



Characterization of fungal aerosol in a landfill and an incineration plants in Guangzhou, Southern China: The link to potential impacts

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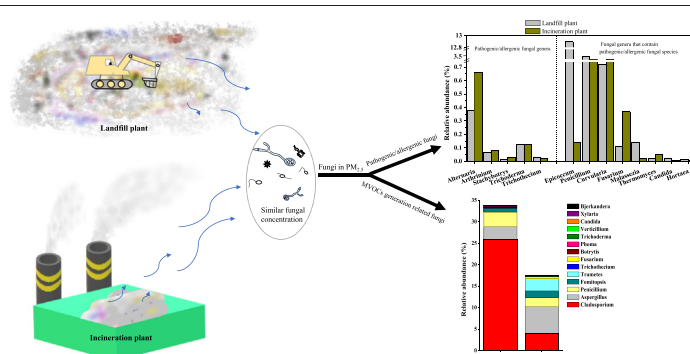
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HIGHLIGHTS

- Culturable concentration and DNA of fungal aerosols were measured in waste plants.
- Culturable airborne fungi predominantly resided in the size range of 2.1–3.3 μm .
- Different fungal communities may be due to waste sorts and treatment procedures.
- Pathogenic and MVOCs related fungi were identified to reveal potential impacts.

GRAPHICAL ABSTRACT



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ABSTRACT

To understand the characteristics and potential impacts of fungal aerosols in waste disposal treatments, we performed observations at a landfill and an incineration plants in Guangzhou, Southern China. Size-segregated airborne fungal concentrations were measured based on culture-dependent method, and fungal compositions in $\text{PM}_{2.5}$ were obtained using high-throughput sequencing method. Concentrations of airborne fungi varied from 376 to 9318 CFU/m^3 in the landfill plant and from 53 to 8491 CFU/m^3 in the incineration plant, respectively. The temporal and spatial variations of fungal aerosols indicate that waste disposal operation, garbage transport, air mixing, and meteorological factors can significantly influence the variations of airborne fungi in the outdoor environment in both plants. Among the meteorological factors, light/moderate rain could significantly increase the airborne fungal concentrations while heavy rain could decrease the concentrations due to wet scavenging. We observed that culturable fungal aerosols predominantly resided in the size range of 2.1–3.3 μm . Different fungal community structures in $\text{PM}_{2.5}$ were found between the landfill and the incineration plants, suggesting the influence of different waste sorts and treatment procedures. We further identified the pathogenic/allergenic fungal taxa (e.g., *Alternaria*, *Epicoecum* sp. and *Stachybotrys* sp.) in the two plants, implying the potential human

Abbreviations: AS, Active sector; LTZ, Leachate treatment zone; CL, Central location; GTE, Garbage truck entrance; OA-O, Office area (outdoor); OA-I, Office area (indoor); AMC, Ascomycota; BMC, Basidiomycota; RH, Relative humidity; T, Temperature; MVOCs, Microbial volatile organic compounds.

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health risks with long-term exposure for on-site workers and surrounding residents. The fungal genera producing microbial volatile organic compounds (MVOCs, e.g., *Cladosporium*, *Fusarium* sp., *Penicillium* sp. and *Candida*) were found in both plants. These MVOCs generation related fungal genera could contribute to the odor in the plants and, more importantly, affect the downwind area after aerosolization and transportation.

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1. Introduction

Fungal aerosol particles are ubiquitous in different environments, originating from soil, vegetation, waste, etc., and can live under extreme conditions (Frohlich-Nowoisky et al., 2009; Yamamoto et al., 2012; Ziaee et al., 2018; Humbal et al., 2018; Elbert et al., 2007). Fungal aerosols could be the sources of microbial volatile organic compounds (MVOCs) and be correlated with odor issue causing unfavorable environmental effects (Shinohara et al., 2018). Exposure to allergenic fungi can cause allergic respiratory diseases, such as allergic sensitization, symptoms of allergy and asthma (Yamamoto et al., 2015; Zhai et al., 2018; Denning et al., 2006). It has been reported that approximately 3–10% of the general population is sensitive to fungal allergens (Ianovici, 2016; Cramer et al., 2006). The pathogenic fungi are related to hypersensitivity, invasive mycoses and respiratory abnormalities (Bush and Portnoy, 2001; Verhoeff and Burge, 1997; Jung et al., 2009). The complex structure of fungal particles can diversify their aerodynamic size, influencing their deposition in human body (Zhai et al., 2018; Després et al., 2012). As an important vector for the transmission of fungi, PM_{2.5} carrying pathogenic/allergenic fungi can penetrate into human alveolar region and blood, and thus initiate various ailments in humans (Xu et al., 2019; Brodie et al., 2007; Xie et al., 2019).

It is well known that landfill and incineration are the most prevailing treatments of solid waste in Guangzhou, Southern China. Waste disposal plants including landfill and incineration are sources of fungal aerosols (Vilavert et al., 2011; Domingo and Nadal, 2009). Fungal aerosol particles could be generated through the processes of waste handling activities (delivery, unloading, dumping and layering) (Vilavert et al., 2011; Chen et al., 2017; He et al., 2019; Madhwal et al., 2020). A variety of studies on the characteristics of fungal aerosols have been conducted in various waste management plants and their vicinities (Patil and Kakde, 2017; Schlosser et al., 2016; Burkowska et al., 2011; Abdel Hameed et al., 2015). Landfill sites generally showed higher loadings of bioaerosol than those of their surroundings, but could have impact on the microbial air quality 1000–1200 m away (Kaźmierczuk and Bojanowicz-Babłok, 2014; Frączek et al., 2014; Breza-Boruta, 2016). The influencing factors of fungal aerosols were studied including season, temperature (T), relative humidity (RH) and rainfall (Patil and Kakde, 2017; Tignat-Perrier et al., 2020; Bragoszewska, 2019).

Although characterization of bioaerosol concentrations and compositions has been studied in waste management plants in previous studies (Burkowska et al., 2011; Abdel Hameed et al., 2015; Morgado Gamero et al., 2018; Zhang et al., 2009; Viegas et al., 2014), comprehensive studies regarding levels, characteristics and impacts of bioaerosol in different waste treatment plants, especially in incineration plants, with multiple techniques and thorough investigation are still necessary.

