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Bioaerosols emission characteristics from wastewater treatment aeration tanks and associated health risk exposure assessment during autumn and winter

Xiao-yan Zhao[1,2], Dong-zi An[3], Man-li Liu[4], Jia-xin Ma[1], Wajid Ali[1], Hao Zhu[5], Ming Li[5], Xiao-jun Ai[5], Zaheer Ahmad Nasir[6], Sonia Garcia Alcega[7], Frederic Coulon[6], Cheng Yan[1,2*]

[1] School of Environmental Studies, China University of Geosciences, Wuhan 430074, PR China

[2] Hubei Key Laboratory of Environmental Water Science in the Yangtze River Basin, China University of Geosciences, Wuhan 430074, PR China

[3] China Construction Eco-Environmental Group Co., Ltd. Beijing 100037, PR China

[4] Department of Hydraulic Engineering, Hubei Water Resources Technical College, Wuhan 430202, PR China

[5] POWERCHINA Hubei Electric Engineering Co., Ltd, Wuhan 430040, PR China

[6] School of Water, Energy and Environment, Cranfield University, Cranfield MK43 0AL, UK

[7] School of Physical Sciences, The Open University, Walton Hall, Milton Keynes MK6 7AA, UK

Corresponding author:

[*]Dr. Cheng Yan

School of Environmental Studies, China University of Geosciences, 388 Lumo Road, Wuhan 430074, PR China

Tel.: +86 027 67883170

Fax: +86 027 67883170

E-mail: cheng_yan@cug.edu.cn

ABSTRACT

Aeration tanks from activated sludge wastewater treatment plants (WWTPs) can release a large amount of bioaerosols that can pose health risks. However, risk characterization of bioaerosols emissions form wastewater treatment plants is currently not systematically carried out and still in its infancy. Therefore, this study investigated emission characteristic of two indicator model bioaerosols *Staphylococcus aureus* and *Escherichia coli*, emitted from aeration tanks of a municipal WWTP. Monte Carlo simulation was then used to quantitatively assess microbial risk posed by different aeration modes under optimistic and conservative estimates. Further to this, two different exposure scenarios were considered during 3 days sampling campaign in autumn and winter. Results showed that the bioaerosol concentration from microporous aeration tank (20–262 CFU m^{-3}) was one order of magnitude lower than rotating disc aeration tank. Average aerosolization rate was 7.5 times higher with mechanical aeration mode. Health risks of exposed populations were 0.4 and 9.6 times higher in winter than in autumn for *E. coli* and *S. aureus* bioaerosol, respectively. Health risks of staff members were 10 times higher than academic visitors. Interesting results were observed for academic visitors without personal protective equipment (PPE) respectively exposed to *S. aureus* and *E. coli* bioaerosol in autumn and winter: while the derived infection risk met the United States Environmental Protection Agency (U.S. EPA) benchmark under optimistic estimation, the disease risk burden was over the World Health Organization (WHO) benchmark under conservative estimation. These revealed that only satisfying one of the two benchmarks didn't mean absolute

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acceptable health risk. This study could facilitate the development of better understanding of bioaerosol quantitative assessment of risk characterizations and corresponding appropriate risk control strategies for wastewater utilities.

Keywords: Quantitative microbial risk assessment; Annual infection risk; Disease burden; Aerosolization ratio; Wastewater treatment plants; Bioaerosols

1 Introduction

Wastewater treatment plants (WWTPs) can emit significant quantities of bioaerosols, which may affect the health of staff members (Lebrero et al., 2011; Niazi et al., 2015; Xu et al., 2020). For example, Li et al. (2016) reported that WWTP staff members are more likely to suffer from lung function impairment and respiratory symptoms than other staff members. These diseases are collectively referred to as "sewage worker's syndrome" (Fracchia et al., 2006). Accordingly, a great deal of research on bioaerosols emissions from WWTPs has been conducted over the last decade (Orsini et al., 2002; Wang et al., 2018; Zhang et al., 2018; Singh et al., 2021). One of the major sources of bioaerosol emissions in WWTPs is the aeration tank (Sánchez-Monedero et al., 2008; Li et al., 2016). There are some correlations between the WWTP design and the emission of bioaerosols. Mechanical and blast aeration modes are the most two common design for aeration modes that equipped WWTP in China, which typical representatives are rotating disc and microporous aeration tanks, respectively (Sánchez-Monedero et al., 2008; Li et al., 2016). Studies have shown that the intense mixing and turbulence during the operation of the aeration tanks cause splashing, bubble bursting and spraying, all contributing to the emission of bioaerosols (Fracchia et al., 2006; Burdsall et al., 2021). The mechanical aeration mode usually produces higher bioaerosol emissions than blast aeration mode as the mechanical agitation is more likely to cause the migration of microorganisms from wastewater to the air (Korzeniewska, 2011; Han et al., 2019; Burdsall et al., 2021).

Therefore, studying the emission characteristics of the wastewater treatment process

and assessing the risk posed by the release of bioaerosols are important for health risk control. Airborne *S. aureu*s and *E. coli* are common microbial pathogens found in municipal sewage (Keisuke, 2012). These two inhaled airborne pathogens can deposit in upper respiratory tract and then be swallowed into stomach which can lead to gastrointestinal diseases, such as vomiting, stomach cramps, and diarrhea (Fuhrimann et al., 2016; Nag et al., 2021). They are usually used as indicators and model bioaerosols for risk assessment and quantification, often termed as quantitative microbial risk assessment (QMRA) (Carducci et al., 2016; Kowalski et al., 2017; Kozajda et al., 2019). QRMA is a model framework for evaluating the health risks caused by specific pathogenic microorganisms (Hamilton and Haas, 2016; Carducci et al., 2016). Classical QMRA consists of four basic steps (National Research Council, 1983; Brooks et al., 2012; Carducci et al., 2016), but determining the dose-response is the most important step to describe the indicator bioaerosol for risk assessment (Haas, 2015). Dose-response model is a presumptive mathematical method employed to generate equations that describe the infection probability as a function of exposure dose (La et al., 2021). Dose-response model quantify the relationship between dose and infection probability, which is broadly used for QMRA (Stellacci et al., 2011; Blanky et al., 2017). The beta-Poisson and exponential dose-response model have been well studied and widely used to characterize infectivity of various airborne bacterial pathogens (Schmidt et al., 2013; Xie et al., 2017). Howerer, there are some limitations for these two dose-response models. For the exponential dose-response model, it is assumed that pathogenic bacterial organisms which land in an appropriate place can cause infection and have

equal chance of independent survival; for the beta-Poisson dose-response model, it is assumed that the probabilities of pathogenic bacterial survival and infection are non-constant, and the probability of survival follows beta distribution (Haas, 2002; Haas, 2015). Monte Carlo simulation is used to build a probabilistic-based risk model for estimating the inherent uncertainty and variability caused by environmental parameters (i.e. temperature, relative humidity, and illumination) of risk assessment; accordingly, the range and likelihood of the risk can be assessed quantitatively (Jahne et al., 2015; Lim et al., 2015). In addition, two widely used health benchmarks are applied to interpret the severity of the risk assessment. These are acceptable annual infection risk level ($\leq 10^{-4}$ per person per year [pppy]) proposed by the U.S. EPA (2005) and the acceptable disease burden ($\leq 10^{-6}$ disability-adjusted life years (DALYs) pppy) by WHO (Haas et al., 1999; Lim et al., 2015; Shi et al., 2018). The health risk is unsatisfied and intolerable when the risk level is beyond the corresponding benchmark; while below the benchmark means opposite.

