCs were identified. These may pose health risks to humans or negatively affect the surrounding environment.
4) The results of the health risk assessment showed that the simulated risk values of the air outlet and biochemical treatment were higher than those of other treatment facilities.
5) This study contributes to the growing body of knowledge on bioaerosols in semiunderground WWTPs, emphasizing the critical need for tailored health risk assessments and mitigation strategies to protect both workers and public health.

ASSOCIATED CONTENT

Data Availability Statement

The data of ITS sequencing in present study are available from the Sequence Read Archive (SRA) (PRJNA944786).

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/envhealth.4c00195.

Comprehensive details on experimental methods, including qPCR protocols, statistical analyses of data, raw data files, diagrams corresponding to experimental results, and sampling layout maps (PDF)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Key Research and Development Program of China (Nos. 2022YFC3704700 and 2023YFC3708203), the National Natural Science Foundation of China (Nos. 22122601 and 22376033), the Science and Technology Commission of Shanghai Municipality (Nos.

21230712800 and 21DZ1202300), and the Natural Science Foundation of Guangdong Province, China (No. 2021A1515010415). Supported by Major Project of Guangzhou National Laboratory, Grant No. GZNL2024A01028.

REFERENCES

(1) Bongomin, F.; Gago, S.; Oladele, R. O.; Denning, D. W. Global and Multi-National Prevalence of Fungal Diseases-Estimate Precision. *J. Fungi* **2017**, *3* (4), 57.
(2) Ianovici, N. Atmospheric concentrations of selected allergenic fungal spores in relation to some meteorological factors, in Timişoara (Romania). *Aerobiologia* **2016**, *32* (1), 139−156.
(3) Liu, Y.; Zhang, Y.; Shi, Y.; Shen, F.; Yang, Y.; Wang, M.; Zhang, G.; Deng, T.; Lai, S. Characterization of fungal aerosol in a landfill and an incineration plants in Guangzhou, Southern China: The link to potential impacts. *Sci. Total Environ.* **2021**, *764*, No. 142908.
(4) Han, Y.; Yang, T.; Xu, G.; Li, L.; Liu, J. Characteristics and interactions of bioaerosol microorganisms from wastewater treatment plants. *J. Hazard. Mater.* **2020**, *391*, No. 122256.
(5) Haas, D.; Unteregger, M.; Habib, J.; Galler, H.; Marth, E.; Reinthaler, F. F. Exposure to Bioaerosol from Sewage Systems. *Water Air Soil Poll* **2010**, *207* (1−4), 49−56.
(6) Chen, Y.-h.; Yan, C.; Yang, Y.-f.; Ma, J.-x. Quantitative microbial risk assessment and sensitivity analysis for workers exposed to pathogenic bacterial bioaerosols under various aeration modes in two wastewater treatment plants. *Sci. Total Environ.* **2021**, *755*, No. 142615.
(7) Yan, C.; Gui, Z.-c.; Wu, J.-t. Quantitative microbial risk assessment of bioaerosols in a wastewater treatment plant by using two aeration modes. *Environ. Sci. Pollut R* **2021**, *28* (7), 8140−8150.
(8) Gao, M.; Qiu, T. L.; Sun, Y. M.; Wang, X. M. The abundance and diversity of antibiotic resistance genes in the atmospheric environment of composting plants. *Environ. Int.* **2018**, *116*, 229−238.
