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RESEARCH ARTICLE

Bioaerosol emissions variations in large-scale landfill region and their health risk impacts

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HIGHLIGHTS

- The airborne bacteria in landfills were 4–50 times higher than fungi.
- Bioaerosols released from the working area would pose risk to on-site workers.
- The safe distance for the working area should be set as 80 m.

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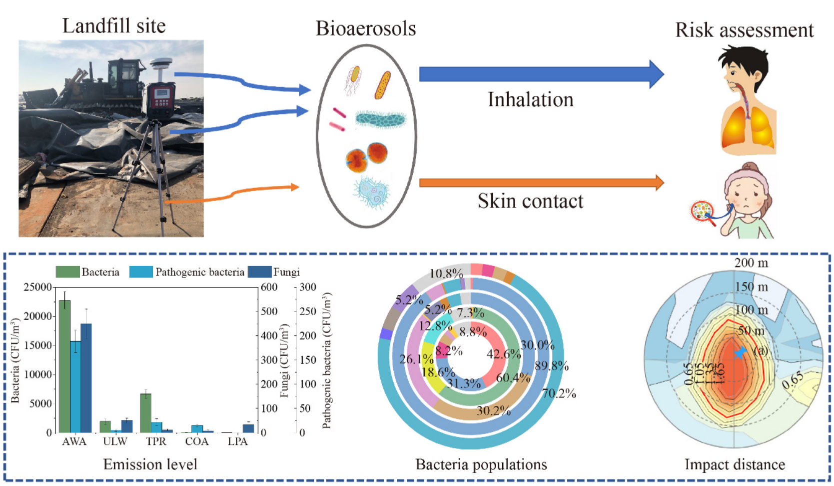
GRAPHIC ABSTRACT



ABSTRACT

Landfills are widely complained about due to the long-term odor and landfill gas emissions for local residents, while the bioaerosols are always neglected as another threat to on-site workers. In this study, bioaerosols samples were collected from the typical operation scenes in the large-scale modern landfill, and the emission levels of airborne bacteria, pathogenic species, and fungi were quantified and co-related. The corresponding exposure risks were assessed based on the average daily dose via inhalation and skin contact. It was found that the levels of culturable bacteria and fungi in all landfill samples were around 33–22778 CFU/m^3 and 8–450 CFU/m^3, and the active-working landfill area and the covered area were the maximum and minimum emission sources, respectively, meaning that the bioaerosols were mainly released from the areas related with the fresh waste operation. *Acinetobacter* sp., *Massilia* sp., *Methylobacterium-Methylorubrum* sp. and *Noviherbaspirillum* sp. were the main bacterial populations, with a percentage of 42.56%, 89.82%, 70.24% and 30.20% respectively in total bioaerosols measured. With regards to the health risk, the health risks via inhalation were the main potential risks, with four orders of magnitude higher than that of skin contact. Active-working area showed the critical point for non-carcinogenic risks, with a hazard quotient of 1.68, where 80 m protection distance is recommended for on-site worker protection, plus more careful protection measures.

1 Introduction

Landfills were widely used to dispose solid waste around

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the world, and around 242.06 million tons domestic waste were disposed in landfills in China (Duan et al., 2021). Landfill gas was released during the municipal solid waste (MSW) biodegradation from the collected pointed to the final disposal area. The odor and greenhouse gas emissions have not been reported yet. Bioaerosols were another concern target from MSW, as the associated byproducts from MSW, which might be generated during the disturbance process, such as the discarded stages, the transport process, and the landfilling (Li et al., 2021; Liu et al., 2021a).

Bioaerosols were air-suspended particles that contain microorganisms, and the main components included biological particles such as bacteria and fungi, which have been widely reported in biological treatment processes, such as wastewater treatment plants, livestock and poultry areas (Tian et al., 2020; Lim et al., 2021; Yan et al., 2021). Bioaerosol were also found in some landfill sites due to the biodegradation of MSW, especially for the unloading areas and the aeration areas for leachate, while most of their works reported were from unsanitary landfill (Uhrbrand et al., 2017; Liu et al., 2021c).