Herein, this study aimed to characterize airborne fungal particles with respect to culturable fungal concentration and the fungal community in PM_{2.5} using both culture-dependent method and high-throughput sequencing. The observations were conducted in a landfill and an incineration plants in Guangzhou during the warm seasons. The influence of meteorological conditions on fungal aerosols was studied. We further investigated the fungi in PM_{2.5} associated with pathogenicity/allergenicity and unfavorable odor MVOCs, and therefore, their potential impacts are discussed.

2. Materials and methods

2.1. Sampling of fungal aerosols

2.1.1. Sampling sites and periods

Airborne fungal particles were collected in a landfill and an incineration plants in Guangzhou, Southern China. The locations of sampling sites in these plants are listed in Table 1.

In the landfill plants, five sampling sites were selected, including active sector (AS), leachate treatment zone (LTZ), central location (CL), garbage truck entrance (GTE) and office area (outdoor) (OA-O). The sampling was conducted during the period of 11th–16th October 2018. The data of Guangzhou Meteorological Station shows that the relative humidity was in the range of 61–99%, and outdoor temperature varied from 17.9 °C to 27.4 °C during sampling campaign.

In the incineration plant, four sampling sites were selected, including AS, LTZ, OA-O and office area (indoor) (OA-I). The sampling was conducted during the period of 25th July–6th August 2019 with a pause from 1st to 4th August due to a typhoon event. The relative humidity ranged from 48% to 98% and outdoor temperature varied from 25.3 °C to 36.7 °C during sampling campaign according to the data of Guangzhou Meteorological Station.

2.1.2. Size-segregated samples collection

A six-stage microbial cascade impactor (JMT-6, Beijing) was employed to aspirate airborne fungi onto Petri dishes containing Sabouraud dextrose agar (Difco, BD, USA). The sampler was positioned at a height of ~1.5 m above the ground for simulating aspiration from the human breathing zone. The particles were captured in particle size (aerodynamic diameter) ranges of >7.0, 4.7–7, 3.3–4.7, 2.1–3.3, 1.1–2.1 and 0.65–1.1 µm. The size-segregated samples were collected at a flow rate of 28.3 L/min for 2 min and triplicate samples were taken during every collection. The apparatus and sampling materials were sterilized with 75% ethanol before sampling. Three size-segregated samples and one blank sample were obtained at each site every day. A total of 75 and 87 samples (blanks not included) were taken in the landfill and the incineration plants respectively.

2.1.3. PM_{2.5} collection

PM_{2.5} were collected on quartz fiber filters (8 × 10, PALL Life Sciences) using two high-volume samplers (Mingye, Guangzhou; XT-1025, Shanghai). PM_{2.5} sampling with a flow rate of 1 m³/min and a duration of 23.5 h was conducted at OA-O and CL in the landfill plant as well as at OA-O in the incineration plant. A total of 10 and 8 PM_{2.5} samples (blanks not included) were collected in the landfill and the incineration plants respectively. To decontaminate organic pollution, the quartz fiber filters were prebaked at 550 °C for 6 h and then stored at prebaked aluminum foil bags prior to sampling. After collection, the samples were stored at –20 °C for the subsequent analysis. Operation blanks and field blanks were collected and analyzed to assure the sampling and analysis.

2.2. Analysis method

2.2.1. Culturable fungal concentration

The size-segregated fungi captured onto Petri dishes were transferred to the laboratory for cultivation at 25–28 °C for ~72 h. After

Table 1
Description of selected sampling sites in the landfill plant and the incineration plant.

Plant	Sampling point (abbreviation)	Outdoor/indoor	Description
Landfill plant	Active sector (AS)	Outdoor	An open area used for waste dumping and compacting. The samples were taken next to the operation area.
	Leachate treatment zone (LTZ)	Outdoor	The zone used for the treatment of leachate. The site was about 1000 m southeast to the AS. The samples were taken outdoors next to the treatment station.
	Central location (CL)	Outdoor	A location approximately in the center of landfill plant. The site was about 300 m east to the AS.
	Garbage truck entrance (GTE)	Outdoor	An entrance where the garbage trucks loaded with wastes entering into the plant and leaving the plant after dumping.
	Office area (outdoor) (OA-O)	Outdoor	An area where administration staff work. It is about 600 m northeast to the AS and 300 m east to the GTE. The site was located on the roof of a three-storied office building.
Incineration plant	Active sector (AS)	Indoor	An area inside the main building where wastes were dumped to a space under negative pressure for incineration handling.
	Leachate treatment zone (LTZ)	Outdoor	The zone used for the treatment of leachate. The samples were taken outdoors next to the treatment station. The site was about 300 m east to the OA-O.
	Office area (outdoor) (OA-O)	Outdoor	An area where administration staff work. The site was located on the roof of a three-storied office building.
	Office area (indoor) (OA-I)	Indoor	The site was located inside the office building. The area was mechanically ventilated and cleaned frequently.

incubation, the fungal concentration at each stage was counted and expressed in colony-forming units (CFU/m³) using positive-hole correction method (Andersen, 1958; Feller, 1950).

2.2.2. DNA extraction and PCR

Fungal DNA was extracted from ~1/8 of the PM_{2.5} quartz fiber filters (~180 m³ of air) using a commercial soil extraction kit (LysingMatrixE, FastDNASpin Kit for Soil, MP Biomedicals). The filters were first cut into small pieces and ground into powders in a sterilized agate mortar. The powder samples were then loaded into a LysingMatrixE tube for DNA extraction. The extracted genomic DNA was dissolved in 70 µL of DES buffer. Concentration and purity of the extracted DNA were estimated with Nanodrop Spectrometer (Nanodrop 2000, Thermo Scientific). The DNA concentrations were in the range of 13–35 ng/µL which were sufficient for sequencing. The ITS1 region (internal transcribed spacer region 1) of the fungal rRNA gene was amplified with the forward primers ITS1F (CTTGGTCATTTAGAGGAAGTAA) and the reverse primers ITS2 (GCTGCGTCTTCATCGATGC). Thermal cycling conditions were as noted below: initial denaturation at 95 °C for 5 min to activate the polymerase, followed by 35 cycles at 95 °C for 30 s, 50 °C for 30 s, 72 °C for 40 s, and a 7 min final extension at 72 °C, then hold at 4 °C. Blank filters were analyzed using the same procedure, and no fungal contaminations were detected. High-throughput sequencing analysis was performed on the Illumina HiSeq 2500 at Biomarker Technologies Corporation (Beijing, China).