(9) Onursal, A.; Icgen, B. Discharges of Wastewater Treatment Plants Needed Further Monitoring to Minimize Potential Risk of Entamoeba and Blastocystis for Public Health. *Environment & Health* **2024**, *2* (11), 838−845.
(10) Kowalski, M.; Wolany, J.; Pastuszka, J. S.; Plaza, G.; Wlazlo, A.; Ulfig, K.; Malina, A. Characteristics of airborne bacteria and fungi in some Polish wastewater treatment plants. *Int. J. Environ. Sci. Te* **2017**, *14* (10), 2181−2192.
(11) Lou, M.; Liu, S.; Gu, C.; Hu, H.; Tang, Z.; Zhang, Y.; Xu, C.; Li, F. The bioaerosols emitted from toilet and wastewater treatment plant: a literature review. *Environ. Sci. Pollut. Res.* **2021**, *28* (3), 2509−2521.
(12) Lee, S.; Hung, R.; Bennett, J. W. An Overview of Fungal Volatile Organic Compounds (VOCs). In *Fungal Associations*, Hsueh, Y.-P., Blackwell, M., Eds.; Springer International Publishing, 2024; pp 83−111.
(13) Zhai, Y.; Li, X.; Wang, T.; Wang, B.; Li, C.; Zeng, G. A review on airborne microorganisms in particulate matters: Composition, characteristics and influence factors. *Environ. Int.* **2018**, *113*, 74−90.
(14) Wang, K. Development of underground wasterwater treatment plant and eco-civilization in China. *Water Wastewater Eng.* **2021**, *47* (8), 7.
(15) Huh, S. Y.; Shin, J.; Ryu, J. Expand, relocate, or underground? Social acceptance of upgrading wastewater treatment plants. *Environ. Sci. Pollut. Res.* **2020**, *27* (36), 45618−45628.
(16) Wei, Q. The Current Status and Prospect of Underground Wastewater Treatment Plant in China. *China Water Wastewater* **2017**, *06*, 18−26.
(17) Zhang, X.; Lu, B. J.; Chen, G.; Wang, L. H.; Lin, B. J.; Peng, Z. L.; Lu, S. L.; Li, D.; Chen, J. M. Culturable and inhalable airborne bacteria in a semiunderground municipal wastewater treatment plant: Distribution, transmission, and health risk assessment. *J. Hazard Mater.* **2023**, *459*, No. 132234.
(18) Huang, T.; Fang, Y.; Feng, Z.; Shen, H.; Nie, Y.; Zheng, X.; Wang, J.; Xu, Z. Bacterial Communities in a Middle School Campus Assessed by Highthroughput Sequencing. *Biotechnol. Bull.* **2020**, *36* (8), 96−103.
(19) Dehghani, M.; Sorooshian, A.; Ghorbani, M.; Fazlzadeh, M.; Miri, M.; Badiee, P.; Parvizi, A.; Ansari, M.; Baghani, A. N.; Delikhoon, M. Seasonal Variation in Culturable Bioaerosols in a Wastewater Treatment Plant. *Aerosol Air Qual Res.* **2018**, *18* (11), 2826−2839.
(20) Jalili, D.; Dehghani, M.; Fadaei, A.; Alimohammadi, M. Assessment of Airborne Bacterial and Fungal Communities in Shahrekord Hospitals. *J. Environ. Public Health* **2021**, *2021*, 1−7.
(21) Steen, A. D.; Crits-Christoph, A.; Carini, P.; DeAngelis, K. M.; Fierer, N.; Lloyd, K. G.; Thrash, J. C. High proportions of bacteria and archaea across most biomes remain uncultured. *Isme J.* **2019**, *13* (12), 3126−3130.
(22) Wang, C.; Song, L.; Zhang, Z.; Wang, Y.; Xie, X. Microwave-induced release and degradation of airborne antibiotic resistance genes (ARGs) from Escherichia coli bioaerosol based on microwave absorbing material. *J. Hazard. Mater.* **2020**, *394*, 122535.