Huge pathogenic bacteria species were presented in the bioaerosol (Wu et al., 2021), including *Aspergillus* sp., *Penicillium* sp., *Acinetobacter* sp., *Pseudomonas* sp., *Enterococcus* sp., *Aeromonas* sp., and *Bacillus* sp. meaning that the exposure of bioaerosols would pose a disease risk to local residents since these bacteria in bioaerosols, such as *Aeromonas* sp., *Penicillium* sp., and *Aeromonas* sp. could cause respiratory and intestinal infections after being breathed into the human body (Thorn et al., 2002; Heinonen-Tanski et al., 2009; Madhwal et al., 2020). In addition, bioaerosols were usually attached to the surface of the particle and suspended in the air for a long time, which would transport for a long distance with the aid of wind (Jahne et al., 2015; Han et al., 2021). Skin contact and inhalation were two main approaches of exposure to bioaerosols, and the average daily dose of skin contact and inhalation was used to assess the possible health risks to humans from airborne bacteria (Reponen, 2011; Kim et al., 2018; Liu et al., 2018).

To understand the impact areas of bioaerosols for the potential risk, the CALPUFF model was widely used to simulate the distributed and impacted of health risk of bioaerosols (Liu et al., 2016; Zhang et al., 2021) and the simulation of the pollutant distributions in the atmosphere with time-varied and space-varied were necessary. As a three-dimensional unsteady Lagrange diffusion model system, it could be predicted for average-times ranging from 1 h to one year. CALPUFF's meteorological preprocessor CALMET was a meteorological model that could calculated hourly wind and temperature fields on a 3D grid modelled domain (Tartakovsky et al., 2013; Capelli and Sironi, 2018; Bezyk et al., 2021).

In this study, bioaerosol samples were collected from the typical operation areas in a modern working landfill, with the treatment capacity of 12 thousand tons waste disposed, and a container collection transport were applied for MSW transfer. High-throughput sequencing was used to determine the bacterial populations of bioaerosols in these samples firstly. The airborne bacteria health risks of skin contact and inhalation were assessed by the average daily dose, and the pathogenic bacteria inhalation health risk was assessed based on the pathogen's dose. The CALPUFF software was used to simulate the impacted distance of bioaerosols, and the potential control measures were finally proposed.

2 Materials and methods

2.1 Overview of the landfill and sample sites

Bioaerosol samples were collected from the Laogang Landfills (China), the largest landfill in Asia. MSW were transported and transferred by the container at the dock directly. Air samples were collected at the different typical landfilling process, including unloading wharf (ULW), transportation road (TPR), active working area (AWA), coverage area (COA) and leachate pool area (LPA). The AWA and ULW were both belong to operation areas, where ULW was closed to the office area. The TPR was used to detect bioaerosols emissions during waste transport. The LPA was the leachate treatment area. The COA was a soil-covered area with a ten-year landfill age. The location of the sampling sites in the landfill was shown in Fig. S1.

2.2 Sample collection methods

Field sampling was carried out on October 2021 during peak working hours (9:00–11:00 am), one of the most likely times occurring bioaerosol pollution. Bioaerosols from landfills were continuously collected using total suspended particles samplers (Laoying-2050, Qingdao, China) at a flow rate of 100 L/min, and the sampling volume was recorded after completion. The quartz membrane (Whatman QM-A, Whatman, China) in the sampler was used for sample collection. Before each sampling, membranes were treated at 500 °C for 4 h, and after drying in a desiccator for 48 h, they were weighed and stored sealed, and the collection device was cleaned with a 75% ethanol solution (AR, Macklin, China). The membranes were removed from the collection device and refrigerated for subsequent microbiological analysis when sampling was completed. Blank samples at each sampling site were also collected as the control case.

2.3 Analysis methods

2.3.1 Microbiological analysis methods

Bacteria, pathogenic bacteria and fungi were cultured in Luria-Bertani (LB) medium, MacConkey medium and

Bengal medium (BR, Shanghai Majorbio Bio-pharm Technology Co.Ltd, China) respectively (Olsen et al., 1997; de Boer, 1998; Tong and Lighthart, 1998). The bacteria and pathogenic bacteria were cultured at 30 °C for 48 h, and fungi were cultured at 25 °C for 72 h. The concentration of bioaerosol was calculated as the number of colonies formed per cubic meter of air (CFU/m^3). For convenience, the composition of LB medium, MacConkey medium and Bengal medium was described in Text S1 of Supplementary Material.

