

# Seasonal variation of quantitative microbial risk assessment for three airborne enteric bacteria from wastewater treatment plant emissions

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## ABSTRACT

Airborne *E. coli*, fecal coliform, and *Enterococcus* are all related to sewage worker's syndrome and therefore used as target enteric bioaerosols about researches in wastewater treatment plants (WWTPs). However, most of the studies are often inadequately carried out because they lack systematic studies reports bioaerosols emission characteristics and health risk assessments for these three enteric bacteria during seasonal variation. Therefore, quantitative microbial risk assessment based on Monte Carlo simulation was utilized in this research to assess the seasonal variations of health risks of the three enteric bioaerosols among exposure populations (academic visitors, field engineers, and office staffs) in a WWTP equipped with rotating-disc and microporous aeration modes. The results show that the concentrations of the three airborne bacteria from the rotating-disc aeration mode were 2–7 times higher than the microporous aeration mode. Field engineers had health risks 1.5 times higher than academic visitors due to higher exposure frequency. Health risks of airborne *Enterococcus* in summer were up to 3 times higher than those in spring and winter. Similarly, health risks associated to *E. coli* aerosol exposure were 0.3 times higher in summer compared to spring. In contrast, health risks associated with fecal coliform aerosol were between 2 and 19 times lower in summer compared to spring and winter seasons. Data further suggest that wearing of N95 mask could minimize health risks by 1–2 orders of magnitude. This research shed light on seasonal variation of health risks associated with bioaerosol emission from wastewater utilities.

## 1. Introduction

Biological particles ranging from 0.05 to 100  $\mu\text{m}$  are defined as bioaerosols and their size distributing below 4.7  $\mu\text{m}$  are mainly found in wastewater treatment plants (WWTPs) (Li et al., 2016; Kowalski et al., 2017; Wang et al., 2018; Han et al., 2021). With increasing wastewater production, the threat posed by bioaerosols in WWTPs has become significant in recent years (Haas et al., 2014; Qu et al., 2019; Han et al., 2021). There are approximately over  $1.9 \times 10^3$ ,  $3.3 \times 10^3$ , and  $4.2 \times 10^3$  municipal WWTPs in the UK, France, and Germany, respectively (OECD, 2021). In comparison, there are approximately  $4.14 \times 10^3$  municipal WWTPs in operation in China (Information Center of the Ministry of Housing and Urban-Rural Development, 2020). However, there is

currently no provision of assessing the health risk of bioaerosol in WWTPs issued by the various governmental authorities. Epidemiology studies showed that sewage worker's syndrome is firmly associated with exposure to high concentrations of airborne pathogenic bacteria in WWTPs (ACGIH, 1989; Kowalski et al., 2017). High contribution to emission of airborne pathogenic bacteria could be attributed to aerator tanks in WWTPs (Korzeniewska, 2011; Han et al., 2021). Enteric bacteria bioaerosols (e.g. *E. coli*, fecal coliform, and *Enterococcus* bioaerosols) are widely recognized as typical airborne pathogenic bacteria causing respiratory diseases, such as asthma and chronic bronchitis (Espigares et al., 2006; Yang et al., 2019; Han et al., 2021). Though as target indicator enteric bioaerosols, these three bioaerosols are frequently found in WWTPs and widely employed in studying the health

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risks of bioaerosols, they lack systematic studies of emission characteristics and health risk assessments (Pascual et al., 2003; Korzeniewska et al., 2009; Popovic et al., 2015; Nascimento et al., 2020; Han et al., 2021). Health risks are usually quantified by the annual probability of infection ( $P_y$ ) and disease burden (DB) (Haas et al., 2014) which are evaluated by quantitative microbial risk assessment (QMRA) (Harb et al., 2017; Yang et al., 2019). Temperature and relative humidity related to seasonal variation are the most important environmental factors affecting bioaerosol emissions in WWTPs (Xu et al., 2020). This is because the survivability of bioaerosols is greatly related to temperature and relative humidity (Maharia et al., 2015; Michaikiewicz, 2018). High relative humidity hinders bioaerosol droplet evaporation and delays airborne microorganisms' die-off because the liquid sorption on their surface will provide protection against extraneous damage (Korzeniewska, 2011). Furthermore, health risks are highly associated with bioaerosols survivability and correlated positively with the concentrations of bioaerosols (Haas, 2015; Lim et al., 2015; Shi et al., 2018; Forde et al., 2019). Thus, to capture seasonal variation, Monte Carlo simulation is often used for QMRA (Lim et al., 2015; Jahne et al., 2016; Liu et al., 2019). By using Monte Carlo simulation, the concentrations of bioaerosols are assumed to follow a logarithmic normal distribution and selected randomly as the input parameter on the basis of probability distributions. However, given the limited knowledge on the emission characteristics and QMRA for bioaerosols in WWTPs during seasonal variation, further systematic studies are required (Yang et al., 2019; Han et al., 2021).

Therefore, based on our previous reports, this study investigated bioaerosols emitted from a WWTP equipped with rotating-disc and microporous aeration modes. As target enteric bioaerosols, *E. coli*, fecal coliform, and *Enterococcus* bioaerosols were employed for the assessment of health risks with QMRA. The health risks ( $P_y$  and DB) of academic visitors, field engineers, and office staffs with or without a N95 mask on and who were exposed to bioaerosols in spring, summer, and winter are discussed. In addition, the effects of the beta-Poisson and exponential dose-response models on the results of calculated health risks were studied. This research gives novel insights on the seasonal variations of health risks under the best-case/worst-case condition in the practical application of QMRA framework. Moreover, the scientific impact of this research can be applied as policy orientation to accelerate the implementation of vigilant precautions for bioaerosol threats to public health from local wastewater utilities.

## 2. Method and materials

### 2.1. Wastewater treatment plants description

Bioaerosols emission from a WWTP located in Central China treating  $1.16 \text{ m}^3/\text{s}$  domestic wastewater were collected for 16 days. The WWTP is composed of a classical activated sludge process with rotating-disc aeration mode and microporous aeration mode (Fig. 1). The two aeration modes were conducted in parallel and treated the same amount of

domestic wastewater ( $0.58 \text{ m}^3/\text{s}$ ).