The studies on the health risk assessment of bioaerosols are fragmentary. Korzeniewska (2011) showed that the type of the aeration system significantly influences the emission degree of bioaerosols: the fungal aerosol concentration produced by mechanical aeration mode was 5.8 times higher than the blast aeration mode. Lou et al. (2020) and Burdsall et al. (2021) exhibited that the staff members of WWTPs are exposed to these bioaerosols through inhalation, skin, or mucosal contact; among them, the inhalation is the main route of infection for staff members. Aghaei et al. (2020) also demonstrated that bioaerosols in WWTPs, when inhaled and deposited

in the lungs, can cause respiratory and gastrointestinal diseases. Previous studies have shown that increased aeration intensity during the autumn and winter seasosns to overcome low biological activities due to low temperature can have substantial impact on bioaerosol emissions from aeration tanks (Wang et al., 2019; Xing et al., 2021). However, the literature on methodical risk assessment associated with the intrinsic indeterminacy and changeability of bioaerosols in various seasons is limited (Dungan, 2014; Haas, 2015). The effects of aeration mode on the bioaerosol emission characteristics and related methodical risk assessment have not received sufficient attention (Xu et al., 2020). Understanding how bioaerosol emission characteristics quantitatively vary can be useful to better evaluate the health risk posed by bioaerosol emissions. In addition, the lack of study to weigh the risk characterization with personal protective equipment (PPE) hinders the popularity of wearing PPE (Haas et al., 2014).

This study systematically analyzed the concentration, aerosolization ratio, and size distribution of two kinds of airborne bacteria, including *S. aureus* and *E. coli*, as indicator bioaerosols emitted from rotating disc and microporous aeration tanks. QMRA was used to quantitatively estimate the risk characterizations of the exposed populations (staff members and academic visitors). The annual infection risk and disease burden of the exposed populations for various exposure scenarios in autumn and winter were estimated by using Monte Carlo simulation to explain how the intrinsic indeterminacy of bioaerosols emission influenced the risk characterization, and the conservative and optimistic exposure estimations.

2 Method and materials

2.1 Description of the wastewater treatment plant

Bioaerosol sampling was performed at a municipal WWTP located in Hubei province, central China with a treatment capacity of 100,000 m^3 wastewater day^{-1} (650,000 People Equivalent [PE]). The influent sewage was typical domestic wastewater from urban residents. The design scale of the first phase was 50,000 m^3 wastewater day^{-1} (325,000 PE). It was equipped with a rotating disc aeration tank and adopted with DE oxidation ditch treatment process. The design scale of the second phase was 50,000 m^3 wastewater day^{-1} (325,000 PE). It was equipped with a microporous aeration tank and adopted with Anaerobic-Anoxic-Oxic process. The remaining treatment units of these two phases were the same (primary/secondary sedimentation tank) or shared (grillage machine and inlet pumping station). The surrounding ambience of WWTP was undisturbed. It was adjacent to a local vocational-technical school and surrounded on three sides by school buildings. The front of the WWTP was a vacancy area.

2.2 Sampling campaign

An Andersen six-stage cascade impactor (FA-3, Hongchangxin Inc., Beijing, China) was used to sample bioaerosols emission from the inverted umbrella and microporous aeration tanks in autumn and winter (19 October, 30 November, and 16 December

2020). Sampling sites are shown in Fig. S1 in the Supplement Material. The ambient atmospheric conditions and specific time during sampling campaigns are provided in Table S1 in the Supplement Material. The impactor was loaded with 6 Petri dishes containing. Mac Conkey agar medium was for *E. coli* and Egg-yolk mannitol salt agar medium was for *S. aureus* (Grisoli et al., 2009; Grzyb and Lenart-Boron, 2019).

The sampling sites were located in the middle of the centre corridor of the second microporous aeration tank and the first rotating disc aeration tank from north to south (Fig. S1). The inlet port of the cascade impactor (flow rate 28.3 L min^{-1}) was 1.5 m above the ground of the middle corridor of each aeration tank (Szyłak-Szydłowski et al., 2016). Following the standard procedure in the literature, the sampling time was 20 min (for *E. coli* bioaerosol) or 5 min (for *S. aureus* bioaerosol) (Hung et al., 2010; Kowalski et al., 2017). The impactor was disinfected with 75% ethanol before and after each sampling to prevent contamination. All samplings were done in triplicate. In order to analyse the microorganism concentration in wastewater for calculating the aerosolization ratio, the 500 ml wastewater samples were took by a sterility water sampling bottle in site. The wastewater samples were done in duplicate.

2.3 Laboratory analysis

Collected samples were transported to the lab immediately in the cold storage box. The Petri dishes were incubated at 37 °C for 24 h following the standard procedure (Bragoszewska and Biedroń, 2018). Bioaerosol colonies were counted using an

automatic colony counter (ICOUNT). Standard procedures were followed according to the manual of the automatic colony counter. Positive-hole corrections were applied and the results were expressed in CFU m^{-3} (Kowalski et al., 2017).

2.4 Quantitative health risk assessment

[Table 1 inserts here]

[Table 3 inserts here]

The classical quantitative health risk assessment approach was used in this study. For the QMRA, quantification of the emission of aerosolized *E. coli* and *S. aureus* from the rotating disc and the microporous aeration tanks were used. The staff members inspecting the two aeration tanks and academic visitors carrying out the sampling were considered as the two main exposed populations. The details of their exposure scenarios are shown in Table 1. In addition, according to the issued standard Technical Requirements for Medical Surgical Masks, the bacterial filtering efficiency of masks should not be less than 95% (Beijing Institute of Medical Device Testing, 2011). Therefore, in this study, it was assumed that the retention rate of bioaerosols were 95% when wearing PPE (i.e. mask) and 0% when not wearing PPE to calculate the exposure dose with or without PPE.

The QMRA calculation parameters are shown in Tables S2 and 3. The exponential and beta-Poisson dose-response model was used as the dose-infection model for *S.*

aureus and *E. coli* bioaerosol, respectively (Shi et al., 2018; Esfahanian et al., 2019). Monte Carlo simulation was conducted to estimate the probability distribution of health risks for quantitatively assessing the range and likelihood of the risks (Shi et al., 2018). The details of classical QMRA process and Monte Carlo simulation are shown in Supplement Material. There were currently no local or national standards of risk benchmark for the health risk assessment in China. Therefore, the U.S. EPA and WHO benchmark approaches were used in this study.

2.5 Aerosolization ratio

The summation of the bioaerosol concentrations for the cascade impactor is the cumulative concentration used for calculating the aerosolization ratio.

The cumulative concentration is calculated using Eq. (1):

$$C' = \sum_{1}^{6} C_{pi} \tag{1}$$

where *C'* is the airborne cumulative concentration of *S. aureus* or *E. coli* (CFU m^{-3} air) (Table 4), and C_{pi} is the bioaerosol concentration of *i* stage of Anderson six-stage impactor (CFU m^{-3}) (Table 3).

The microorganism concentration in wastewater is calculated using Eq. (2):

$$C'_{W} = \frac{\sum_{1}^{3}\left(C_{Wi}N_{i}\right)}{3} \times 10^{6} \tag{2}$$

where C_{W}' is the *S. aureus* or *E. coli* concentration in wastewater (CFU m^{-3} wastewater) (Table 4), and C_{Wi} is the number of colonies after dilution (CFU ml^{-1}) , and N_i is diluted multiples (10^3, 10^4 and 10^5).

The aerosolization ratio (CFU m^{-3} air / CFU m^{-3} wastewater) is calculated by

dividing the microorganism concentration in wastewater into the airborne cumulative concentration (Bauer et al., 2002).