(23) Uhrbrand, K.; Schultz, A. C.; Koivisto, A. J.; Nielsen, U.; Madsen, A. M. Assessment of airborne bacteria and noroviruses in air emission from a new highly-advanced hospital wastewater treatment plant. *Water Res.* **2017**, *112*, 110−119.
(24) Andriana, Y.; Widodo, A. D. W.; Endraswari, P. D. A Correlation between the Number of Airborne Bacteria and Fungi using the Settle Plate Method with Temperature and Relative Humidity at the Clinical Microbiology Laboratory of Dr. Soetomo Regional General Hospital Surabaya, Indonesia. *J. Pure Appl. Microbio* **2023**, *17*, 942.
(25) Andersen, A. New Sampler for the Collection, Sizing, And Enumeration of Viable Airborne Particles. *J. Bacteriol.* **1958**, *76* (5), 471−484.
(26) Mbareche, H.; Veillette, M.; Bilodeau, G.; Duchaine, C. Comparison Of The Performance Of Its1 And Its2 As Barcodes In Amplicon-Based Sequencing of bioaerosols. *PeerJ.* **2020**, *8*, No. e8523.
(27) Rousk, J.; Bååth, E.; Brookes, P. C.; Lauber, C. L.; Lozupone, C.; Caporaso, J. G.; Knight, R.; Fierer, N. Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J.* **2010**, *4* (10), 1340−1351.
(28) *WHO Fungal Priority Pathogens List to Guide Research, Development and Public Health Action*; World Health Organization, 2022.
(29) Jahne, M. A.; Rogers, S. W.; Holsen, T. M.; Grimberg, S. J.; Ramler, I. P. Emission and Dispersion of Bioaerosols from Dairy Manure Application Sites: Human Health Risk Assessment. *Environ. Sci. Technol.* **2015**, *49* (16), 9842−9849.
(30) Jahne, M. A.; Rogers, S. W.; Holsen, T. M.; Grimberg, S. J. Quantitative microbial risk assessment of bioaerosols from a manure application site. *Aerobiologia* **2015**, *31* (1), 73−87.
(31) Han, Y.; Yang, K.; Yang, T.; Zhang, M.; Li, L. Bioaerosols emission and exposure risk of a wastewater treatment plant with A2O treatment process. *Ecotox. Environ. Safe.* **2019**, *169*, 161−168.
(32) Michałkiewicz, M. Wastewater Treatment Plants as a Source of Bioaerosols. *Polym. J. Environ. Stud.* **2019**, *28* (4), 2261−2271.
(33) Yang, K. X.; Li, L.; Wang, Y. J.; Xue, S.; Han, Y. P.; Liu, J. X. Airborne bacteria in a wastewater treatment plant: Emission characterization, source analysis and health risk assessment. *Water Res.* **2019**, *149*, 596−606.
(34) Xu, P. C.; Zhang, C. M.; Mou, X.; Wang, X. C. C. Bioaerosol in a typical municipal wastewater treatment plant: concentration, size distribution, and health risk assessment. *Water Sci. Technol.* **2020**, *82* (8), 1547−1559.
(35) Wang, R.-n.; Li, X.; Yan, C. Seasonal fluctuation of aerosolization ratio of bioaerosols and quantitative microbial risk assessment in a wastewater treatment plant. *Environ. Sci. Pollut. Res.* **2021**, *28* (48), 68615−68632.
(36) Han, Y. P.; Yang, K. X.; Yang, T.; Zhang, M. Z.; Li, L. Bioaerosols emission and exposure risk of a wastewater treatment plant with A(2)O treatment process. *Ecotox. Environ. Safe.* **2019**, *169*, 161−168.
(37) Rolph, C. A.; Gwyther, C. L.; Tyrrel, S. F.; Nasir, Z. A.; Drew, G. H.; Jackson, S. K.; Khera, S.; Hayes, E. T.; Williams, B.; Bennett, A. Sources of Airborne Endotoxins in Ambient Air and Exposure of Nearby Communities—A Review. *Atmosphere* **2018**, *9*, 375.