2.2.3. Sequence analysis

According to the overlapping among paired-end reads, the raw sequences obtained through Illumina sequencing were merged into raw tags using FLASH v1.2.7, and raw tags with low quality were discarded using Trimmomatic v0.33. Chimeric sequences identification and removal were conducted using UCHIME v4.2. The obtained effective tags were clustered into operational taxonomic units (OTUs) at a level of 97% similarity using UCLUST. Low-abundant OTUs (lower than 0.005% for abundance) were removed. The representative sequences of OTU were compared to reference Database (UNITE) for phylotypes annotation. Alpha and beta diversity analysis were carried out with Mothur v.1.30, R scripts and QIIME v1.8.0. Line Discriminant Analysis Effect Size (LEfSe) analysis was also utilized in present study to single out fungal taxa that mainly attribute to fungal community disparity among groups.

2.2.4. Identification of pathogenic/allergenic fungi

Pathogenic fungi were determined according to the directory of humans infecting pathogenic microorganisms promulgated by the Ministry of Health of China and the list of pathogenic fungi proposed by Makimura (2001). Allergenic fungi were characterized with reference to Simon-Nobbe et al. (2008).

2.2.5. Identification of fungi related to MVOCs generation

Fungi related to MVOCs emission were identified by the mVOC 2.0 Database (<http://bioinformatics.charite.de/mvoc/>) (Lemfack et al., 2018).

2.2.6. Meteorological conditions

The data of meteorological conditions were obtained from the website of Guangzhou Meteorological Station (<http://www.tqyb.com.cn/>). According to the standard of rainfall intensity used by China Meteorological Administration, light, moderate and heavy rain was defined as the rainfall of <5, 5–14.9 and 15–29.9 mm per 12 h.

3. Results and discussion

3.1. Concentration of culturable fungal aerosols

In the landfill plant, the results of culturable fungal concentrations are shown in Table 2. CFU concentrations of airborne culturable fungi generally ranged from 376 CFU/m³ to 9318 CFU/m³. Gentle spatial variations were observed, with the highest count at GTE, an average of 3173 ± 2421 CFU/m³. The high loading of airborne fungi at GTE was caused by the release of fungi during garbage transport. The lowest fungal concentration was found at AS which had an average of 2047 CFU/m³. OA-O (2920 CFU/m³), CL (2500 CFU/m³) and LTZ (2336 CFU/m³) were the second highest, third highest and second lowest fungal concentrations, respectively. Overall, the similar fungal concentration levels at different sites shows that the atmospheric fungal concentrations were relatively uniform in the whole landfill plant due to the favorable conditions of air mixing and dispersion in open environment, which may not be necessarily elevated by dumping or land filling operation.

Airborne culturable fungal concentrations generally ranged from 53 to 8491 CFU/m³ at all of the sites of incineration plant (Table 2). AS was the confined indoor environment that generated fungal aerosols and further easily promoted the accumulation of airborne fungi. The average fungal concentration recorded at AS was up to 4081 ± 2714 CFU/m³. The lowest concentration (810 ± 789 CFU/m³) was measured at OA-I. Compared to the outdoor fungal concentration in office area of 1907 CFU/m³, the indoor value reduced by a factor of ~2. Bragoszewska (2019) reported a similar outdoor/indoor ratio (~1.7) in a waste-sorting plant in Poland. It could be due to the close-door environment with effective ventilation systems and frequent cleaning. The airborne fungal concentration at LTZ was similar to that at OA-O.

There was no significant difference in fungal concentration levels between the two waste management plants ($P > 0.05$). The fungal concentrations at outdoor areas were similar. The results of both plants are comparable to other reports that have measured in landfill plants around the world (Breza-Boruta, 2016; Morgado Gamero et al., 2018; Huang et al., 2002). For example, Zhang et al. (2009) reported that the

Table 2
Airborne fungal concentrations in the landfill plant and the incineration plant (CFU/m³).

Plant	Sampling date	AS*	LTZ*	CL*	GTE*	OA-O*	OA-I*
Landfill plant	12 th Oct 2018	508 ± 147	1287 ± 370	1488 ± 936	964 ± 148	834 ± 204	
	13 th Oct 2018	470 ± 73	1981 ± 179	1181 ± 207	2833 ± 1204	1262 ± 56	
	14 th Oct 2018	608 ± 155	1355 ± 147	777 ± 349	1274 ± 123	376 ± 236	
	15 th Oct 2018	6237 ± 1116	5158 ± 1024	6199 ± 1158	6970 ± 1640	9318 ± 1070	
	16 th Oct 2018	2413 ± 81	1897 ± 417	2853 ± 898	3822 ± 854	2806 ± 66	
	Average	2047 ± 2481	2336 ± 1608	2500 ± 2210	3173 ± 2421	2920 ± 3692	
Incineration plant	26 th July 2019	718 ± 31	920 ± 80			652 ± 94	419 ± 90
	27 th July 2019	1423 ± 10	646 ± 113			680 ± 188	183 ± 80
	28 th July 2019	2511 ± 657	3990 ± 1619			4597 ± 1062	738 ± 108
	29 th July 2019	8491 ± 752	602 ± 396			535 ± 18	353 ± 446
	30 th July 2019	4536 ± 1701	1152 ± 101			1578 ± 92	2340 ± 283
	31 st July 2019	5435 ± 1800	–			–	1697 ± 331
	5 th Aug 2019	–	53 ± 47			843 ± 172	590 ± 104
	6 th Aug 2019	5455 ± 1753	6629 ± 1013			4467 ± 803	160 ± 36
	Average	4081 ± 2714	1999 ± 2410			1907 ± 1825	810 ± 789

* Mean ± SD standard deviation.

average fungal concentrations in a landfill plant of Beijing were ~1750 CFU/m³ at both AS and LTZ. The average fungal concentration in a landfill plant of India was 4582.75 ± 1358.25 CFU/m³ (Madhwal et al., 2020). Additionally, the concentrations of fungal aerosols could be higher in the two plants than in some urban areas of Guangzhou which had the fungal concentrations in the range of 462–584 CFU/m³ (Ouyang et al., 2006). It should be noted that the fungal concentration at AS was much higher in the incineration plant compared to that in the landfill plant. The significant increase of fungal aerosols at AS in the incineration plant should draw public attentions to the health of the waste treatment plant workers.