2.6 Statistical analyses

All statistical analyses were performed using SPSS software (SPSS 26). Multiple comparisons of the average bioaerosol concentrations for the three sampling days was determined by analysis of variance (ANOVA) followed by Duncan's multiple range tests to determine the significance ($P=0.05$).

3 Results and discussion

[Table 2 inserts here]

[Table 3 inserts here]

[Table 4 inserts here]

[Fig. 1 inserts here]

3.1 Emission characteristics

The average concentrations of aerosolized *S. aureus* and *E. coli* during the sampling campaigns are shown in Table 2. The bioaerosol concentrations from the microporous aeration tank were not significantly different ($P>0.05$); in contrast, the bioaerosol emissions from the rotating disc aeration tank were significantly different ($P<0.05$).

This result indicated that the release of bioaerosols in the rotating disc aeration tank was more susceptible to external factors than that in the microporous aeration tank.

Table 3 shows the average concentrations of aerosolized *S. aureus* and *E. coli* under various particle size distribution ranges from the two aeration modes based on the samplings of the cascade impactor. The airborne cumulative concentrations of bioaerosol are showed in the Table 4.

For autumn and winter, the concentrations of *S. aureus* and *E. coli* bioaerosols in the microporous aeration tank (blast aeration mode) (*S. aureus*: 1678 CFU m^{-3} in autumn and 1104 CFU m^{-3} in winter; *E. coli*: 78 CFU m^{-3} in autumn and 118 CFU m^{-3} in winter) were lower by one order of magnitude than those in the rotating disc aeration tank (mechanical aeration mode) (*S. aureus*: 262 CFU m^{-3} in autumn and 228 CFU m^{-3} in winter; *E. coli*: 22 CFU m^{-3} in autumn and 20 CFU m^{-3} in winter) (Table 4). Many studies have come up with similar findings: the effect of mechanical aeration mode on the production of bioaerosols was more noteworthy than that of blast aeration mode (Brandi et al., 2000; Sánchez-Monedero et al., 2008; Singh et al., 2021). In general, the aeration mode highly affected the bioaerosol emissions (Filipkowska et al., 2000; Korzeniewska, 2011). In mechanical aeration mode, the rotating disk rotation can intensely disturb the water body and send wastewater flying into the air to become droplets. Then, the splashing wastewater droplets procedure a large number of bioaerosols after aerosolization during this disturbance (Fracchia et al., 2006; Wang et al., 2019). However, the microporous aeration mode elevated aeration efficiency and facilitated mixing of the wastewater through the creation of fine/medium-sized bubbles

by mild blast aeration. These bubbles broke into small fragments or droplets when they reached the wastewater surface, and then, the bacteria in the wastewater were dispersed from the liquid interface to the air interface to form bioaerosols (Han et al., 2019; Yang et al., 2019; Han et al., 2020).

Table 4 displays the mean aerosolization ratio values, airborne concentration, and concentration in wastewater of *S. aureus* and *E. coli* from the two aeration modes. For *S. aureus* bioaerosols, the aerosolization ratio in winter was 2.2 times higher than that in autumn, while the aerosolization ratio of *E. coli* bioaerosols in autumn was 8.9 times higher than that in winter. The aerosolization ratio in the rotating disc aeration tank was 5.4 and 11.6 times higher for *S. aureus* and *E. coli,* respectively, than that in microporous aeration tank. This finding indicated that microorganism in wastewater was more likely to be aerosolized under mechanical aeration mode (Sánchez-Monedero et al., 2008). Overall, this provides a novel perspective which can expediently visualize the bioaerosol generating capacity of aeration model by calculating aerosolization ratio without labor-intensive sampling work onsite.

For the two aeration modes, the concentrations in autumn of *S. aureus* were in the same order of magnitude as those in winter under all of the size distribution ranges, except for the size distribution range of 0.65 – 1.1 μm (stage 6) for the rotating disc aeration tank (Table 3). However, *S. aureus* aerosolized concentrations in winter were always lower than those in autumn under the size distribution ranges of 2.1 – 3.3 μm (stage 4), 1.1 – 2.1 μm (stage 5), and 0.65 – 1.1 μm (stage 6) in the two aeration modes. This finding indicated that less fine bioaerosol particles (0.65 – 3.3 μm in stage 4 – 6)

were detected in winter (Fig. 1a). The fine bioaerosol particles in autumn were 2.2 – 5 (in the rotating disc aeration tank) or 1.1 – 2.1 (in the microporous aeration tank) times higher than those in winter (Table 3). This result should be related to the ambient atmospheric conditions. According to Table S1 in the Supplement Material, the relative humidity in winter was higher than that in autumn, while the temperature and illumination were lower. In general, the relative humidity was highly important for the survival of bioaerosols in different seasons (Karra and Katsivela, 2007; Kumar et al., 2020; Xing et al., 2021). Bioaerosols generated from wastewater source were usually formed with a thin layer of moisture surrounding the airborne microorganisms and consisted of aggregates of several other microorganisms (Kumar et al., 2020; Xing et al., 2021). These bioaerosol aggregates were exposed to a higher relative humidity in winter (Table S1) and then tended to indicate increased water sorption, which can provide protection to airborne microorganisms against adverse external influence, e.g. UV-induced inactivation (Peccia and Hernandez, 2001; Peccia et al., 2001; Reinthaler et al., 2003; Karra and Katsivela, 2007). Therefore, single fine bioaerosol microorganisms were enmeshed or attached to each other to make larger units of aggregated bioaerosol particles in winter (Millner, 2009; Vestlund et al., 2014). As a result, less fine bioaerosol particles were identified in winter. The study of Han et al. (2019) also discovered that the size of bioaerosol particles emitted in spring and summer were larger than in autumn and winter. And the bioaerosol concentrations in warm seasons (spring and summer) were generally higher than those in cold seasons (autumn and winter) (Kozajda et al., 2020; Talepour et al., 2020).

The higher concentrations of aerosolized *E. coli* from the two aeration modes were under the size distribution ranges of 3.3 – 4.7 μm (stage 3), 2.1 – 3.3 μm (stage 4), 1.1 – 2.1 μm (stage 5), and 0.65 – 1.1 μm (stage 6) (Fig. 1b). According to Kowalski et al. (2017), bioaerosol particles with an aerodynamic diameter size smaller than 4.7 μm (ranging from stage 3 to stage 6) were classified as respirable particles. The small size of these particles meant that they could enter the lungs easily if inhaled and be highly threatening to the upper respiratory tract (nasal passage and bronchioles) of the exposed populations (Korzeniewska, 2011; Qiu, 2012). In general, the bioaerosol concentrations were higher in winter than in autumn, except for the fine particles (size distribution range of 1.1 – 2.1 μm (stage 5) or 0.65 – 1.1 μm (stage 6)). This result can be explained by the related meteorological factors that the relative humidity in winter was higher but the illumination and temperature were lower than those in autumn (Table S1). Law et al. (2001) reported that the survival of bacteria in air was greatly affected by relative humidity and higher relative humidity led to higher concentrations. The study of Stellacci et al. (2011) also showed that low temperature, high humidity, and low illumination tended to favor airborne microorganism survival. In general, the environment in winter was more suitable for the survival of *E. coli* bioaerosol from the two aeration modes due to higher humidity and lower temperature and illumination.

In this study, it was assumed that the ambient air from surrounding buildings/infrastructures (mainly schools) did not influence the level of bioaerosols measured from the WWTP and therefore the influence of exogenetic aerosol pollutants including transfer of airborne pathogens from the surroundings was disregarded in this

study.