(38) Li, Y.; Feng, Y.; Feng, W. Deeply fluorinated multi-wall carbon nanotubes for high energy and power densities lithium/carbon fluorides battery. *Electrochim. Acta* **2013**, *107*, 343−349.
(39) Morgado Gamero, W. B.; Ramírez, M. C.; Parody, A.; Viloria, A.; López, M. H. A.; Kamatkar, S. J. Concentrations and Size Distributions of Fungal Bioaerosols in a Municipal Landfill. *Lecture Notes in Computer Science* **2018**, *10943*, 244−253.
(40) He, P.; Wei, S.; Shao, L.; Lü, F. Aerosolization behavior of prokaryotes and fungi during composting of vegetable waste. *Waste Management* **2019**, *89*, 103−113.
(41) Gou, H.; Lu, J.; Li, S.; Tong, Y.; Xie, C.; Zheng, X. Assessment of microbial communities in PM1 and PM10 of Urumqi during winter. *Environ. Pollut.* **2016**, *214*, 202−210.
(42) Fröhlich-Nowoisky, J.; Burrows, S. M.; Xie, Z.; Engling, G.; Solomon, P. A.; Fraser, M. P.; Mayol-Bracero, O. L.; Artaxo, P.; Begerow, D.; Conrad, R.; et al. Biogeography in the air: fungal diversity over land and oceans. *Biogeosciences* **2012**, *9* (3), 1125−1136.
(43) Wery, N. Bioaerosols from composting facilities-a review. *Front. Cell. Infect. Microbiol.* **2014**, *4*, 9.
(44) Zhang, J.; Xia, L.-j.; Du, W.-l.; Wang, J.-a.; Zhang, C.-r.; Ji, S.-s.; Yu, B. Community Structure and Time-Space Distribution Characteristic of Airborne Fungi in a Municipal Landfill Site. *Huanjing Kexue* **2009**, *30* (11), 3184−3189.
(45) Maharia, S.; Srivastava, A. Influence of seasonal variation on concentration of fungal bioaerosol at a sewage treatment plant (STP) in Delhi. *Aerobiologia* **2015**, *31* (2), 249−260.
(46) Fathi, S.; Hajizadeh, Y.; Nikaeen, M.; Gorbani, M. Assessment of microbial aerosol emissions in an urban wastewater treatment plant operated with activated sludge process. *Aerobiologia* **2017**, *33* (4), 507−515.
(47) Maziarz, E. K.; Perfect, J. R. Cryptococcosis. *Infect. Dis. Clin. North Am.* **2016**, *30* (1), 179−206.
(48) Fisher, M. C.; Henk, D. A.; Briggs, C. J.; Brownstein, J. S.; Madoff, L. C.; McCraw, S. L.; Gurr, S. J. Emerging fungal threats to animal, plant and ecosystem health. *Nature* **2012**, *484* (7393), 186−194.
(49) Broaddus, V. C. Aspergillus. In *Murray & Nadel's Textbook of Respiratory Medicine*, 7th ed.; Elsevier, 2022.
(50) Korzeniewska, E.; Harnisz, M. Culture-Dependent and Culture-Independent Methods in Evaluation of Emission of from Sewage to the Air and Surface Water. *Water Air Soil Poll* **2012**, *223* (7), 4039−4046.
(51) Blatzer, M.; Latgé, J. P. Fungal spores are future-proofed. *Nat. Microbiol* **2021**, *6* (8), 979−980.
(52) Duan, J.; Ding, R.; Li, M.; Qi, J.; Yin, P.; Wang, L.; Sun, Z.; Hu, Y.; Zhou, M. Subnational Evidence for the Attributable Burden of Respiratory Infections in China's Population under 20: Challenges from Particulate Matter Pollution. *Environ. Health* **2024**, DOI: 10.1021/envhealth.4c00137.
(53) Zhang, L.; Yao, M. S. Unique atmospheric microbiota patterns for 31 major Chinese cities. *Atmos. Environ.* **2023**, *315*, No. 120143.
(54) Zuraimi, M. S.; Fang, L.; Tan, T. K.; Chew, F. T.; Tham, K. W. Airborne fungi in low and high allergic prevalence child care centers. *Atmos. Environ.* **2009**, *43* (15), 2391−2400.
(55) RodrÍGuez-Rajo, F. J.; Iglesias, I.; Jato, V. Variation assessment of airborne Alternaria and Cladosporium spores at different bioclimatical conditions. *Mycol. Res.* **2005**, *109* (4), 497−507.
(56) Brągoszewska, E. Exposure to Bacterial and Fungal Aerosols: Microorganism Indices in A Waste-Sorting Plant in Poland. *Int. J. Environ. Res. Public Health* **2019**, *16*, 3308.
(57) van de Veerdonk, F. L.; Gresnigt, M. S.; Romani, L.; Netea, M. G.; Latge, J. P. Aspergillus fumigatus morphology and dynamic host interactions. *Nat. Rev. Microbiol.* **2017**, *15* (11), 661−674.
(58) Matysik, S.; Herbarth, O.; Mueller, A. Determination of microbial volatile organic compounds (MVOCs) by passive sampling onto charcoal sorbents. *Chemosphere* **2009**, *76* (1), 114−119.
(59) Lemfack, M. C; Gohlke, B.-O.; Toguem, S. M. T.; Preissner, S.; Piechulla, B.; Preissner, R. mVOC 2.0: a database of microbial volatiles. *Nucleic Acids Res.* **2018**, *46* (D1), D1261−D1265.
(60) Schleibinger, H.; Laussmann, D.; Brattig, C.; Mangler, M.; Eis, D.; Ruden, H. Emission patterns and emission rates of MVOC and the possibility for predicting hidden mold damage? *Indoor Air* **2005**, *15*, 98−104.
(61) Kanchiswamy, C. N.; Malnoy, M.; Maffei, M. E. Chemical diversity of microbial volatiles and their potential for plant growth and productivity. *Front. Plant Sci.* **2015**, *6*, No. 00151, DOI: 10.3389/fpls.2015.00151.
(62) Han, Y. P.; Yang, K. X.; Yang, T.; Zhang, M. Z.; Li, L. Bioaerosols emission and exposure risk of a wastewater treatment plant with A2O treatment process. *Ecotoxicol. Environ. Safety* **2019**, *169*, 161−168.
(63) Wang, Y. J.; Lan, H. C.; Li, L.; Yang, K. X.; Qu, J. H.; Liu, J. X. Chemicals and microbes in bioaerosols from reaction tanks of six wastewater treatment plants: survival factors, generation sources, and mechanisms. *Sci. Rep-Uk* **2018**, *8*, 9362.