After mixing TSP filter with sterile membrane water and filtering through a 0.22 μm filter (47 mm, Titan, China), the membrane with residue was used for microbial structure analysis. The primer sets 515F and 907R were used to amplify the V3–V4 regions of the bacterial 16S rRNA gene. Each sample was amplified in triplicates (Breza-Boruta, 2016; Liu et al., 2021b). PCR products were sent to the Illumina MiSeq platform for sequencing. The primer sequences of 515F and 907R was described in Text S2 of Supplementary Material.

The QIIME program (version 1.9.1) was used to decompose and filter the original FASTQ files. Each sequence was classified according to the SILVA database using the RDP classifier (version 2.2), with a confidence threshold of 70% (Yoo et al., 2017; Li et al., 2021).

2.3.2 Bioaerosols health risks impact assessment

The 3D hourly meteorological data, including temperature, solar radiation, wind speed, relative humidity, and wind direction, was processed using the WRF (Weather Research and Forecasting) model with a resolution of 1 km from August to November within the study area (MacIntosh et al., 2010). The dimensions of horizontal simulation grids were 18000 m × 18000 m and a resolution of 100 m. Terrain elevation and land use data were used to construct 3D wind farms. Diffusion process without considered the wet-dry deposition and chemical transformation.

2.4 Risk assessment of bioaerosols

The average daily dose (ADD) for inhalation and skin contact was calculated by Eqs. (1) and (2), respectively.

$$ADD_{\text{inhalation}} = \frac{C \times IR \times EF \times ET_{\text{inhalation}}}{BW \times AT}, \tag{1}$$

$$ADD_{\text{skin}} = \frac{C \times S_{\text{A}} \times DAF \times EF \times ET_{\text{skin}}}{BW \times AT}, \tag{2}$$

where, $ADD_{\text{inhalation}}$ and ADD_{skin} were the average daily doses for inhalation and skin contact (CFU/kg/d), C was the bioaerosols concentration (CFU/m^3), IR was the inhalation rate (m^3/d), EF was the frequency for exposure (d/a), $ET_{\text{inhalation}}$ and ET_{skin} were the duration of exposure time by inhalation and skin contact (year), S_{A} was the area of skin contact (m^2), DAF was the dermal absorption factor (m/h), BW was the body weight (kg) and AT was the averaging time (d) (Li et al., 2013; Li et al., 2021).

For the pathogenic bacteria, specific formulae were used to calculate inhalation dose. The pathogen dose (PD) was evaluated based on the followed Eq. (3).

$$PD_{\text{inhalation}} = PBC \times BR \times T \times PBR, \tag{3}$$

where, $PD_{\text{inhalation}}$ was the inhalation pathogenic bacteria dose, PBC was the airborne pathogenic bacteria concentration (CFU/m^3), BR was the breathing rate (m^3/h), T was the duration of inhalation exposure of the pathogen (h), PBR was the pathogenic bacteria absorption rate. The breathing rate was set to 0.61 m^3/h, T was set to 24 h, PBR was set to 0.1 (Dungan, 2014).

The non-carcinogens risk assessment was the ratio of exposure dose to the reference dose (R_fD) to assess the human health risks of pollutants (Eqs. (4) and (5)).

$$HQ_{\text{bacteria}} = \frac{ADD}{R_f D}, \tag{4}$$

$$HQ_{\text{pathogenic}} = \frac{PD}{R_f D}, \tag{5}$$

where, HQ_{bacteria} and $HQ_{\text{pathogenic}}$ represent the bacteria hazard quotient and pathogenic bacteria hazard quotient. R_fD was the reference dose, represented the maximum acceptable daily dose. The concentration of 500 CFU/m^3 for R_fD was taken by the recommendations of the American Conference of Governmental Industrial Hygiene Experts (ACGIH) (Han et al., 2019). When $HQ < 1$, the non-carcinogens risks was relatively low. When $HQ > 1$, bioaerosols would have adverse health risks (Chen and Carter, 2017). The value of related paraments was explained in Table S1 of Supplementary Material.

3 Results and discussion

3.1 Bioaerosol emissions and populations

3.1.1 Emission levels of bioaerosols

The average concentrations of airborne bacteria and fungi were within the range of 33–22778 CFU/m^3 and 8–450 CFU/m^3, and the emission level of airborne bacteria was much higher than that of airborne fungi in landfill areas (Fig. 1).