### 2.2. Bioaerosol sampling regime

Seasonal bioaerosols emissions from the rotating-disc aerator tank and microporous aerator tank (Supplementary material Tables 1, 2, and 3) was carried using an Anderson six-stage impactor (ZR-2000B) and Microbio Air sampler (ZR-2050). Specifically, the airborne concentrations of *E. coli*, fecal coliform, and *Enterococcus* were determined as previously described by Fang et al. (2008), Johnsen et al. (2010) and Grzyb et al. (2019). The Anderson six-stage impactor (flow rate  $28.3 \text{ L/min}$ ) and Microbio Air sampler (flow rate  $8.33 \times 10^{-4} \text{ m}^3/\text{s}$ ) worked simultaneously for 10 min (Fang et al., 2008; Johnsen et al., 2010; Malakootian et al., 2013). The particle size ranges of the Andersen impactor are as follows: Stage 1 ( $\geq 7.0 \mu\text{m}$ ), Stage 2 ( $4.7\text{--}7.0 \mu\text{m}$ ), Stage 3 ( $3.3\text{--}4.7 \mu\text{m}$ ), Stage 4 ( $2.1\text{--}3.3 \mu\text{m}$ ), Stage 5 ( $1.1\text{--}2.1 \mu\text{m}$ ), and Stage 6 ( $0.65\text{--}1.1 \mu\text{m}$ ) (Kowalski et al., 2017). A Petri dish, which was previously prepared and sterilized in an autoclave, containing agar medium was placed in each stage of the impactor. MacConkey Agar medium, Tergitol-7 Agar medium, and membrane-filter *Enterococcus*-selective agar, in accordance with method of Slanetz and Bartley, were utilized for *E. coli*, fecal coliform, and *Enterococcus* bioaerosols, respectively (Pascual et al., 2003; Korzeniewska et al., 2009; Popovic et al., 2015).

Samples were collected in the middle of the corridor of the aerator tanks and in front of the office building (Fig. 1) from 1.5 m above the ground level corresponding to the average human breathing height (Brooks et al., 2004; Grzyb et al., 2019). Sampling was carried out in triplicate. Other details for sampling regime are demonstrated in the Supplementary material Tables 1, 2, and 3. The Petri dish and agar medium were sterilized in an autoclave before samplings, and the two devices were sterilized by alcohol between samplings.

### 2.3. Laboratory analysis

Laboratory analyses were conducted following the standard procedures (Pascual et al., 2003; Korzeniewska et al., 2009). After each sampling, the samples were sealed and stored in a refrigerated box. All samples would be transferred to the laboratory immediately when the work of sampling on site was finished. The agar medium of *E. coli* and *Enterococcus* bioaerosols were incubated at  $37^\circ\text{C}$  for 24 and 72 h, respectively (Pascual et al., 2003; Korzeniewska et al., 2009). Another incubator was used to culture the fecal coliform bioaerosol at  $44^\circ\text{C}$  for 24 h (Pascual et al., 2003). Positive-hole method was employed to determine the bioaerosol concentration of each stage which have been counted colony by an automated colony counter (HiCC-B) in triplicate (Lawless, 2000). The bioaerosol concentrations sampled by Anderson six-stage impactor and Microbio Air sampler (ZR-2050) were utilized to investigate the characteristics of bioaerosol emission and the assessment of health risks about bioaerosols, respectively.

### 2.4. Quantitative microbial risk assessment

The target enteric bacteria bioaerosols in this research were bioaerosols of *E. coli*, fecal coliform, and *Enterococcus* from the rotating disc aerator tank and microporous aerator tank. These enteric bacteria bioaerosols are the most widely used target indicators in the literatures about bioaerosols in WWTPs (Nascimento et al., 2020; Han et al., 2021). The exposure scenarios investigated in this research are showed in Table 1. Field engineers maintained the aerator tanks on-site and office staffs worked indoors were both exposed to bioaerosols on weekdays. Academic visitors were exposed to bioaerosols due to collecting samples near the two aerator tanks once a week. All exposure populations were adults within 18–60 years old. The inhaled breathing rate factor is shown in the Supplementary material Table 5.

Basing on our previous reports and literatures (Haas et al., 2014, 2017; Chen et al., 2020), the exposure dose for exposure assessment and

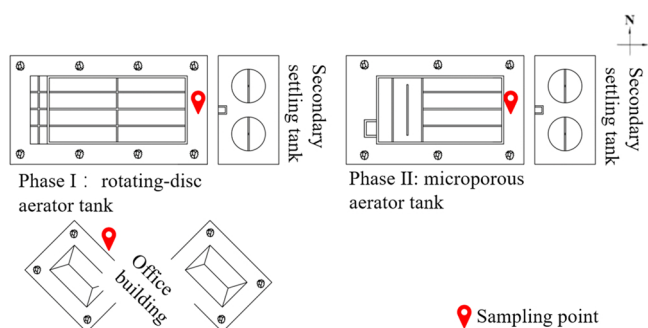


Fig. 1. Distribution of sampling points.

**Table 1**

Exposure scenarios.

| Items              | Academic visitors                                                                | Field engineers                                                                      | Office staffs                  |
|--------------------|----------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|--------------------------------|
| Exposure issue     | Temporary entry and sampling in the two aerator tanks                            | Maintaining the two aerator tanks                                                    | Working in the office building |
| Exposure time      | Rotating-disc aerator tank: 3 h per day<br>Microporous aerator tank: 3 h per day | Rotating-disc aerator tank: 1.5 h per day<br>Microporous aerator tank: 1.5 h per day | Office building: 8 h per day   |
| Exposure frequency | 52 days for work per year                                                        | 250 days for work per year                                                           | 250 days for work per year     |

the health risks ( $P_y$  and DB) for risk characterization were calculated. The dose response assessment is a quantifiable index for the estimate of health risks (Haas, 2002, 2015; Xie et al., 2017). The beta-Poisson and exponential dose-response models are two models that are based on the needs for the QMRA dose response relationships (Teunis et al., 2000; Haas, 2014; Xie et al., 2017). There are however some limitations due to the models' assumptions which restrict their applicability. For the exponential dose-response model: Poisson distribution is applicable for the dose of pathogenic bacteria; one pathogenic bacteria organism can cause an infection if it lands in a proper place, and possess equal chances of independent survival during this process (Haas, 2002, 2015). For the beta-Poisson dose-response model, assumptions same as the exponential model except: the probabilities of survival and infection of pathogenic bacteria are non-constant; the probabilities of survival obey beta distribution (Haas, 2002, 2015). In addition, we suppose that the wastewater utilities could have 95% reduction in bioaerosols inhalation by wearing a N95 mask. All parameters for QRMA were showed in the [Supplementary material Table 5](#).