3.2. Size distribution of culturable fungal aerosols

The size distributions of airborne culturable fungi are shown in Fig. 1. The highest loading of airborne culturable fungi was observed in Stage 4

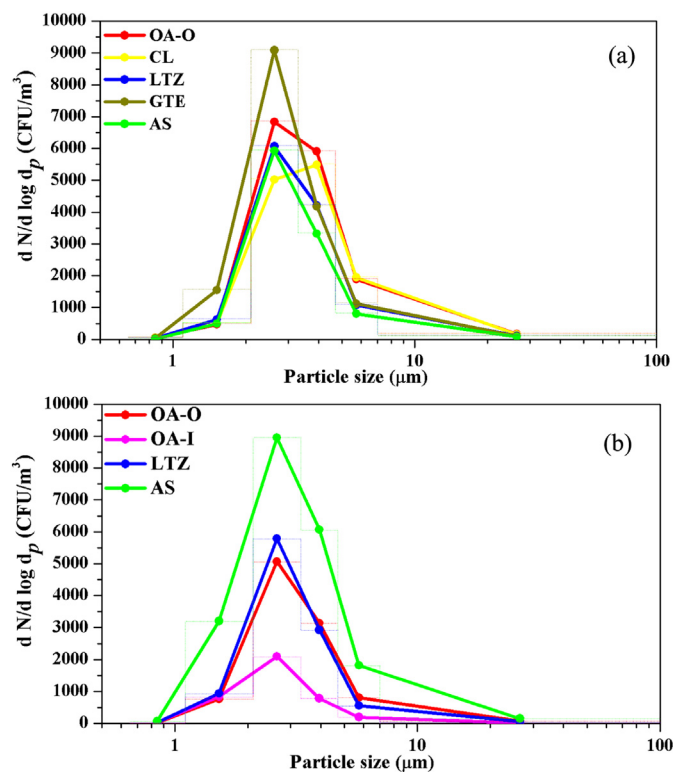


Fig. 1. (a) Size distributions of airborne culturable fungi at the five sampling sites in the landfill plant; (b) Size distributions of airborne culturable fungi at the four sampling sites in the incineration plant.

with the aerodynamic size range of 2.1–3.3 μm, similar to an observation of a landfill plant in Colombia (Morgado Gamero et al., 2018). The characteristics of the airborne culturable fungal particle size distribution was similar to that observed in cars, sewage treatment plants and feedstuff-manufacturing factories (Wang et al., 2013; Maharia and Srivastava, 2014; Kim et al., 2009). It is consistent with previous studies showing that the size of airborne fungi may vary from ~1 to ~50 μm but mainly accumulate in the range of 2–10 μm (Després et al., 2012).

The proportions of fine mode fungal aerosols with the size smaller than 3.3 μm in total fungal aerosols are shown in Table 3. The incineration plant had higher abundances of fungi in fine particles (67–80% on average) than those of the landfill plant (50–69% on average). The different size contributions could be attributed to the different fungal communities in the two plants. It is known that fine mode particles containing fungi could pose health risk when they are inhaled by human body. The fungal aerosols emitted in these waste treatment plants may contain hazardous and pathogenic genera which can trigger adverse human health effects on on-site workers and the downwind residents when they are dispersed and transported (see more details in Section 3.3 and 3.5). Therefore, the loading of fungi in fine particles in the two waste treatment plants should arise public concerns on fungal aerosol emission and community of waste treatment plants.

3.3. Fungal community in PM_{2.5}

The constructed Rarefaction Curves of observed OTUs are shown in Fig. S1, suggesting that further increases in sample size will hardly yield even more species. Sample α diversity metrics are summarized in Table S1, there was no statistically significant difference of fungal

Table 3
The percentages of fine mode fungal aerosols (<3.3 μm) in total fungal aerosols.

Plant	Sampling date	AS	LTZ	CL	GTE	OA-O	OA-I
Landfill plant	12 th Oct 2018	74%	84%	50%	63%	56%	
	13 th Oct 2018	56%	74%	60%	76%	75%	
	14 th Oct 2018	57%	58%	50%	65%	80%	
	15 th Oct 2018	64%	51%	37%	69%	45%	
	16 th Oct 2018	64%	46%	55%	71%	54%	
	Average	63%	63%	50%	69%	62%	
Incineration plant	26 th July 2019	64%	73%			74%	86%
	27 th July 2019	81%	80%			50%	77%
	28 th July 2019	75%	90%			64%	80%
	29 th July 2019	64%	67%			64%	78%
	30 th July 2019	88%	90%			84%	88%
	31 st July 2019	53%	–			–	65%
	5 th Aug 2019	–	67%			77%	88%
	6 th Aug 2019	53%	54%			53%	78%
	Average	68%	74%			67%	80%

diversity levels between the landfill and the incineration plants (Student's t -test, $P > 0.05$). Fig. 2 (a) illustrated the high proportional distribution of *Ascomycota* (AMC) and *Basidiomycota* (BMC) across all PM_{2.5} samples collected from the two plants. This observation is quite consistent with those reported in different environments (He et al., 2019; Boreson et al., 2004; Gou et al., 2016). Fröhlich-Nowoisky et al. (2012) have reported that AMC and BMC were presented as the dominant phyla of fungi in aerosol particles across the continental, coastal and marine sites and they were both the frequently observed fungal taxa in soil, aquatic, and air habitats. *Dothideomycetes*, *Sordariomycetes*,

Agaricomycetes and *Eurotiomycetes* were the four abundant classes in both of the landfill and the incineration plants (Fig. 2 (b)). *Dothideomycetes* is parasitic or saprophytic class and can be found in woody debris, decaying leaves and dung (Haridas et al., 2020; Mbareche et al., 2019). Members of *Sordariomycetes* and *Eurotiomycetes* are decomposers involved in the decomposition of organic matter (Maharachchikumbura et al., 2015; Geiser et al., 2006; Mbareche et al., 2017). *Agaricomycetes* can play roles in wood decaying activities and in ectomycorrhizal symbiosis (Hibbett and Matheny, 2009; Morgenstern et al., 2008). The high relative abundances of the four