AWA was the maximum value among all the sample sites, with the average airborne bacteria concentration of 22778 CFU/m^3, which was much higher than that detected in COA (57 CFU/m^3 on average). The concentrations of airborne bacteria detected in AWA was much higher, and 399 times higher than those of the COA. It seemed that AWA released higher concentration of airborne bacteria, compared to those landfills in

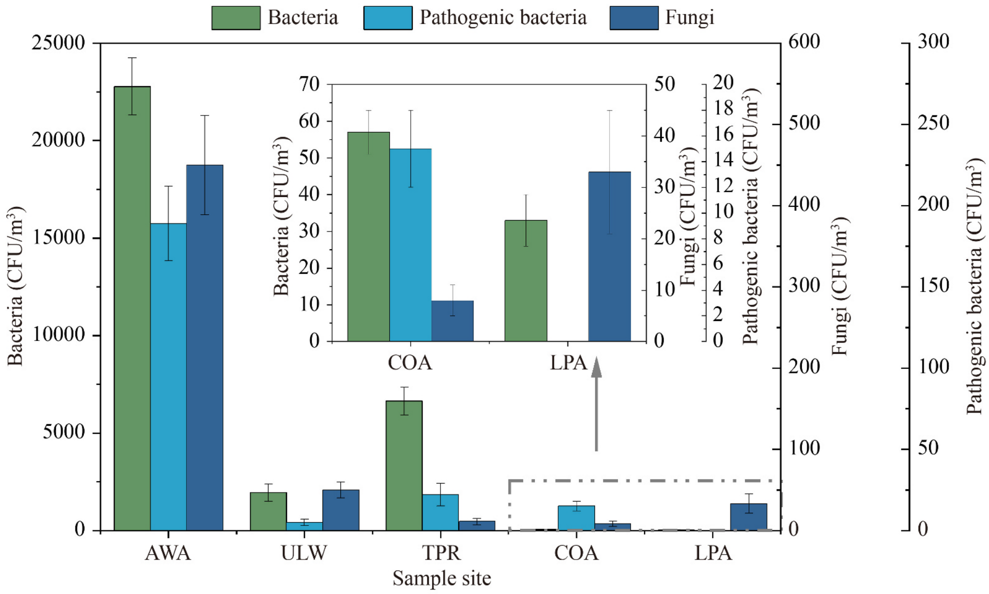

Fig. 1 Bioaerosols emission level at five sampling sites. (AWA-active working area, ULW-unloading wharf, TPR-transportation road, COA-coverage area, LPA-leachate pool area).

northern Poland and North China, with 10000 CFU/m^3 and 10883 CFU/m^3 (Breza-Boruta, 2016; Li et al., 2021). The concentration of microorganisms in the air was related to meteorological conditions, such as temperature and humidity (Tignat-Perrier et al., 2020). The average temperature and landfill operation area were different between the three cities, which may be the reason for the difference in bioaerosols concentration. Airborne bacteria in AWA was still higher than the bacterial threshold in most countries, with the ranges of 500–10000 CFU/m^3, and < 10000 CFU/m^3 was considered clean air (Rocchi and Reboux, 2022). Compared to the Chinese bioaerosol standard stipulates, all other sample sites, except for AWA, were below the threshold, i.e. bacteria of 7000 CFU/m^3 and fungi of 1000 CFU/m^3 (Zhang et al., 2020), meaning that this landfill showed a good performance, and more measures should be considered for AWA.

The concentrations of pathogenic bacteria were within the range of 0–189 CFU/m^3. The maximum value was observed in AWA, with an average pathogenic bacteria concentration of 189 CFU/m^3. LPA was not detected pathogenic bacteria exist. Pathogenic bacteria could enter the human body through breathed or skin contact, causing organ infections. Compared with other sample sites, the airborne fungi of AWA reached 450 CFU/m^3, and those of COA was 8 CFU/m^3, which was lower than those reported in landfills located in Poland and France, with the range of 112–16445 CFU/m^3 and up to 10^3 CFU/m^3 (Schlosser et al., 2016; Frączek et al., 2017). It should be pointed out that the fungi concentration exceeding 100 CFU/m^3 was considered to pose a risk to human health (Heida et al., 1995), and the AWA area was still at a higher level, since during the dumping process of fresh domestic waste, the previously compacted waste was disturbed, and the microorganisms were released into the atmosphere. The organic matter of the domestic waste in AWA increased, and the average temperature in landfill compartments was higher than 60 °C, and the landfill operation area was larger than those reported, which resulted in the higher value of the airborne bacteria concentration. In previous studies, the quantity and variety of bacteria in domestic waste were usually much larger than fungi (Gandolfi et al., 2015; Fan et al., 2019). Therefore, the concentrations of airborne bacteria were more than that of fungi in the landfill working area. In the coverage area, a large amount of soil was covered on the domestic waste surface. A large amount of soil covered makes it difficult for the microorganisms in the domestic waste to enter the atmosphere. It showed that landfills could inhibit the bioaerosols released and reduced the health hazards to the surrounding environment.