### 3. Results and discussion

#### 3.1. Emission characteristics of bioaerosols

The concentrations of the three enteric bacteria bioaerosols from the two source aerator tanks and the one downwind location office building sampled by the Anderson six-stage impactor are showed in [Table 2](#). For all size distribution ranges, the concentrations of the three enteric bacterial bioaerosols in the microporous aeration mode and office building were always lower than those in the rotating-disc aeration mode (except the Stage 5 of *Enterococcus* bioaerosol). Furthermore, the concentrations of the three bioaerosols from the rotating-disc aeration mode were 2.96–7.78 times as big as the microporous aeration mode. This is because agitation is the main emission mechanism for bioaerosols in aeration tanks; and the agitation of the rotating-disc aeration mode is much more violent (Korzeniewska, 2011; Kowalski et al., 2017). Similar results had been demonstrated in previous literatures. Comparable results were obtained by Sánchez-Monedero et al. (2008), who affirmed that the quantity of bioaerosol emission from the aeration mode of

mechanic is three orders of magnitude more than the aeration mode of fine bubble micropore. Wang et al. (2019a), (2019b) also stated parallel results, showing that the aeration mode using mechanics would lead to higher concentration of bioaerosols produced. It is suggested that designers should consider retrofitting WWTP by employing microporous aeration mode rather than mechanic aeration mode to reduce the occupational health risks of sewage workers.

For *E. coli* bioaerosol, the particles emitted from the microporous aeration mode ranging from 0.65 to 1.1  $\mu\text{m}$  (Stage 6) and those emitted from the office building ranging from 4.7 to 7 (Stage 2), 1.1–2.1 (Stage 5), and 0.65–1.1  $\mu\text{m}$  approached zero. We considered that sampling of bioaerosols in such a short period of time were disturbed easily owing to short-term concentration variations (Fang et al., 2008). Thus, the zero values in [Table 2](#) might be unnecessarily to reflect no emission of bioaerosols (Anne et al., 2005). Notably, the *Enterococcus* bioaerosol concentrations for the size distribution ranges of greater than 7 (Stage 1) and 3.3–4.7  $\mu\text{m}$  (Stage 3) at the office building were higher than those from the microporous aeration mode. Bioaerosol concentration in the office building was determined by the combined influence of the bioaerosol emission and diffusion of the two aerator tanks (Karra et al., 2007; Korzeniewska, 2011; Han et al., 2021). Furthermore, distance from the two aeration tank sources played a key role in reducing the concentrations of bioaerosols at the office building (Dungan, 2014; Nascimento et al., 2020). Therefore, the summed concentration of the office building can be higher than that of the microporous aeration tank due to the possibility that the rotating-disc aeration mode contributed substantially more bioaerosol concentration than the microporous aeration mode.

The highest concentrations of *E. coli* bioaerosol from the two aeration modes (rotating-disc aeration mode: 7.04 CFU/m<sup>3</sup>, microporous aeration mode: 1.52 CFU/m<sup>3</sup>) and office building (1.27 CFU/m<sup>3</sup>) were all in the Stage 1 (greater than 7  $\mu\text{m}$ ). This outcome implies that particles of *E. coli* airborne particles may cause harm to the nasal and oral regions of the exposure populations (Qiu, 2012). For fecal coliform bioaerosol, the highest concentration distribution both ranged from 3.3  $\mu\text{m}$  to 4.7  $\mu\text{m}$  (Stage 3) from the two aeration modes (rotating-disc aeration mode: 4.66 CFU/m<sup>3</sup>, microporous aeration mode: 0.89 CFU/m<sup>3</sup>). The result demonstrates that the mainly effects of fecal coliform bioaerosols on the exposure populations were the infection of weasand and the first bronchus (Dungan, 2010; Pahari et al., 2016). For *Enterococcus*, the results were very same as those for *E. coli*. No significant differences were observed between the emission characteristics of size distribution for the two aeration modes. In general, the diameter of the most bioaerosols in the size distribution ranges were less than 4.7  $\mu\text{m}$ . The results agree well with other literatures, especially with the paper published by Korzeniewska (2011), who demonstrated that the bioaerosol particles size distribution ranges were mainly below 4.7  $\mu\text{m}$  in a WWTP. Thus, most of *E. coli*, fecal coliform, and *Enterococcus* bioaerosols turn out deposition particles in the weasand and bronchus of the exposed populations. Remarkably, the bioaerosols classified the particles under the size distribution range less than 4.7  $\mu\text{m}$  (Stages 3–6) as respirable particles, which meant that these particles could be harmful to health though

**Table 2**Annual mean value  $\pm$  standard deviation of bioaerosol concentration (CFU/m<sup>3</sup>) of each stage of the Anderson six-stage impactor.

| Items                              |                            | Anderson six-stage impactor |                 |                 |                 |                 |                 |
|------------------------------------|----------------------------|-----------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                    |                            | Stage 1                     | Stage 2         | Stage 3         | Stage 4         | Stage 5         | Stage 6         |
| <i>Escherichia coli</i> bioaerosol | Rotating-disc aerator tank | 7.04 $\pm$ 6.10             | 5.17 $\pm$ 6.25 | 6.10 $\pm$ 5.88 | 6.52 $\pm$ 5.62 | 2.28 $\pm$ 4.00 | 0.68 $\pm$ 2.16 |
|                                    | Microporous aerator tank   | 1.52 $\pm$ 3.35             | 0.76 $\pm$ 2.36 | 1.35 $\pm$ 2.44 | 1.44 $\pm$ 3.23 | 0.51 $\pm$ 1.56 | 0               |
|                                    | Office building            | 1.27 $\pm$ 3.39             | 0               | 1.27 $\pm$ 3.39 | 0.38 $\pm$ 1.37 | 0               | 0               |
| Fecal coliform bioaerosol          | Rotating-disc aerator tank | 2.76 $\pm$ 3.80             | 3.46 $\pm$ 4.88 | 4.66 $\pm$ 6.70 | 2.76 $\pm$ 4.28 | 0.69 $\pm$ 1.97 | 0.30 $\pm$ 1.22 |
|                                    | Microporous aerator tank   | 0                           | 0.33 $\pm$ 1.29 | 0.89 $\pm$ 2.43 | 0.33 $\pm$ 1.29 | 0.33 $\pm$ 1.29 | 0               |
|                                    | Office building            | –                           | –               | –               | –               | –               | –               |
| <i>Enterococcus</i> bioaerosol     | Rotating-disc aerator tank | 3.22 $\pm$ 4.41             | 2.37 $\pm$ 3.85 | 2.46 $\pm$ 4.34 | 1.61 $\pm$ 3.42 | 0.25 $\pm$ 1.13 | 0.85 $\pm$ 2.61 |
|                                    | Microporous aerator tank   | 1.27 $\pm$ 3.66             | 0.42 $\pm$ 1.89 | 0.25 $\pm$ 1.13 | 1.27 $\pm$ 3.11 | 0.42 $\pm$ 1.89 | 0               |
|                                    | Office building            | 1.52 $\pm$ 3.21             | 0.42 $\pm$ 1.89 | 0.93 $\pm$ 2.88 | 0               | 0.42 $\pm$ 1.89 | 0.44 $\pm$ 1.94 |

entering the respiratory system deeply (Korzeniewska, 2011; Kowalski et al., 2017). Further researches about how these respirable particles can pose higher infection probability and even more serious sewage work's syndrome are very important. However, there is a limitation that these research contents are not involved in this study.