[Fig. 2 inserts here]

[Fig. 3 inserts here]

3.2 Health risks

Figs. 2 and 3 present the health risks (annual infection risk and disease burden) from the Monte Carlo exposed to bioaerosols in the WWTP. For each exposure scenario, the health risks of females were permanently lower than those of males, but they were still in the same order of magnitude. This was because of the lower inhaled breathing rate of females (Table S2), which can highly affect the health risks (Brooks et al., 2012). Therefore, the exposure concentration of females, which was calculated by inhaled breathing rate and exposure time in Supplement Material Eq. (2), was lower than that of males under the same exposure time. The lower health risks of females were then related with this descending exposure concentration (Brooks et al., 2005; Shi et al., 2018).

Since the staff members' exposure time was considerably longer (Table 1), their health risks were 10 times higher than those of the academic visitors. Considerable relevant literature confirmed a tendency that the health risks increased with the progress in exposure time (Carducci et al., 2016; Carducci et al., 2018). The health risks of using PPE for all the exposed populations (academic visitors and staff members) were

reduced by one order of magnitude compared with those of the exposure scenario without PPE (Figs. 2 and 3). PPE was responsible for reducing direct contact with pathogens and the inhaled dose and thus the health risks (Nascimento et al., 2020).

The health risks in winter for all exposure scenarios (Table 1) discussed above were always between 1 and 2 orders of magnitude higher than those in autumn (Figs. 2 and 3). However, this result of the health risks was contrary to the findings of *S. aureus* bioaerosol concentrations between these two seasons (Table 4). This was due to that the deposition coefficient, rather than the cumulative concentration (Table 4), was considered when calculating the inhaled bioaerosol dose in the health risk quantitative assessment framework (Table S2 and Eq. (1) in the Supplement Material). In addition, the health risk of the exposed population was correlated with the aerosolization rate. Although the health risks were influenced by many factors, the higher the aerosolization rate, the higher the dose and the higher the health risks associated with the same exposure scenario (Havelaar et al., 2012; Shi et al., 2018; Esfahanian et al., 2019).

3.2.1 Health risks for S. aureus bioaerosol

The health risks (annual infection risk and disease burden) of the academic visitors in winter without PPE and staff members in autumn and winter without PPE exposed to *S. aureus* bioaerosol were between 1 and 2 orders of magnitude respectively higher than the U.S. EPA benchmark ($\leq 10^{-4}$ pppy) and WHO benchmark ($\leq 10^{-6}$ DALYs pppy). However, after wearing PPE, these health risks values were reduced by 1 – 2 orders of magnitude, and most of them were lower than the benchmark values (Fig. 2). Wearing PPE allowed to reduce inhalation dose and therefore reducing the health risk of the

academic visitors from unacceptable to a level below of the benchmark. Similar results were reported by Heinonen-Tanski et al. (2009) and Teixeira et al. (2013) which both emphasized that the use of PPE was essential for reducing the health risk of exposing to bioaerosol. Although the U.S. EPA and WHO benchmarks used reflect infection cases, caution should be taken in extrapolating this information as the infection cases may not results in an increase health outcome. That means even if the health risks of exposed populations surpass the corresponding benchmark, the risks do not necessarily translate to higher health outcome.

The health infection risk of the staff members with PPE in winter generally satisfied the U.S. EPA benchmark, but it unsatisfied this benchmark under the conservative estimation (Fig. 2a). This finding displayed that constant vigilance was still necessary when working in the WWTPs even if wearing PPE for the worst case scenario. On the other hand, the health risk burden of the academic visitors without PPE in autumn was generally above the WHO benchmark, but it turned to satisfy the benchmark under optimistic estimation (Fig. 2b). This presented that the health risks might still be acceptable in the best scenario for certain exposure scenarios even if no PPE was worn.

For the academic visitors without PPE exposed to *S. aureus* bioaerosol in autumn, an interesting result was observed: their health infection risk satisfied the U.S. EPA benchmark (Fig. 2a), while their health risk burden exceeded the WHO benchmark under conservative estimation (Fig. 2b). Similarly, the health infection risk of the staff members with PPE exposed to *S. aureus* bioaerosol in winter met the U.S. EPA benchmark value under the optimistic estimation (Fig. 2a), but their health burden risk

unsatisfied the WHO benchmark value (Fig. 2b). The two pairs of results indicated that satisfying one benchmark didn't ensure absolute security or mean acceptable health risk (Teixeira et al., 2013; Haas, 2015). Thus, the health risks of exposed populations should not be easily dismissed only based on satisfying one benchmark.

3.2.2 Health risks for E. coli bioaerosol

For all exposure scenarios to *E. coli* bioaerosol, the health risks of academic visitors were one order of magnitude lower than those of staff members (Fig. 3). Similar to that of *S. aureus* bioaerosol, the reason was that the exposure duration of the academic visitors was lower (Tables 1 and S2). Although the health risks in winter for all exposure scenarios were always 0.4 times higher than those in autumn, they were all at the same order of magnitude (Fig. 3). This finding might be due to that the concentration of *E. coli* bioaerosol in the rotating disc aeration tank in autumn was 0.7 times lower than that in winter with the same other parameters (Tables S2 and 4). In the exposure scenarios without PPE, the health risks of staff members were ranging between 1.8 – 22.6 times and 10.6 – 129.6 times higher than the U.S. EPA benchmark ($\leq 10^{-4}$ pppy) and WHO benchmark ($\leq 10^{-6}$ DALYs pppy), respectively (Fig. 3). Meanwhile, the health risk burden of academic visitors without PPE was above the WHO benchmark. However, the health risks of exposed populations with PPE were reduced by one order of magnitude. The use of PPE could effectively reduce the health risks of exposed populations (Yang et al., 2019).

However, when wearing PPE was considered, the health risk burden of staff members still exceeded the WHO benchmark (Fig. 3b), even though the rest exposure scenarios

satisfied the benchmark. This finding showed that the health risks of the staff members should not be ignored even in the exposure scenario of wearing PPE. PPE did not mean absolute security; thus, risk prevention should still be considered (Majchrzycka et al., 2016; Kozajda et al., 2019). Moreover, the health risk burden of the academic visitors without PPE in winter exceeded WHO benchmark, while their health infection risk met the U.S. EPA benchmark under optimistic estimation (Fig. 3a). This result indicated that the absence of PPE did not certainly equate to an unacceptable risk in the best scenario for certain exposure scenarios (Shi et al., 2018).

4 Conclusion

The concentration and aerosolization rate of *S. aureus* and *E. coli* from the mechanical aeration mode were higher than those from the blast aeration mode. The bioaerosol concentrations were also higher in winter than in autumn, except for the size distribution range of 1.1 – 2.1 μm or 0.65 – 1.1 μm. Our study further demonstrated that infection risk and disease burden may not satisfy the corresponding benchmark at the same time. Therefore, satisfying one of the two benchmarks may not always ensure absolute safety or be indicative of acceptable health risk. Nevertheless, wearing PPE can significantly reduce the health risks, and this can be up to one order of magnitude.