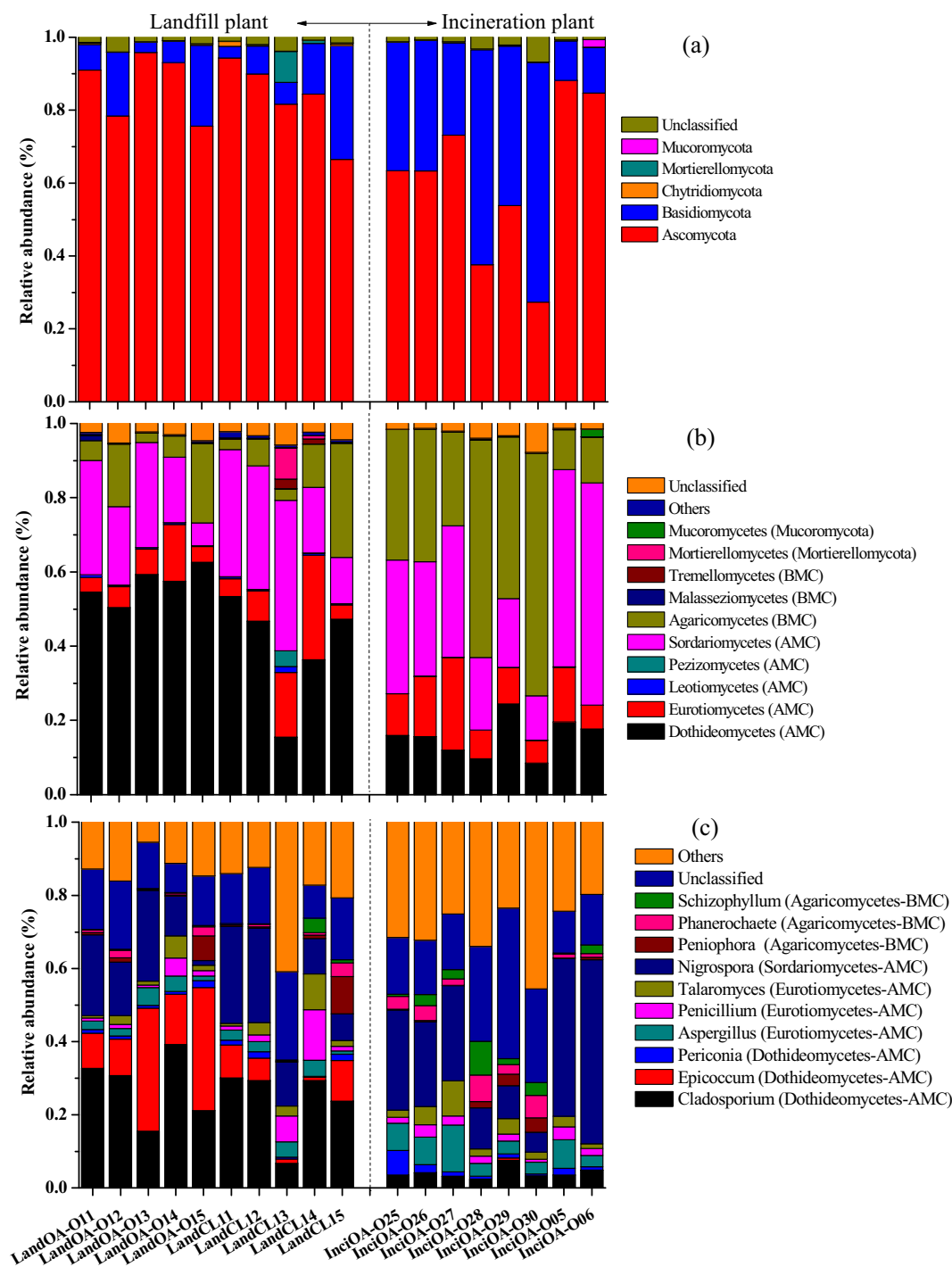


Fig. 2. Fungal aerosol population distribution: (a) phylum level; (b) class level; (c) genus level (Land represents Landfill plant; Inci represents Incineration plant; CL represents Central location; OA-O represents Office area (outdoor); 11–15 represents sampling date from 11th to 15th Oct in the landfill plant; 25–06 represents sampling date from 25th July to 06th Aug in the incineration plant).

classes might be related to the decaying process and the fermentation phase of solid waste in the treatment plants. At genus level, 243 and 218 fungal genera were recognized in the landfill and the incineration plants, respectively (Fig. 2 (c)). In the landfill plant, *Cladosporium* (7–39%), *Nigrospora* (1–26%), *Epicoccum* (1–34%) and *Penicillium* (1–14%) were the top four fungal genera. In the incineration plant, the primary genera of fungi were *Nigrospora* (5–50%), *Aspergillus* (3–13%), *Cladosporium* (2–8%) and *Talaromyces* (1–10%). *Cladosporium* and *Aspergillus* are saprotrophic fungi. The presence of *Cladosporium* has been reported in different regions (Asif et al., 2018; Wei et al., 2018), and some species of *Cladosporium* are capable of emitting odorous MVOCs. The observed high percentage of *Cladosporium* may contribute to the odors generation in the treatment plants (see more discussion in Section 3.5). Similar to this study, Huang et al. (2002) and Zhang et al. (2009) reported the highest levels of *Cladosporium* in two landfill plants in Taiwan and Beijing. *Penicillium*, which was one of the dominant genus of fungi in the landfill plant, has been recognized as important causative agents of extrinsic bronchial asthma (Maharia and Srivastava, 2014). The occurrence of *Penicillium* was believed to be associated with the decomposition process of waste as it was also recorded to be the dominant fungal taxa in composting plants (Mehdinejad et al., 2014; Wéry, 2014).

Principal Coordinate Analysis (PCoA) of the fungal populations using binary jaccard distance revealed a significant separation in fungal communities by the two plants (Fig. 3). LEfSe analysis was conducted to single out the biomarkers that differentiate the fungal community structures (Fig. S2). The primary biomarkers in the landfill plant at phylum, class and genus level were AMC, *Dothideomycetes*, *Cladosporium* and *Epicoccum*, respectively. The relative abundances of these fungal taxa were considerably higher in the landfill plant than in the incineration plant. The primary biomarkers in the incineration plant at phylum, class and genus level were BMC, *Agaricomycetes* and *Aspergillus*, respectively, which were obviously more abundant in the incineration plant compared to the landfill plant. In addition to the divergence of community structures between the two plants, FUNGuild used to taxonomically parse fungi by ecological functions also showed the difference in fungal ecological function between the two plants. In the incineration plant, most of the fungi were divided into two guilds: wood saprotroph and

plant pathogen-wood saprotroph. In the landfill plant, wood saprotroph, ectomycorrhizal and endophyte were the most frequently detected fungal trophic modes (Fig. S3). The difference between the two waste disposal plants in airborne fungal community structure and ecological function might partly be caused by the different disposed waste (e.g., with/without kitchen waste in the landfill/incineration plant) and waste treatment procedures.