### 3.2. Annual probability of infection

Figs. 2 and 3 present the results of average, median, 5th percentile (best-case condition), and 95th percentile quantiles (worst-case condition) of  $P_y$  employing the Monte Carlo simulations to run  $1 \times 10^4$  trials for various exposure scenarios in three seasons. The  $P_y$  in Figs. 2 and 3 were calculated by beta-Poisson and exponential dose-response models, respectively.

For all exposure scenarios, several consistent conclusions were achieved. First, the male consistently had a little higher infection risk than female in spite of the values of risks in the same order. This finding was on account of that male had considerably higher rate of respiration than

female (Ministry of Environmental Protection, 2013). The most common routes of enteric bacteria bioaerosol infection are exposure to wastewater through inhalation and dermal exposure from epidemiological studies' point of view (Korzeniewska et al., 2009; Kowalski et al., 2017; Rasheduzzaman et al., 2019). However, dermal exposure and more potent inhalation exposure differ by about five orders of magnitude (Fathi et al., 2017). Thus, a great risk of infection is related to exposure to enteric bacteria bioaerosols through inhalation from epidemiological perspective (Korzeniewska et al., 2009; Rasheduzzaman et al., 2019). Accordingly, the exposure populations who have a higher inhaled breathing rate will have greater risk of infected with epidemic on account of more inhalational enteric bacteria under the same exposure scenario (Lim et al., 2015; Kowalski et al., 2017). In contrast, when wearing a mask, all the exposure populations' infection risks were 19 times lower than that before when they had no mask on. As a useful way to prevent bioaerosol infection, wearing of mask is an effective means of protection for inhalation exposure to enteric bacteria bioaerosols (Brooks et al., 2004). For field engineers, their infection risks were

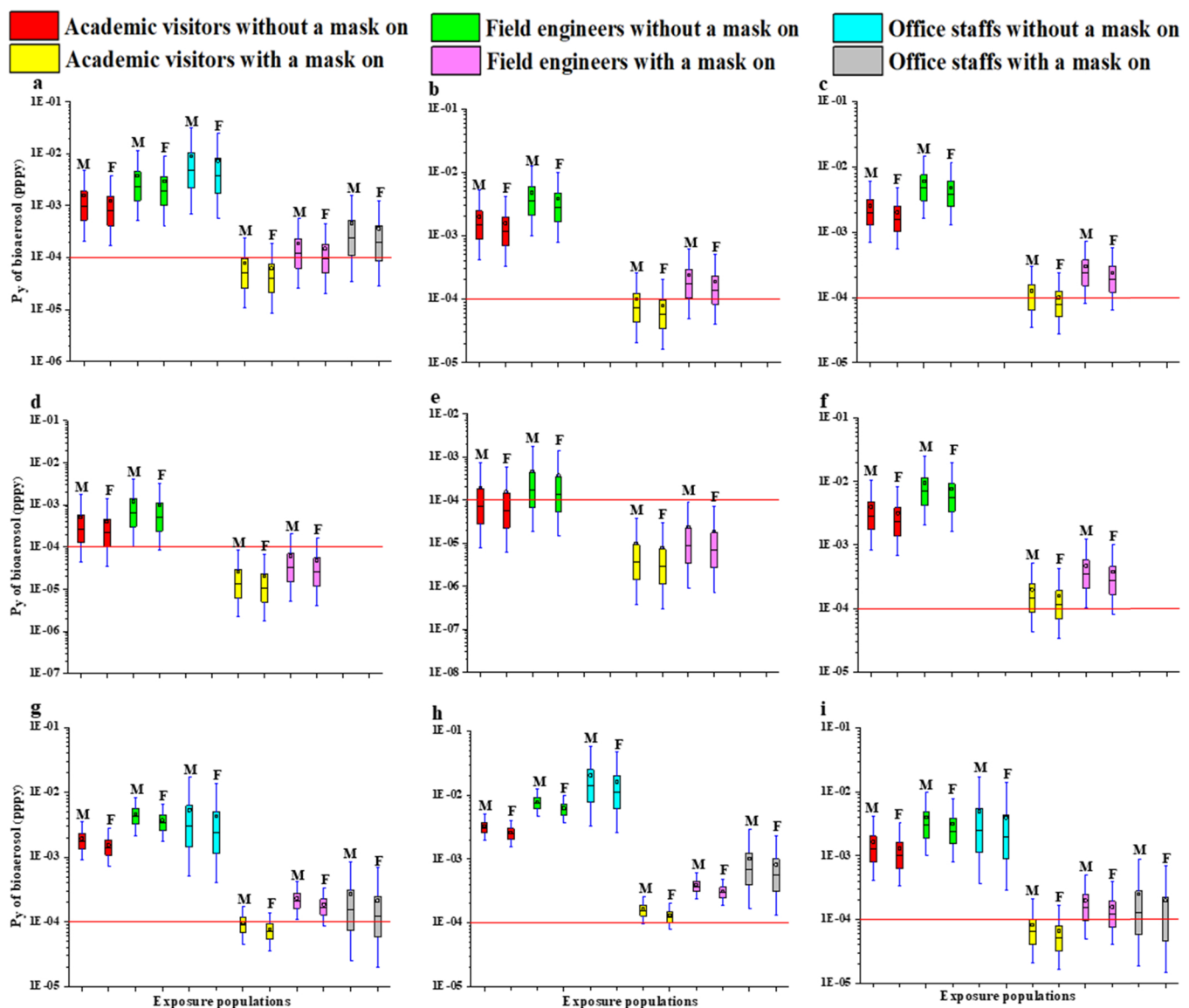


Fig. 2. Annual probability of infection ( $P_y$ ) of various exposure scenarios based on the beta-Poisson dose-response model in three seasons: (a)  $P_y$  of *Escherichia coli* bioaerosol in spring, (b) summer, and (c) winter; (d)  $P_y$  of fecal coliform bioaerosol in spring, (e) summer, and (f) winter; (g)  $P_y$  of *Enterococcus* bioaerosol in spring, (h) summer, and (i) in winter. The bottom and top of the box respectively represent the first and third quartiles (25th and 75th percentile values), the band inside the box denotes the second quartile (median), and the tetragon inside the box refers to the average value. The bottom and top of the whiskers respectively represent the 5th percentile values (best-case condition) and 95th percentile values (worst-case condition). M: Male F: Female.