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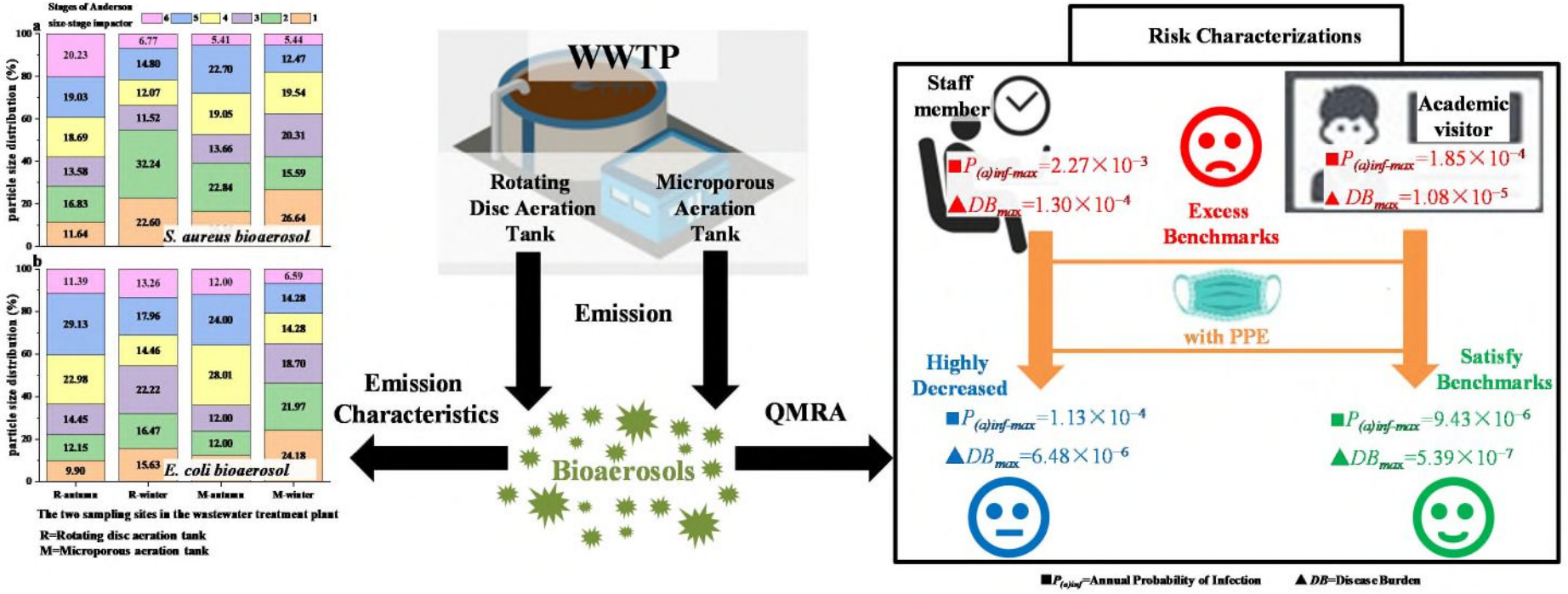
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Graphical abstract



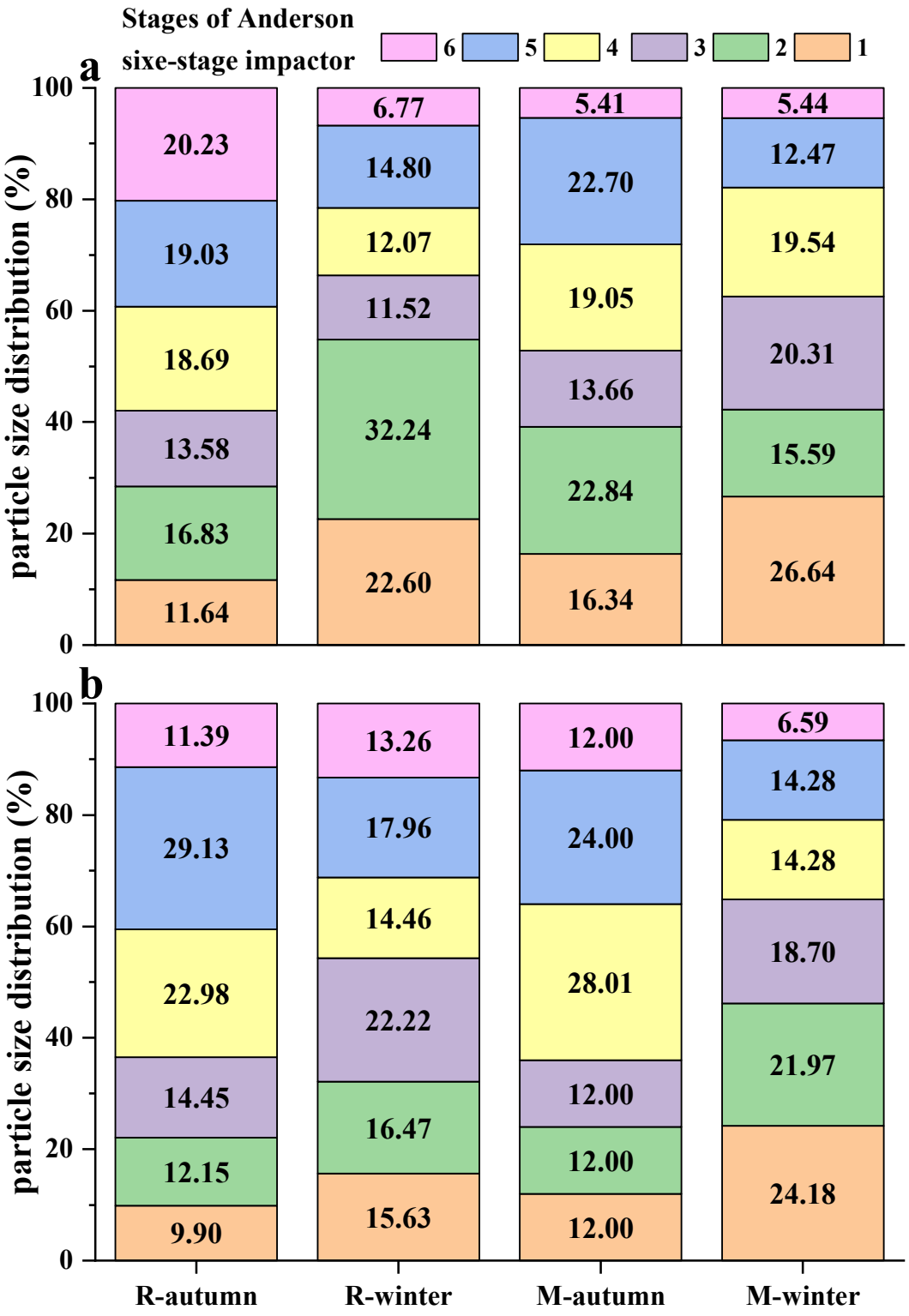


Fig. 1 Proportion of average size distribution of (a) *Staphylococcus aureus* and (b) *Escherichia coli* bioaerosol particles during sampling campaign in two seasons for the two aeration modes of wastewater treatment plant.

R=Rotating disc aeration tank.

M=Microporous aeration tank.