Both the concentration of airborne bacteria and fungi were 33 CFU/m^3 in LPA, lower than the airborne bacteria concentration around rotating discs in the leachate pool of 4726 CFU/m^3 (Li et al., 2011), and 6900 CFU/m^3 of airborne bacterial and 3900 CFU/m^3 of airborne fungi were detected in the wastewater treatment plants (Kowalski et al., 2017). The lower concentration of LPA resulted from the membrane-covered leachate pool, and most of the water stream and biogas were collected in the biogas collection system, which could reduce the discharge of microorganisms to the surrounding air to form bioaerosols.

3.1.2 Bacterial populations in bioaerosols

Bacterial populations in bioaerosols show that *Acinetobacter* sp., *Massilia* sp., *Methylobacterium-Methylorubrum* sp. and *Noviherbaspirillum* sp. were the dominant bacteria at five sample sites (Fig. 2).

Acinetobacter sp., *Massilia* sp. and *Pseudomonas* sp. were detected in AWA with percentages of 42.56%, 31.34% and 8.18%, respectively. *Methylobacterium-Methylorubrum* sp. (70.24%) and *Burkholderia-Caballeronia-Paraburkholderia.* (5.23%) were the main bacterial populations detected in the surrounding air of LPA. *Massilia* sp. (89.82%) and *Methylobacterium-Methylorubrum* sp. (3.58%) were the main bacterial populations detected in the surrounding air of COA. The gram-negative bacteria such as *Acinetobacter* sp., *Massilia* sp. and *Pseudomonas* sp. were accounted for about 70.91% (Wei et al., 2019), which slightly lower than our study (accounted for 82.08%), indicating that the different meteorological conditioned may be possible reason. A study reported that *Bacillus sp.* accounted for 8.01%–22.05% of airborne bacteria from a landfill in northern Poland (Breza-Boruta, 2016). *Cohnella* sp. was detected during landfill and composting, and played an important role in degraded cellulosic materials (Wushke et al., 2013).

Acinetobacter sp. and *Bacillus* sp. were common pathogenic bacteria among the detected bacterial populations (Xie et al., 2021). Madhwal et al. investigated pathogenic bacteria in bioaerosols at an open landfill in Dehradun, India, and found that the bioaerosol species composition showed that the potential pathogens were mainly *Aspergillus* sp., *Penicillium* sp., *Cladosporium* sp., *Alternaria* sp. and gram-negative bacteria (such as *Bacillus* sp., *Streptobacillus* sp.) (Madhwal et al., 2020). *Acinetobacter* sp. was gram-negative bacterial, could exist on human skin, wounds, respiratory tract and oral mucosa, and easily caused respiratory and urinary tract infections (Abo-Zed et al., 2020; Akeda, 2021). In addition, *Bacillus* sp. was a well-known genus of pathogenic bacteria that could enter the human body through human respiration, which causes infection of human organs and harms human health (Xu et al., 2019; Yang et al., 2019).

3.2 Risk assessment of bioaerosols

The risk assessment provided insight into the hazard of bioaerosols to human health. Airborne bacterial enter the human body mainly through breathed and skin contact, so the risks of inhalation and skin contact were calculated for adults and children (Fig. 3).