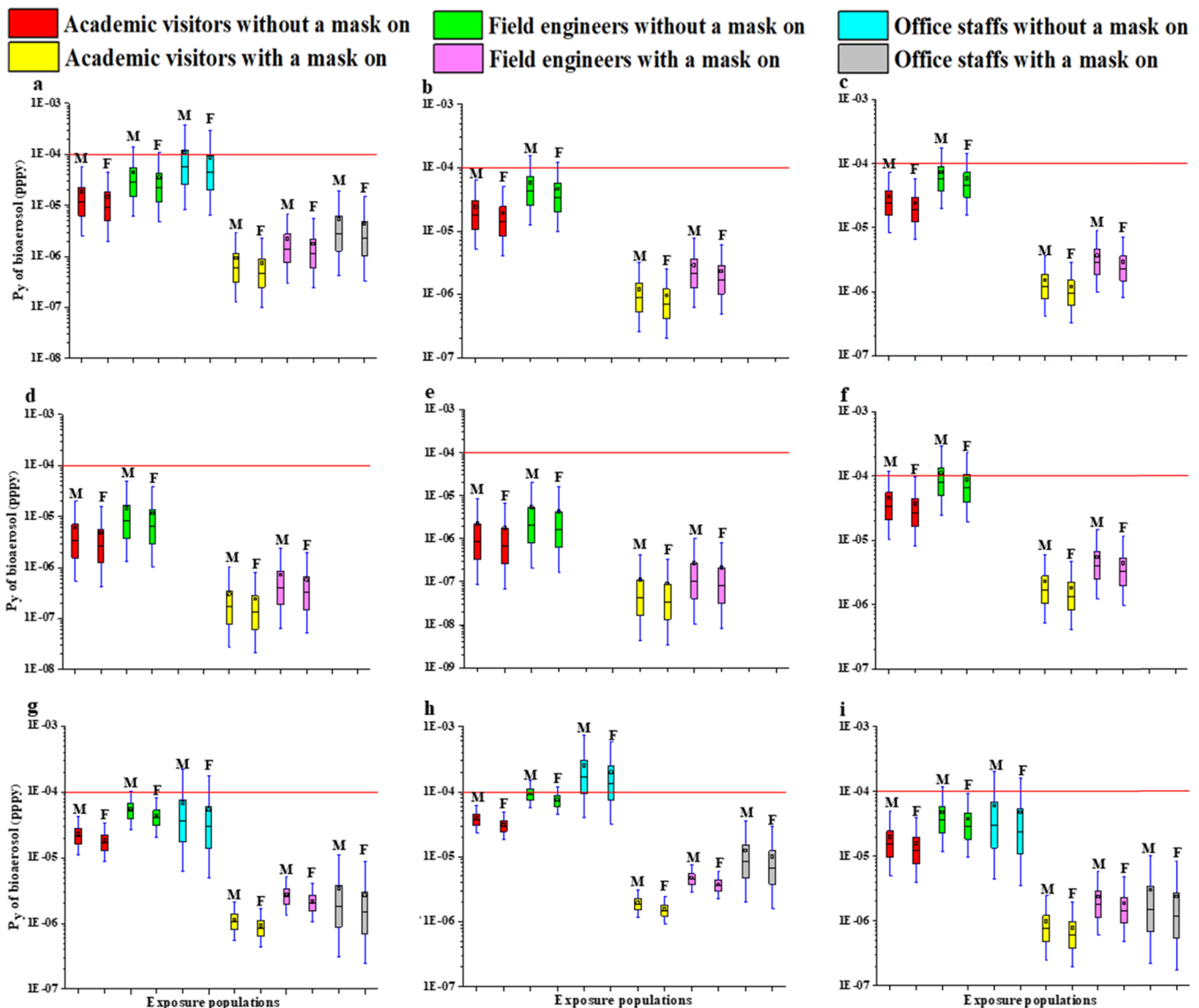


**Fig. 3.** Annual probability of infection ( $P_y$ ) of various exposure scenarios based on the exponential dose-response model in three seasons: (a)  $P_y$  of *Escherichia coli* bioaerosol in spring, (b) summer, and (c) winter; (d)  $P_y$  of fecal coliform bioaerosol in spring, (e) summer, and (f) winter; (g)  $P_y$  of *Enterococcus* bioaerosol in spring, (h) summer, and (i) in winter. The bottom and top of the box respectively represent the first and third quartiles (25th and 75th percentile values), the band inside the box denotes the second quartile (median), and the tetragon inside the box refers to the average value. The bottom and top of the whiskers respectively represent the 5th percentile values (best-case condition) and 95th percentile values (worst-case condition). M: Male F: Female.

consistently 1.40 times higher than those of the academic visitors because the former's exposure frequency is significantly higher (Table 1). The annual probability of bioaerosol infection risk is mainly determined by exposure duration and bioaerosol exposure concentration (Jahne et al., 2015a; Pasalari et al., 2019); a greater risk of infection may occur due to increased exposure frequency under the same bioaerosol exposure concentration. In addition, rotating-disc aeration mode could pose higher infection risk on account of its more violent agitation and consequent higher concentration of bioaerosol emission (Jahne et al., 2015a; Kowalski et al., 2017; Pasalari et al., 2019).