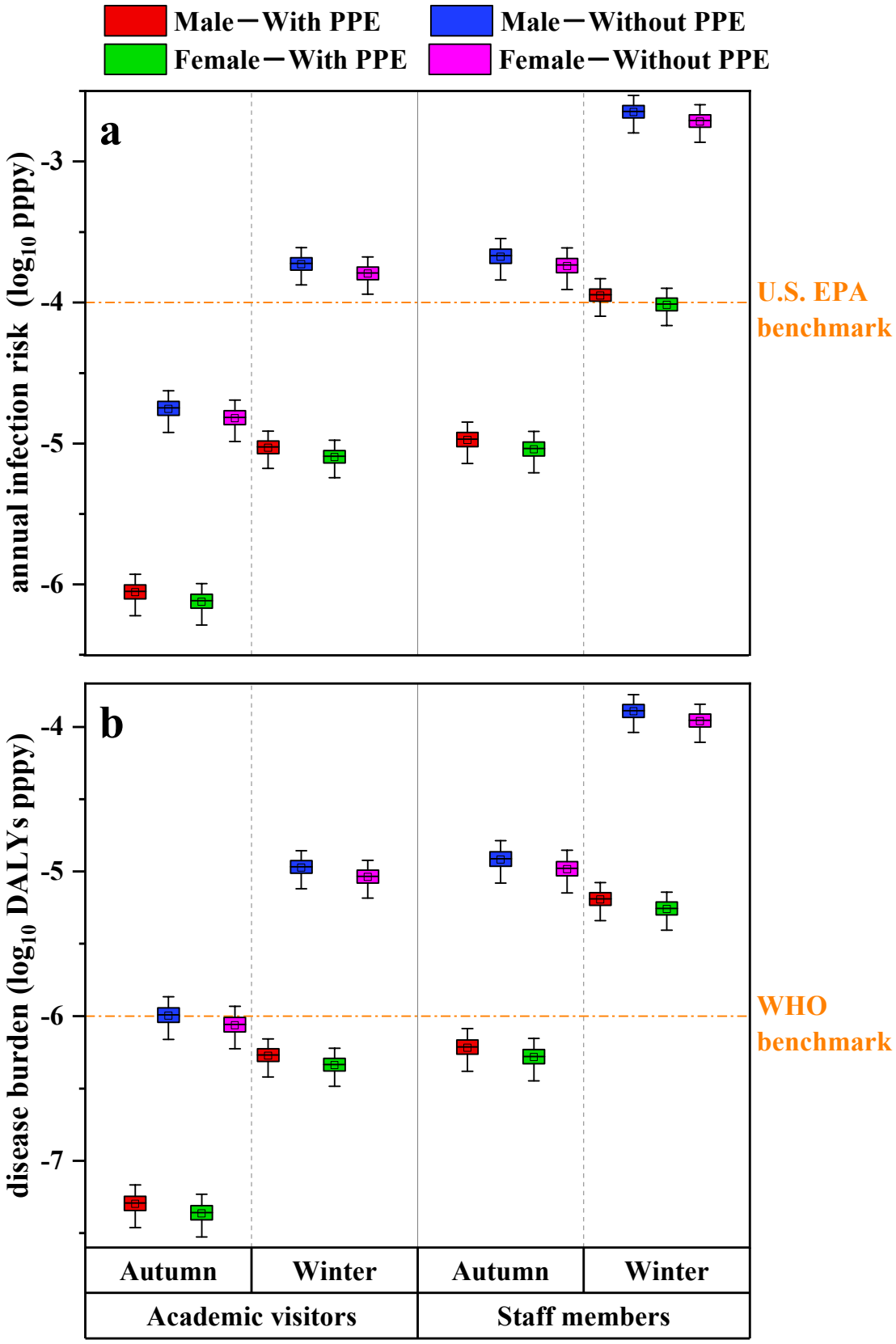


Fig. 2 Box-and-Whiskers diagram of (a) annual infection risk ($P_{(a)inf}$) and (b) disease burden (DB) exposed to *Staphylococcus aureus* bioaerosol in the wastewater treatment

plant.

The bottom and top of the box represent the first and third quartiles (25th and 75th percentile values), the band inside the box represents the second quartile (median), and the tetragon inside the box represents the average value. The bottom and top of the whiskers respectively represent the 5th percentile values (optimistic estimate in best case scenario) and 95th percentile values (conservative estimate in worst case scenario).

PPE=Personal Protective Equipment.

U.S. EPA=United States Environmental Protection Agency.

WHO=World Health Organization.

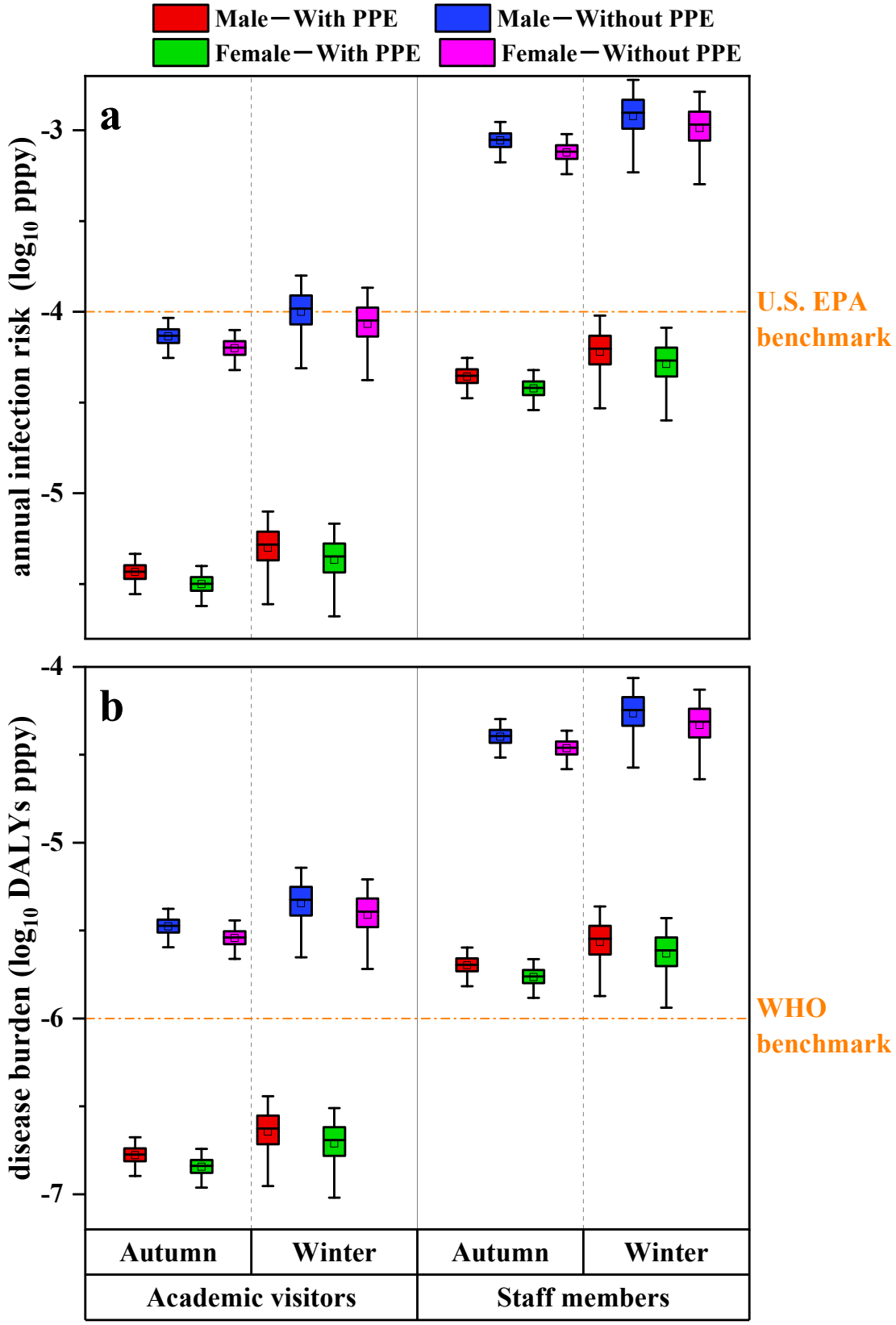


Fig. 3 Box-and-Whiskers diagram of (a) annual infection risk ($P_{(a)inf}$) and (b) disease burden (DB) exposed to *Escherichia coli* bioaerosol in the wastewater treatment plant. The bottom and top of the box represent the first and third quartiles (25th and 75th

percentile values), the band inside the box represents the second quartile (median), and the tetragon inside the box represents the average value. The bottom and top of the whiskers respectively represent the 5th percentile values (optimistic estimate in best case scenario) and 95th percentile values (conservative estimate in worst case scenario).