Pathogenic bacteria invade the human body mainly through inhalation. The results of inhalation risk of pathogenic bacteria for adults and children were shown in Fig. S2. The HQ of pathogenic bacteria via inhalation for adults and children at five sample sites were both less than 1 (Fig. S2). The health risks via inhalation were about four orders of magnitude higher than skin contact, indicating that inhalation was the main route of exposure to airborne bacteria. Except for the site of AWA, the HQ of airborne bacteria via inhalation for both adult and children were less than 1. The HQ was proportional to bioaerosols concentration. In unloading wharf and transportation road, closed carriages make it difficult for microorganisms from the domestic waste to be released into the atmosphere. Because of the existence of pathogenic bacteria, the airborne bacterial inhalation risks were nonnegligible. The HQ of adult males and adult females were 2.35 and 1.92, respectively, which were both greater than 1 at AWA, indicating that exposure to airborne bacteria could pose a non-carcinogenic risk for the adult. The calculated HQ in the working area of the landfill was also greater than 1 (Li et al., 2021). Therefore, the workers in the landfill site should take effective protective measures to reduce the harm of bioaerosols to the human body.

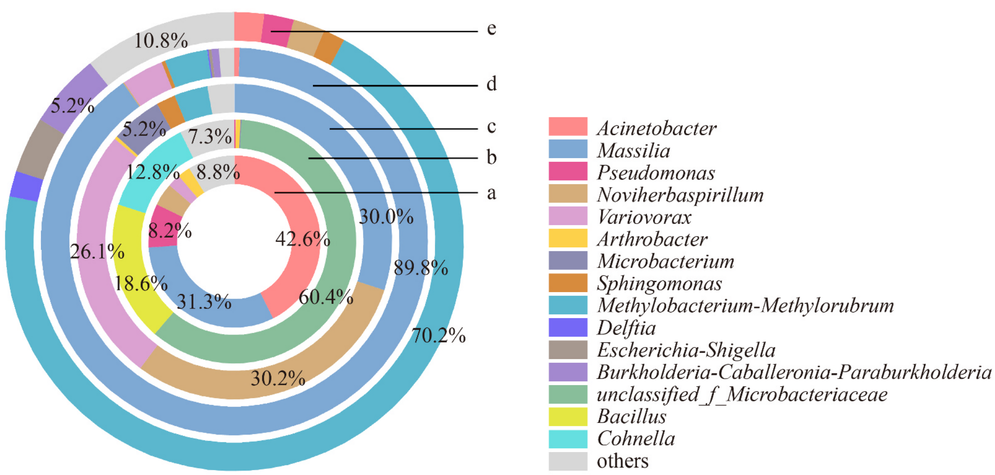

Fig. 2 Bacterial populations at five sampling sites. (a-active working area, b-unloading wharf, c-transportation road, d-coverage area, e-leachate pool area).

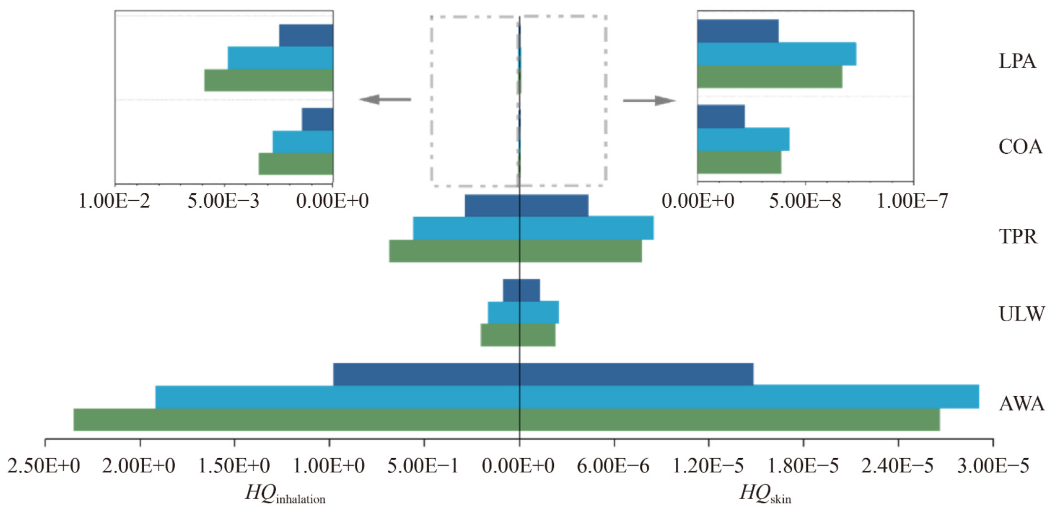

Fig. 3 *HQ* of airborne bacterial by inhalation or skin contact. (AWA-active working area, ULW-unloading wharf, TPR-transportation road, COA-coverage area, LPA-leachate pool area).