The infection risks of *Enterococcus* bioaerosol in summer were up to 3 times higher than those in spring and winter (Fig. 2 g, h, i, Fig. 3g, h, and i). Similarly, the infection risks of *E. coli* bioaerosol were 0.3 times higher in summer compared to spring (Fig. 2a, b, Fig. 3a and b). Epidemiology studies showed that the higher infection risks should be attributed to the significantly higher bioaerosol concentrations in summer than that in spring and winter (Table S4) (Kowalski et al., 2017; Shi et al., 2018; Esfahanian et al., 2019). This was ascribed to that the

temperature of summer were higher than the other two seasons (Tables S1–S3) (Forde et al., 2019). A similar conclusion was reached by Miroslaw et al. (2016), who observed that the highest emissions of bioaerosols occurred in summer throughout the year. Yang et al. (2019) also reported that infection risks during summer were higher than those during spring and winter. However, the infection risks of fecal coliform bioaerosol were between 2 and 19 times lower in summer compared to spring and winter (Fig. 2d, e, f, Fig. 3d, e, and f) given that the seasonal variation of bioaerosol concentrations reversed. A number of factors can be at play here, as temperature and relative humidity are the most important environmental factors affecting bioaerosol infection in WWTPs (Karra et al., 2007; Xu et al., 2020). Bioaerosol concentration strongly corresponds with air temperature but has a negative correlation with relative humidity (Forde et al., 2019). However, the lack of chemical/physical factors of wastewater influence on seasonal variations of bioaerosol concentrations is a limit in this research. Although a high temperature was observed in summer, a high relative humidity was also observed due to the increased amount of rainfall at interval periods

of the sampling campaign (Supplementary material Tables 1, 2, and 3). The instantaneous bioaerosol emission is sensitive to short-term fluctuations of aeration rate and wastewater feed of the aerator tank in summer (Jahne et al., 2016). In addition, the infection risks of bioaerosols in spring were consistently lower than those in winter (Fig. 2a, c, d, f, Fig. 3a, c, d, and f). The exception was the infection risks of *Enterococcus* bioaerosol in spring, which were higher than those in winter (Fig. 2g, i, Fig. 3g, and i), although the risks still had the same order of magnitude. In a word, the seasonal variations of weather conditions have a significant influence on bioaerosol-related infection risks. However, this influence is rarely reported, and the changes in these infection risks with regard to seasonal variations is unpredictable without analyses given that its variability depends on the worksites and time (Anne et al., 2005; Han et al., 2019).

For calculation by the beta-Poisson dose-response model, the infection risks of all the exposure populations without a mask generally exceeded the U.S. EPA benchmark ( $\leq 10^{-4}$  pppy) (Fig. 2). The exception was that the infection risk of academic visitors and field engineers exposed to fecal coliform bioaerosol in spring and summer satisfied the

benchmark in the best-case condition (5th percentile quantiles) (Fig. 2d and e). In the circumstances of wearing mask, all the infection risks were below the benchmark or at least at the same order of magnitude. However, in the worst-case condition (95th percentile quantiles), the infection risk of the office staffs, who wore a mask, exposures to *E. coli* and *Enterococcus* bioaerosols still exceed the benchmark by an order of magnitude in spring and summer, respectively (Fig. 2a and h). The infection risks of academic visitors in the best-case condition (Fig. 2d), field engineers in the worst-case condition (Fig. 2e), field engineers in the worst-case condition (Fig. 2f and g), and academic visitors in the best-case condition (Fig. 2h) all roughly agreed with the benchmark in same order when they wore a mask. Therefore, the infection risks were still largely in worst-case condition despite the wearing of mask. On the other hand, exponential dose-response model adopted for calculation, the infection risks generally satisfied the benchmark under all exposure scenarios (Fig. 3). The exception was that the infection risk of office staffs exposed to *Enterococcus* bioaerosol in summer (Fig. 3h) exceeded the benchmark in spite of these values in the same order. The infection risks of field engineers (Fig. 3a, g, and i) and academic visitors in the