3.4. Variation of airborne fungi and influencing factors

The period of July–October is generally accompanied by rainfall caused by typhoon. Thereby exploring the airborne fungal variations at such typical meteorological conditions in Guangzhou is favorable for us to understand the controlling factors of fungal aerosols. Fig. 4 (a) presented the temporal variations of airborne fungal concentrations during the landfill plant sampling period. We observed slight increase of airborne fungal concentrations on 13th and 16th October and a strong increase on 15th October. According to the meteorological classification, light rain occurred on both 13th and 16th October and moderate rain occurred on 15th October. It suggests that rainfall could promote the release of fungal spores. Similar increase of fungal aerosol concentrations on the days with light/moderate rain were observed at OA-O and LTZ sites in the incineration plant (Fig. 4 (b)). Previous studies recorded similar increase of fungal aerosol concentrations during rainy weather and rainfall was proposed to accelerate fungal growth, germination and hyphal growth (Bragoszewska, 2019; Njokuocha et al., 2018; Zuraimi et al., 2009; Prenni et al., 2013; Huffman et al., 2013). Within a dry environment, the metabolism and physiological activities of fungi were depressed while the increase of humidity can lead to the clumping of cells and elevate the odds of cell survival (Zuraimi et al., 2009; Rodríguez-Rajo et al., 2005). The release of fungal aerosols can be triggered and promoted by rain splashing and mechanical agitation. In addition, fungal aerosols may be less affected by ultraviolet radiation due to its decreasing intensity in rainy days (Heo et al., 2014; Rathnayake et al., 2017). We also observed a decrease of fungal aerosol concentrations on the day with heavy rain on 29th July (Fig. 4 (b)). Maharia and Srivastava (2014) have pointed out that continuous rain washout in monsoon season was a major factor for the decrease of fungal concentrations. The decrease of fungal concentrations during heavy rain event in present study could be attributed to the effect of wet scavenge.

Moreover, according to PCoA analysis, fungal communities from the incineration plant before the typhoon event were more similar to each other than to communities of after the typhoon event (Fig. 5). Sample α diversity metrics shows that the diversity of before-typhoon samples were significantly higher than those of after-typhoon samples (Table S1). The difference of fungal aerosols between before-typhoon and after-typhoon samples in the community structure and diversity could be attributed to the increased turnover by aerosolization, scavenging and transmission which were caused by convective instability of air currents, transformation of RH and T, torrential rain and strong wind.

3.5. Potential impact of fungal aerosols in waste disposal plants

To understand the potential health and environment impacts of fungal aerosols, we further investigated on the pathogenic/allergenic and odor MVOCs related fungi in these waste disposal plants.

From a health-effects perspective, the pathogenic/allergenic fungi in PM_{2.5} at genus level were identified in Fig. 6, making up on average ~18% and ~6% of the fungal sequences in the landfill and the incineration plants, respectively. The dominant pathogenic/allergenic genus was *Alternaria* which can trigger severe asthma symptoms (Corden and Millington, 2001; Green et al., 2003; Grewling et al., 2019) and activate the innate immune system (Gabriel et al., 2016). *Alternaria* accounts for 0.38% and 0.68% of the fungal sequences in the landfill and the incineration plants, respectively. Among the fungal genera containing

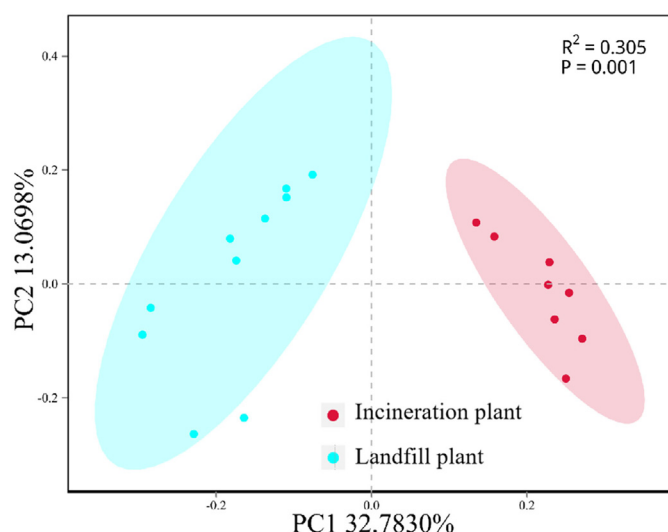


Fig. 3. Principle coordinate analysis (PCoA) of all PM_{2.5} samples in the two plants (Red point: samples of incineration plant; Blue point: samples of landfill plant). The plot was made based on binary jaccard distance matrix, the statistical analysis of the variance using PERMANOVA reflects that there were significant differences in fungal community between the landfill plant and the incineration plant samples ($P < 0.05$).