3.3 Bioaerosols health risks impact area

The HQ value was used to simulate the health risk distribution of bioaerosols through CALPUFF software (Fig. 4).

The results show that the impact range of bioaerosols health risks was centered on the sampled site and spread around. In this simulation of bioaerosols health risk distribution, the HQ value of the sample center was the highest, and the impact of bioaerosol health risks on the surrounding environment decreased as the distance increased. In the high-risk area, the ranged of HQ was within the range of 0.25–1.68, and the HQ was greater than 1 in the range of about 80 m, which indicated that the bioaerosols in this area would pose a health risk to landfill workers. The HQ values within 200 m around the medium-risk area and low-risk area were both less than 1. Because of the existence of pathogenic bacteria, the health risk of bioaerosols to the human body were nonnegligible. The HQ value was proportionated to the level of bioaerosols emission at five sample sites. The higher the level of bioaerosols emission, the higher the HQ value, which would cause greater health risks to surround workers. Therefore, within a distance of 80 m around the high-risk area, workers must effectively protect themselves from exposure to high concentrations of bioaerosols to reduce health hazards. The possible effective measures include setting up protective barriers and covering the surface of the waste with films.

4 Conclusions

The highest level of bioaerosol emission in landfills investigated was in active working area, with the average concentrations of 22778 CFU/m^3 and 450 CFU/m^3 in terms of airborne bacteria and airborne fungi. In coverage area and leachate pool area, the average concentration of airborne bacteria and airborne fungi were both very low. *Acinetobacter* sp., *Massilia* sp., *Methylobacterium-Methylorubrum* sp. and *Noviherbaspirillum* sp. were the main bacteria at each sample site. The HQ value of the sample center was the highest, and the impact of bioaerosols health risks on the surrounding environment

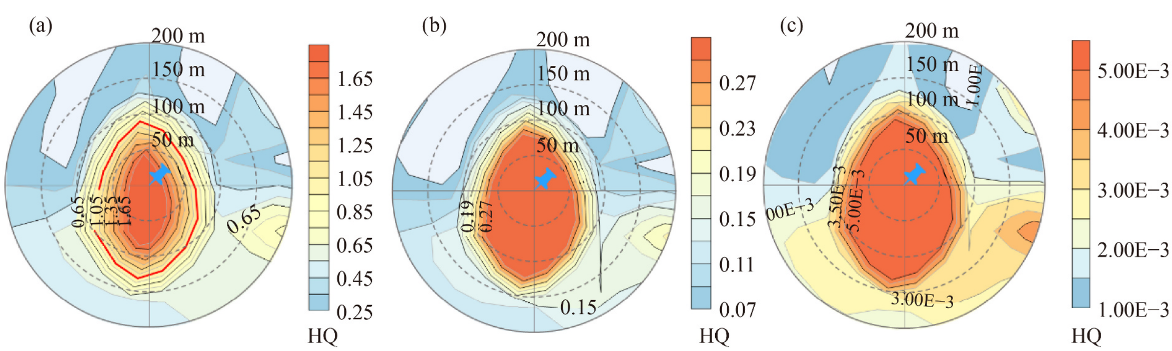

Fig. 4 Health risk distribution of bioaerosols in high-risk area (a), medium-risk area (b) and low-risk area (c).

decreased as the distance increased. For landfill employees, inhalation was the main route of exposure to bioaerosols. Long-term inhalation of bioaerosols around active working area posed a health risk to landfill workers. In the high-risk area, the HQ was greater than 1 in the range of about 80 m. Therefore, appropriate measures such as wearing protective masks must be taken to protect workers from the health hazards of high concentrations of bioaerosols.

Abbreviation List

MSW	Municipal solid waste
ULW	Unloading wharf
TPR	Transportation road
AWA	Active working area
COA	Coverage area
LPA	Leachate pool area
TSP	Total suspended particles samplers
LB	Luria-Bertani
WRF	Weather research and forecasting
PD	Pathogen dose
HQ	Hazard quotient

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CRediT Authorship Contribution Statement
Yanfeng Yang: Investigation, Writing-original draft, Writing-review & editing. **Ruina Zhang:** Methodology, Visualization. **Ziyang Lou:** Resources, Supervision.

Declaration of Interests
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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