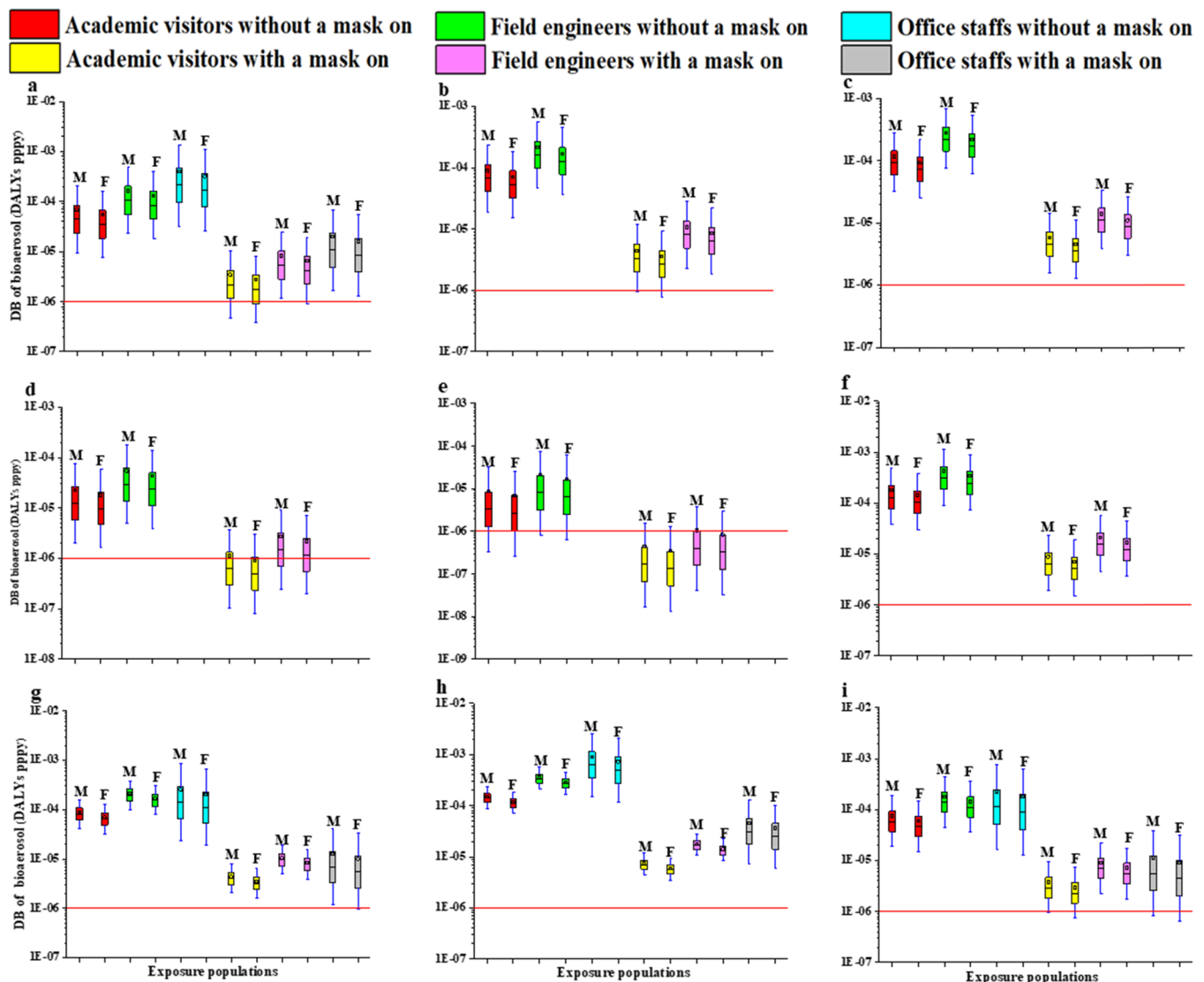


**Fig. 4.** Disease burden (DB) of various exposure scenarios based on the beta-Poisson dose-response model in three seasons: (a) DB of *Escherichia coli* bioaerosol in spring, (b) summer, and (c) winter; (d) DB of fecal coliform bioaerosol in spring, (e) summer, and (f) winter; (g) DB of *Enterococcus* bioaerosol in spring, (h) summer, and (i) in winter. The bottom and top of the box respectively represent the first and third quartiles (25th and 75th percentile values), the band inside the box denotes the second quartile (median), and the tetragon inside the box refers to the average value. The bottom and top of the whiskers respectively represent the 5th percentile values (best-case condition) and 95th percentile values (worst-case condition). M: Male F: Female.

best-case condition (Fig. 3 f) generally agreed with the benchmark in same order when they wore no mask. The infection risks calculated by beta-Poisson dose-response model were always elevated by one or two orders of magnitude compared to the calculations by exponential dose-response model under the same exposure scenario. These findings imply that the choice of the dose-response model exercises considerable influence over the results of infection risks. The two dose-response models are superior at assessing the  $P_y$  and DB for populations exposing to pathogenic bioaerosols (Haas et al., 2014; Haas, 2015; Lim et al., 2015; Xie et al., 2017). The beta-Poisson dose-response model is mathematical complexity and usually employed to describe variability in the interaction between the dose of bioaerosols and exposure populations (Teunis et al., 2000; Haas, 2015; Xie et al., 2017). While, the exponential dose-response model is the simplest and often applied to avoid adverse effects of variability on the actual numbers of bioaerosols that delivered to exposure populations (Haas, 2002, 2015; Xie et al., 2017). The so-called the most suitable or universal dose-response relationship is nonexistent due to the diversity of employed measuring methods and sampling procedures for bioaerosols on-site, and also the lack of

knowledge on specific environmental-health and exposure scenarios (Walser et al., 2015; Mbareche et al., 2019).

### 3.3. Disease burden

Figs. 4 and 5 present results of the average, medium, 5th percentile quantiles (best-case condition), and 95th percentile quantiles (worst-case condition) of DB employing the Monte Carlo simulations to run  $1 \times 10^4$  trials for various exposure scenarios in three seasons. The DBs in Figs. 4 and 5 were calculated by beta-Poisson and exponential dose-response models, respectively. The estimation results for DBs were similar with those for the infection risk (Figs. 2 and 3).

Especially, for calculation with the beta-Poisson dose-response model, all the DBs of *E. coli* and *Enterococcus* bioaerosols in the three seasons exceeded the WHO benchmark ( $\leq 10^{-6}$  DALYs pppy) in various exposure scenarios (Fig. 4a, b, c, g, h, and i). However, for the fecal coliform bioaerosol in winter, DBs exceeded the benchmark only when the exposure populations had no mask (Fig. 4 f). In general, the exposure populations not using masks had serious disease health burden but