PPE=Personal Protective Equipment.

U.S. EPA=United States Environmental Protection Agency.

WHO=World Health Organization.

Table 1 Exposure scenarios

Items	Academic visitors	Staff members
Exposure issue	Sampling in the two aeration tanks	Inspection of the two aeration tanks
Exposure duration	Daily exposure time: Rotating disc aeration tank=3 h day^{-1} Microporous aeration tank=3 h day^{-1} Exposure frequency: 52 days for work per year*	Daily exposure time: Rotating disc aeration tank=1.5 h day^{-1} Microporous aeration tank=1.5 h day^{-1} Exposure frequency: 250 days for work per year**

*The exposure frequency: Work 1 day per week.

**The exposure frequency: 365 days – non-working days (weekend) – annual leave (10 days).

Table 2 Mean value ± SD of total bioaerosol concentration (CFU m^{-3})

Items	*S. aureus* bioaerosol		*E. coli* bioaerosol	
	Rotating disc aeration tank	Microporous aeration tank	Rotating disc aeration tank	Microporous aeration tank
19 October	1678±52[a]	262±52[a]	78±10[b]	15±8[a]
30 November	-	-	184±56[a]	8±6[a]
16 December	1004±85[b]	228±85[a]	44±0[b]	17±3[a]

Values with different superscript letters in the same column of the average bioaerosol concentrations indicate a significant difference at $P<0.05$ according to Duncan's multiple range tests.

Table 3 Mean value ± SD of bioaerosol concentration (CFU m^{-3}) of each stage of the Anderson six-stage impactor

Items			The size distribution of the Anderson six-stage impactor (μm)					
			>7.0	4.7−7.0	3.3−4.7	2.1−3.3	1.1−2.1	0.65−1.1
			Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
S. aureus bioaerosol	Rotating disc aeration tank	Autumn	195±61	282±59	228±31	314±219	319±115	339±246
		Winter	227±100	324±194	116±47	121±53	149±19	68±30
	Microporous aeration tank	Autumn	43±26	60±44	36±37	50±12	59±3	14±10
		Winter	61±19	36±11	46±11	45±18	28±17	12±13
E. coli bioaerosol	Rotating disc aeration tank	Autumn	8±7	9±1	11±2	18±8	23±1	9±1
		Winter	18±14	19±17	26±15	17±12	21±14	16±7
	Microporous aeration tank	Autumn	3±3	3±3	3±3	6±1	5±0	3±3
		Winter	5±1	4±3	4±4	3±3	3±2	1±2

Table 4 Aerosolization ratio

Items			Airborne cumulative concentration (CFU m^{-3})	Concentration in wastewater (CFU m^{-3})	Aerosolization ratio (CFU m^{-3} air / CFU m^{-3} wastewater)
S.aureus bioaerosol	Rotating disc aeration tank	Autumn	1678	2.62×10^{-12}	6.40×10^{-10}
		Winter	1004	6.11×10^{-11}	1.64×10^{-9}
	Microporous aeration tank	Autumn	262	1.80×10^{-12}	1.46×10^{-10}
		Winter	228	9.00×10^{-11}	2.53×10^{-10}
E. coli bioaerosol	Rotating disc aeration tank	Autumn	78	1.98×10^{-6}	3.93×10^{-5}
		Winter	118	9.01×10^{-10}	1.31×10^{-9}
	Microporous aeration tank	Autumn	22	2.20×10^{-10}	1.01×10^{-9}
		Winter	20	1.78×10^{-11}	1.13×10^{-10}

Supplement materials

Table S1 Ambient atmospheric conditions during sampling campaigns

Items	Autumn 19 October (10:03−15:46)	Winter 30 November (13:32−16:37)	Winter 16 December (9:58−16:28)
Temperature (°C)	24.3−26.6	13.8−25.3	6.1−7.5
Relative humidity (%)	34.2−44.4	47.1−67.1	44.7−80.6
Illumination (lux)	18370−65340	4500−49150	2174−15800
*Air Quality Index	61 (Good)	78 (Good)	65 (Good)
Wind direction	North-eastern	Northern	North-eastern
(Wind speed [m s^{-1}])	(1)	(2)	(2)
Weather	Sunny	Sunny	Cloudy
PM 2.5 (μg m^{-3})	38	55	45
PM 10 (μg m^{-3})	70	81	72

*According to the Ambient Air Quality Standards (GB 3095-2012) issued by the Ministry of Ecology and Environment, P.R. China and the weather forecast from the National Meteorological Information Center, P.R. China.

Table S2 Computing methods and parameters for quantitative microbial risk assessment

Items	Description	Unit	Values	References
C_{Pi}	Bioaerosol concentration of each stage of the Anderson six-stage impactor	CFU m^{-3}	Table 3	-
DC	Deposition coefficient of each stage of the Anderson six-stage impactor	-	Stage1=1 Stage2=1 Stage3=0.98 Stage4=0.94 Stage5=0.85 Stage6=0.54	Heyder et al. (1986)
C_d	Bioaerosol concentration	CFU m^{-3}	By calculation	-
IR	Inhaled breathing rate	m^3 day^{-1}	Male=18.65 female=14.80	Ministry of Environmental Protection (2013)
EF	Exposure frequency	day a^{-1}	Table 1	-
ED	Exposure duration	a	40	Ministry of Environmental Protection (2013)
ET	Daily exposure time	h	Table 1	-
BW	Body weight	kg	Male=68.80 Female=58.95	Ministry of Environmental Protection (2013)
AT	Average exposure time	a	Lifetime of male=73.64, lifetime of female=79.43	National Bureau of Statistics of China (2019)
H	The number of hours in a day	h	24	-
α	D=(1−α)×ALDD	-	Without a mask on=0, wearing a mask=0.95	Beijing Institute of Medical Device Testing (2011)

S. aureus bioaerosol	Exponential dose-response model (dose-infection model)	-	$k=8.05\times10^{-8}$	Esfahanian et al. (2019)
	Prevalence	-	$P_{ill/inf}=1$	Busgang et al. (2018)
	Health burden (HB)	DALYs case^{-1}	5.72×10^{-2}	Li (2020)
E. coli bioaerosol	beta-Poisson dose-response model (dose-infection model)	-	$\beta = \frac{D_{50}}{2^{\frac{1}{\alpha}}}$ $\alpha=0.155$ $D_{50}=2.11\times10^{6}$	Shi et al. (2018)
	Prevalence	-	$P_{ill/inf}=1$	Allison et al. (2018)
	Health burden (HB)	DALYs case^{-1}	4.55×10^{-2}	Havelaar et al. (2015)