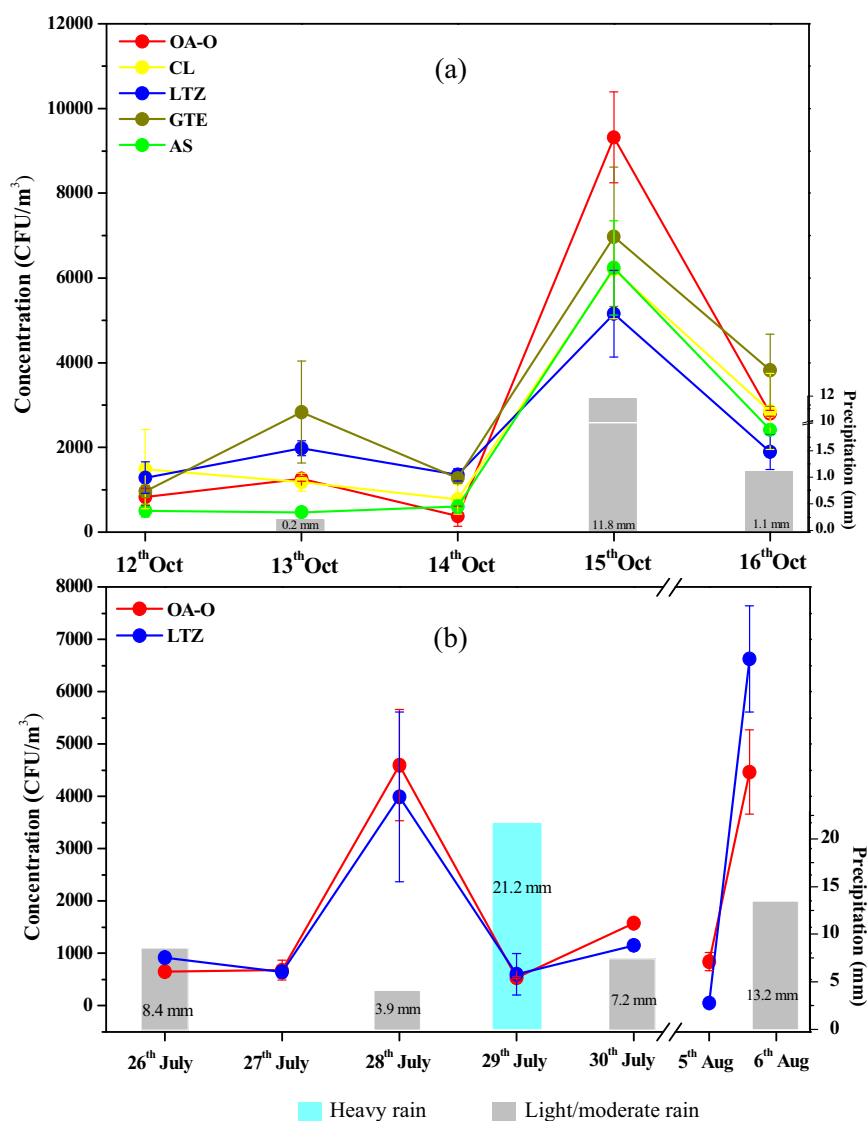


Fig. 4. Temporal variations of (a): fungal concentrations in the landfill plant; (b): fungal concentrations in the outdoor sites of incineration plant.

pathogenic/allergenic fungal species, *Epicoecum* accounts for much higher abundance in the landfill plant (12.89%) than that observed in the incineration plant (0.14%). A species of *Epicoecum* has been identified as an important aeroallergen causing respiratory disorders in 5–7% of population and shows cross-reactivity with other fungi (Bisht et al., 2002a; Dixit et al., 1992; Bisht et al., 2002b). Besides, *Trichoderma*, *Arthrimum*, *Trichothecium* and *Stachybotrys* were also recognized as pathogenic/allergenic fungi in the two plants. For example, *Stachybotrys chartarum*, whose aerosolized conidia and hyphal fragments contained mycotoxins, is of concern to human health (O’Gorman and Fuller, 2008). Although the abundances of pathogenic/allergenic fungi in PM_{2.5} were low, these fungi can still be inhaled into the respiratory system and blood along with fine and ultrafine particles when the on-site workers exposed in the waste processing areas for long time.

On the other hand, fungi can produce MVOCs in the process of decomposing complex organic materials (Matysik et al., 2009). The emitted MVOCs includes some odorous compounds which could cause unpleasant experience to on-site workers and inhabitants in the surrounding areas of the waste treatment plants. From an environment-effects perspective, identification of MVOCs generation related fungi could be of interest and valuable for the subsequent studies because

exposure to fungi-producing MVOCs was significantly high in waste disposal plants due to the extremely high microbial activities in the waste substrates (Fischer et al., 1999). *Cladosporium*, *Aspergillus*, *Penicillium*, *Fusarium* and *Trametes* were the most predominant MVOCs generation related fungal genera, totally accounting for on average ~33% in the landfill plant and ~17% in the incineration plant, respectively (Fig. 7). Proportions of MVOCs generation related fungal genera were higher in the landfill plant than the incineration plant. It supports the fact that stronger unpleasant odor could be smelled in the landfill plant rather than in the incineration plant. Among MVOCs generation related fungi, *Cladosporium*, the most abundant MVOCs related fungi in the landfill plant, can emit MVOCs such as decane, caprinaldehyde, 3-methylfuran, dimethylacetone, nonanone and methylbenzoate (Lemfack et al., 2018; Kanchiswamy et al., 2015). *Aspergillus* sp., the most dominant MVOCs generation related fungi in the incineration plant, can release MVOCs such as 1,3-benzothiazole, 2-methylbutanal, 2-methylpropanal, and benzaldehyde. The other two predominant fungi, *Penicillium* sp. and *Fusarium* sp., are capable of emitting dimethyldisulfide (Schleibinger et al., 2005) which is a widespread natural odorous compound with an unpleasant, garlic-like odor. The concentrations of dimethyldisulfide has been reported to exceed its olfactory limit value by 3600× at a composting facility, suggesting that

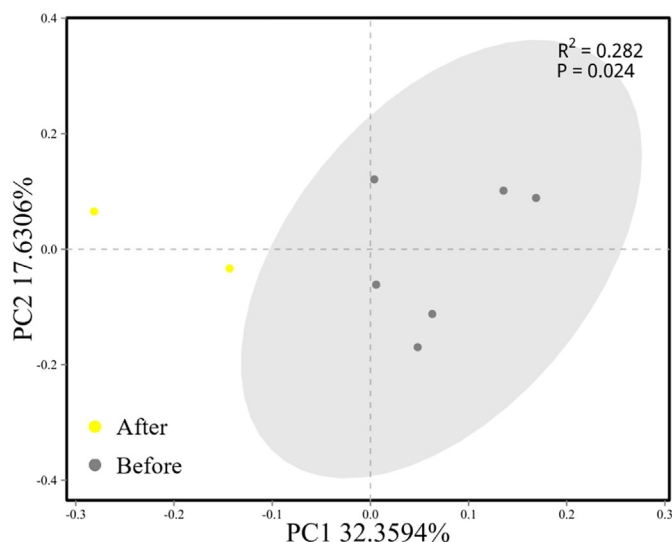


Fig. 5. Principle coordinate analysis (PCoA) of all PM_{2.5} samples in the incineration plant (Yellow point: after-typhoon samples; Gray point: before-typhoon samples). The plot was made based on binary jaccard distance matrix, the statistical analysis of the variance using PERMANOVA reflects that there were significant differences in fungal community between after-typhoon and before-typhoon samples ($P < 0.05$).

its occurrence should be attributed to waste compost (Müller et al., 2004). *Candida* sp., *Trichoderma* sp. and *Xylaria* sp., which are able to release methanethiol, 1,3-benzothiazole and 2-isocyanatopropane respectively, were also identified in both plants. The above-mentioned MVOCs associated fungal taxa in waste could be aerosolized in waste disposal processes and further transported to their vicinities and downwind areas, which undergo odor release. Besides, the odorous MVOCs derived from biodegradation of waste in the function of these fungi could spread around the plants.