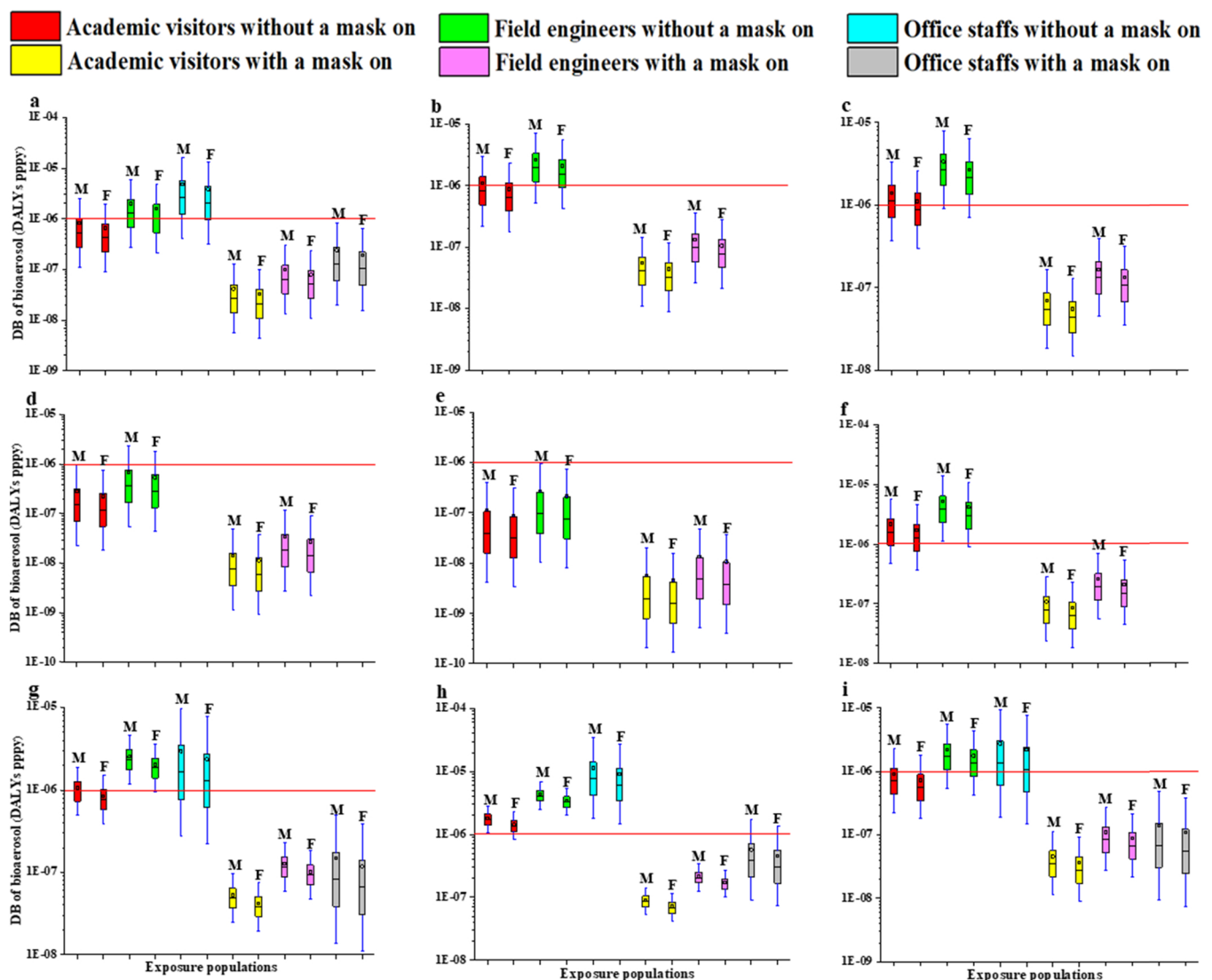


Fig. 5. Disease burden (DB) of various exposure scenarios based on the exponential dose-response model in three seasons: (a) DB of *Escherichia coli* bioaerosol in spring, (b) summer, and (c) winter; (d) DB of fecal coliform bioaerosol in spring, (e) summer, and (f) winter; (g) DB of *Enterococcus* bioaerosol in spring, (h) summer, and (i) in winter. The bottom and top of the box respectively represent the first and third quartiles (25th and 75th percentile values), the band inside the box denotes the second quartile (median), and the tetragon inside the box refers to the average value. The bottom and top of the whiskers respectively represent the 5th percentile values (best-case condition) and 95th percentile values (worst-case condition). M: Male F: Female.

wearing of mask can largely relieve the burden to satisfy the benchmark or at least achieve the same order of magnitude as (Fig. 4). An exception was observed in the worst-case condition for exposure to *E. coli* and *Enterococcus* bioaerosols in the three seasons (Fig. 4a, b, c, g, h, and i) and fecal coliform bioaerosol in winter (Fig. 4f). The DB values,  $9.23 \times 10^{-3}$ – $7.56 \times 10^{-6}$  DALYs pppy, were all over the benchmark despite the wearing of mask. However, wearing mask was still an effective measure to minimize disease health burden 1–2 orders of magnitude through reducing inhalation exposure to enteric bacteria bioaerosols. Therefore, administrations ought to provide proper PPE (e. g. N95 masks) for exposure populations to reduce airborne microbial risk in WWTPs.

For calculation by the exponential dose-response model, the DBs of field engineers (Fig. 5e, f, and g) and academic visitors in best-case condition (Fig. 5h) roughly agreed with the benchmark in same order when they had no mask. Moreover, all the DBs of *E. coli* and *Enterococcus* bioaerosol in the three seasons (Fig. 5a, b, c, f, g, and h) and fecal coliform bioaerosol in winter (Fig. 5i) roughly failed to satisfy the benchmark when wearing no mask for the exposure populations. However, their risks of infection still satisfied the U.S. EPA benchmark (Fig. 3a, b, c, f, g, h, and i). This opposite results of estimation regarding the two benchmarks indicates that whether or not satisfying a benchmark, nothing is absolute security (Haas, 2015). The WHO and U.S. EPA benchmarks ought to be employed in reference for each (Lim et al., 2015).

Meanwhile, DB, expressed in disability adjusted life years per-person-per-year (DALYs pppy), is mainly estimated by the annual infection risk and health burden (Havelaar et al., 2015; Haas et al., 2017). Annual infection risks can be converted to DALYs with the help of disease surveillance data (Lim et al., 2015). However, both of these two risk levels have limitations (Shi et al., 2018). For DB, the disease surveillance data was regionally bounded, and lack of data and knowledge of the portion of ill subjects to support its development (Lim et al., 2015; Shi et al., 2018). Moreover, considering that some exposure populations may not enroll in medical establishment due to their minimally symptomatic or even asymptomatic disease after getting infection, the disease surveillance data could not be accurate because they are derived solely from medical records (Gaunt et al., 2011; Lim et al., 2015). For annual infection risk, it assumed that all airborne pathogenic bacteria pose the same effect on exposure population (Gibney et al., 2013; Shi et al., 2018). Furthermore, the risk levels of the DB and  $P_y$  are both conservative and should be employed as complements rather than oppositions for the interpretation of QMRA results (Mara et al., 2011; Shi et al., 2018).

#### 4. Conclusion

The concentrations of three enteric bacteria bioaerosols in the aeration mode of the rotating-disc were 2.96–7.78 times as big as the microporous aeration mode. Bioaerosol particles less than  $4.7 \mu\text{m}$  (Stages 3–6) accounted for more than half of the total in most scenarios. Moreover, health risks of the three types of bioaerosols varied greatly during reversal of season indicating that temperature and relative humidity are the most important environmental factors for QMRA. The health risks estimated by the beta-Poisson dose-response model always exceeded one or two orders of magnitude comparing to the exponential dose-response model. When wearing mask, all the exposure populations' health risks were approximately 19 times lower than before when they wore no mask showing that wearing of mask is an effective means of protection in inhalation exposure to enteric bacteria bioaerosols. Generally, health risks were still unacceptable despite wearing of mask in the worst-case condition. On the contrary, in the best-case condition, the health risks satisfied (or at least were in the same order of magnitude) with the benchmarks when wearing of mask. Especially, for exposing to *E. coli* and *Enterococcus* bioaerosols in the three seasons and fecal coliform bioaerosol in winter, their DBs generally exceeded the

benchmark of WHO from the perspective of the endpoints of premature mortality and disability when they had no mask, although their  $P_y$  still satisfied the U.S. EPA benchmark from the perspective of infection endpoint. Therefore, these two benchmarks should be utilized as complements rather than oppositions for the interpretation of QMRA results. This research further enriches the knowledge base of assessment for seasonal variation of health risks in local wastewater utilities under best-case/worst-case condition.

#### CRedit authorship contribution statement

**Zi-cheng Gui:** Conceptualization, Sampling and data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Xiang Li:** Conceptualization, Sampling and data curation, Investigation. **Man-li Liu:** Conceptualization, Sampling and data curation, Investigation. **Zhang-di Peng:** Sampling and data curation, Investigation, Methodology. **Cheng Yan:** Conceptualization, Sampling and data curation, Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Zaheer Ahmad Nasir:** Supervision, Writing – review & editing. **Sonia Garcia Alcega:** Supervision, Writing – review & editing. **Frederic Coulon:** Supervision, Writing – review & editing.

#### 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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.113689.

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