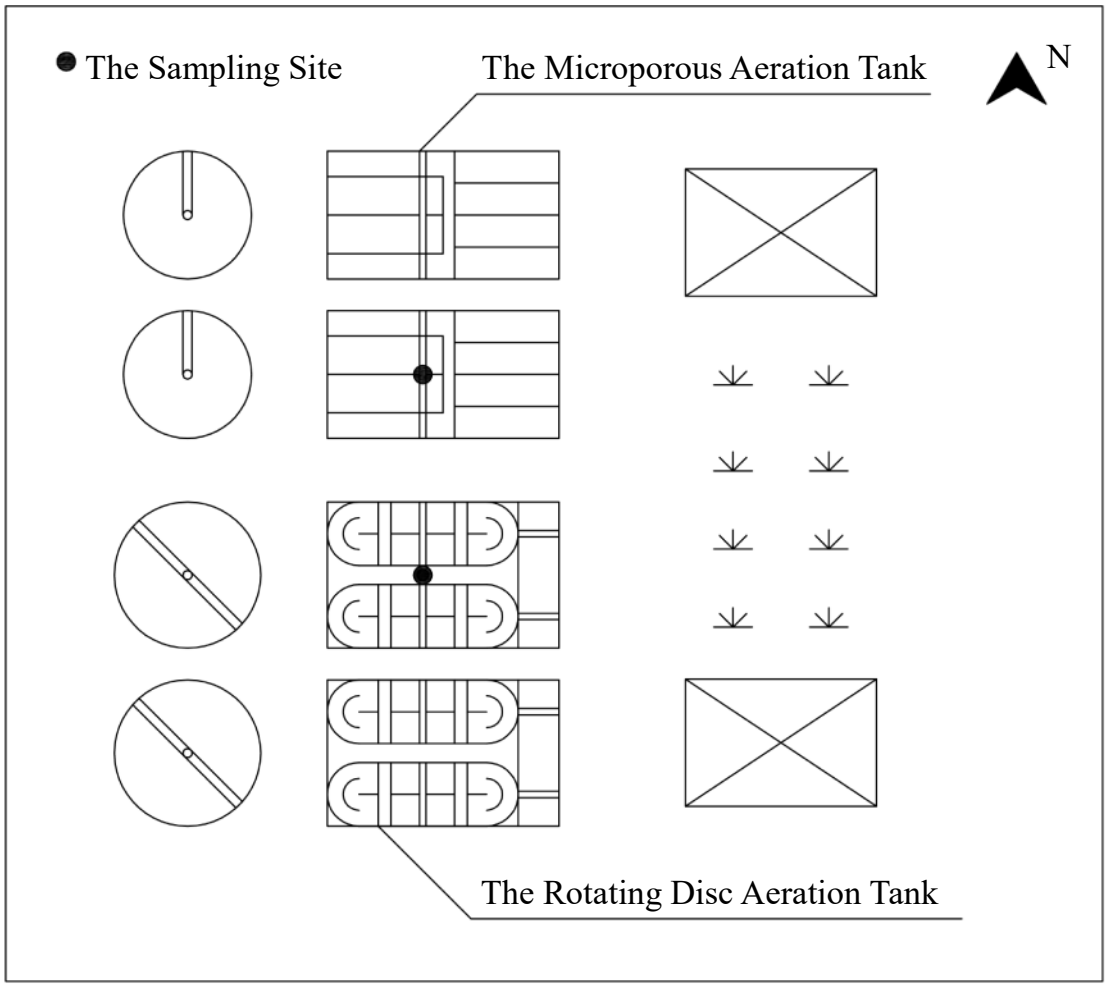


Fig. S1 Diagram of sampling sites in the wastewater treatment plant

QMRA process

(1) Hazard identification

The pathogens of concern in this research were two kinds of indicator bioaerosols (*S. aureus* and *E. coli* bioaerosols) from the rotating disc aeration tank and the microporous aeration tank in the WWTP. Both of the staff members inspecting in the aeration tank and the academic visitors sampling in the aeration tank were faced with potentially high health risks.

(2) Exposure assessment

The exposure scenarios of this research are showed in Table 1. Table 2 lists the computing methods and parameters for QMRA in each exposure scenario. Taking into account the different probability of bioaerosol deposition in the human body with different particle diameters, the bioaerosol concentration is calculated using Eq. (1) (Heyder et al., 1986):

$$C_d = \sum_1^6 (C_{pi} \times DC) \quad (1)$$

where C_d is the bioaerosol concentration of *S. aureus* bioaerosol or *E. coli* bioaerosol (CFU m^{-3}), C_{pi} is the bioaerosol concentration of *i* stage of Anderson six-stage impactor (CFU m^{-3}) (Table 3), and *DC* is the deposition coefficient of each stage of Anderson six-stage impactor (Table 2).

The average lifetime of daily exposure dose (ALDD) is calculated using Eq. (2):

$$ALDD = \frac{C_d \times IR \times EF \times ED \times ET}{H \times BW \times AT} \quad (2)$$

where *ALDD* represents the average lifetime of daily exposure dose (CFU (kg day) $^{-1}$), *IR* is the inhaled breathing rate ($m^3 \cdot day^{-1}$) (Table 2), *EF* is the exposure frequency (the number of work days) for each scenario ($day \cdot a^{-1}$) (Table 1), *ED* is the exposure duration (the number of years in career) for each scenario (a) (Table 2), *ET* is the daily exposure time (h) (Table 1), *H* is the number of hours in a day (h) (Table 2), *BW* is body weight (kg) (Table 2), and *AT* is the average exposure time (lifetime) (a) (Table 2).

The exposure dose is calculated using Equation (3):

$$d = (1 - \alpha) \times ALDD \quad (3)$$

where α represents the parameter of the model equation (Table 2).

(3) Dose-response models

S. aureus bioaerosol uses the exponential dose-response model as the dose-infection model (Eq. (4)) (Esfahanian et al., 2019):

$$P_{(d)inf} = 1 - e^{-dk} \quad (4)$$

where $P_{(d)inf}$ is the daily infection risks, and *k* is the dose-response parameter. The specific value is shown in Table 2.

E. coli bioaerosol uses the beta-Poisson dose-response model as a dose-infection model (Eq. (5)) (Shi et al., 2018):

$$P_{(d)inf} = 1 - (1 + \frac{d}{\beta})^{-\alpha} \quad (5)$$

where $P_{(d)inf}$ is the daily infection risks, α and β are the model parameters (Table 2).

(4) Risk characterization

The annual infection risk is calculated in Eq. (6) (Haas et al., 2014; Sales-Ortells and Medema, 2014):

$$P_{(a)inf} = 1 - (1 - P_{(d)inf})^{n} \quad (6)$$

where $P_{(a)inf}$ is the annual probability of infection per person per year (pppy), and n is the annual exposure frequency (Table 1).

To keep the analysis more conservative, it is assumed that the probability of infection is equal to the probability of illness (P_{inf}=P_{ill}) (Busgang et al., 2018; Allison et al., 2018). The probability of illness, as a conditional of infection, is calculated in Eq. (7) (Carducci et al., 2018):

$$P_{(a)ill} = P_{(a)inf} \times P_{ill/inf} \quad (7)$$

The specific potential disease burden (DALYs pppy) illness caused by exposure to *S. aureus* or *E. coli* bioaerosol is estimated in Eq. (8) (Havelaar et al., 2012):

$$DB = P_{(a)ill} \times HB \quad (8)$$

where DB is the disease burden, and the HB is the health burden that is expressed in DALYs per illness case (DALYs/case) (Table 2).

(5) Monte Carlo simulation

The Monte Carlo simulation was used to estimate the probability distribution of health risks, so that the range and likelihood of the risks were assessed quantitatively (Shi et al., 2018). All calculations were conducted by using Oracle Crystal Ball and Microsoft Excel 2010 (Devleesschauwer et al., 2014; Liu et al., 2019). It was assumed that the concentration of bioaerosol in each stage of Anderson six-stage impactor obeyed lognormal distribution and the mean value and standard deviation was taken as the input parameter of Monte Carlo simulation. The input parameter was randomly selected from probability distributions. The output parameters (annual infection risks and disease burdens) were computed over 10,000 iterations so that the distributions can reach a steady state (Lim and Jiang, 2013; Shi et al., 2018).

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