4. Conclusion

The characteristics of fungal emissions and potential impacts in a landfill and an incineration plants in Guangzhou, Southern China were investigated. The results demonstrated that the fungal concentrations were comparable between the two plants. The variations of fungal aerosol concentrations among the sampling sites suggested that not only waste disposal operation but also other processes such as garbage

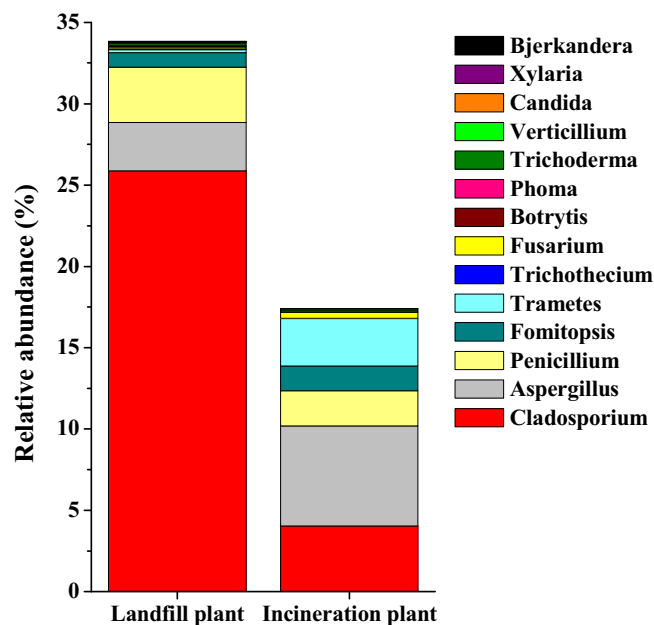


Fig. 7. Relative abundances of fungal genera associated with MVOCs generation in the two plants.

transport, air mixing and so on may contribute to the release of fungal aerosols. The penetration of fungal aerosols could be significantly prevented in indoor environment inside these fields especially with effective ventilation and cleaning. The size distributions of culturable fungal aerosols showed a unimodal distribution with a peak at the range of 2.1–3.3 μm in both plants. The size distributions suggested the important connection between fungal taxa and PM_{2.5} which should raise a concern on human health risk due to inhalation of fine particles. Temporal variations of fungal aerosols were observed and were suggested to be influenced by the precipitation and severe weather event. Light/moderate rain was found to promote the emissions of airborne fungi while heavy rain could substantially conduct wet scavenge on atmospheric fungal particles. The strong convective weather of typhoon had a significant impact on fungal diversity and community structure. The fungal community structures in PM_{2.5} were different between the two waste treatment plants, which could possibly be caused by the disparity of disposed waste and treatment procedures between the two plants. The potential impacts of fungal aerosols were studied by the investigation on pathogenic/allergenic fungi (e.g., *Alternaria*, *Epicoccum* sp. and *Stachybotrys* sp.) and odor MVOCs related fungi (e.g., *Cladosporium*, *Fusarium* sp., *Penicillium* sp. and *Candida*) in PM_{2.5}. We proposed that long-term exposure of these pathogenic/allergenic fungi could have human health risk on allergy, respiratory diseases and other chronic diseases. Furthermore, the aerosolization and transportation of odor MVOCs related fungi could contribute to the unpleasant odor in these waste treatment plants. Overall, the occurrence of fungi associated with pathogenicity/allergenicity and MVOCs in waste treatment plants pointed to the important potential impacts of human health risks and offensive odor on on-site workers and adjacent residents. Studies on the occurrence and fate of fungal aerosols in solid waste treatment plants are still expected to be performed in the future, especially to understand the cause and extent of the potential risks related to the on-site workers working in the waste treatment plants and residents living in the surrounding areas.

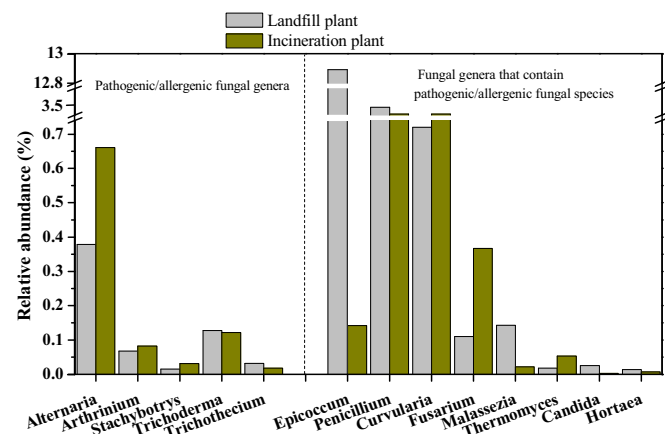


Fig. 6. Relative abundances of pathogenic/allergenic fungal taxa in the two plants.

CCRediT authorship contribution statement

Ye Liu: Conceptualization, Methodology, Software, Investigation, Data curation, Writing - original draft. **Yingyi Zhang:** Data curation,

Writing - review & editing, Validation, Formal analysis. **Yuting Shi:** Visualization, Investigation. **Fangxia Shen:** Supervision, Writing - review & editing. **Ying Yang:** Writing - review & editing. **Meijuan Wang:** Investigation. **Guangyang Zhang:** Investigation. **Tao Deng:** Resources. **Senchao Lai:** Data curation, Writing - review & editing, Funding acquisition, Project administration.

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.scitotenv.2020.142908>.

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