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RESEARCH ARTICLE

Aerosol exposure assessment during reclaimed water utilization in China and risk evaluation in case of *Legionella*

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HIGHLIGHTS

- The Chinese population exposure habits were surveyed.
- The risks of three scenarios of reclaimed water utilization were evaluated by QMRA.
- The risks were markedly higher than the threshold (10^{-4} pppy) recommended by WHO.
- The risks were age-, educational background-, region- and gender-specific.

GRAPHIC ABSTRACT

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ABSTRACT

Reclaimed water utilization provides an effective way to alleviate water shortage. However, the residual pathogens in the recycled water like *Legionella*, could be spread into the air as aerosols through water-to-air transmission process. Inhaling the aerosols by the people nearby increases their susceptibility to diseases. For estimating the health risks associated with the potential exposure of airborne *Legionella* emitted from the urban use of reclaimed water in China, nationwide questionnaire was designed to investigate the exposure habits of Chinese population in different scenarios. Quantitative microbial risk assessment (QMRA) served as the suitable explanatory tool to estimate the risk. The results indicated that annual infection probability of populations exposed to *Legionella* for three scenarios, 0.0764 (95% CI: 0.0032–0.6880) for road cleaning, 1.0000 (95% CI: 0.1883–1.0000) for greenfield irrigation, 0.9981 (95% CI: 0.0784–1.0000) for landscape fountain, were markedly higher than the threshold recommended by WHO (10^{-4} per person per year (pppy)) according to the concentration distribution of *Legionella* in the reclaimed water. An age-, educational background-, region- and gender-specific data in annual infection probability also showed different tendencies for some subpopulations. This study provides some detailed information on the health risks from the water reuse in China and will be useful to promote the safe application of reclaimed water in water-deficient areas.

1 Introduction

Water scarcity is a worldwide challenge due to industrialization, urbanization and population growth. To alleviate the conflict between water supply and demand, wastewater is reclaimed to meet some particular applications (U. S. EPA, 2015a). Reclaimed water has been widely used in agriculture irrigation and landscaping, dust control, road surface cleaning, and municipal water supply (U. S. EPA, 2015b). However, residual pathogens and chemicals in the recycled water have been reported (Liu et al., 2018). And pathogenic microorganisms are identified as the main source of health risks for wastewater reuse (Troldborg et al., 2017). So the paramount need is to ensure the safety of all potential end users. At present, however, there is no unified international standards for reclaimed water. Current standards are based mainly on the guidelines formulated by WHO or U. S. EPA, which only focus on indicator microorganisms (Hong et al., 2020). Whereas some studies have shown that there is no strong correlation between the indicator organisms and the pathogenic organisms in

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#These authors contributed equally to this work reclaimed water (Harwood et al., 2005), which means that the current standards may not be conservative enough to protect the population from pathogen on water reuse.

The exposure routes to waterborne pathogens during reuse are ingestion, inhalation and dermal contact (de Man et al., 2014; Han et al., 2020; Lu et al., 2020; Douglas et al., 2021; Jiang et al., 2021). Many water recreational activities can lead to the ingestion of reclaimed water, such as boating, fishing and swimming (Dorevitch et al., 2011; Xie et al., 2020). Researches on water ingestion are sufficient and methodically, the volume of water and the amount of microbes can be estimated by self-report data or chemical tracer (U. S. EPA, 2019). As to dermal contact, pathogens can hardly penetrate the skin and do harm to health (Nielsen and Jiang, 2020). For inhalation, relevant studies are rare and the amount of reclaimed water inhaled was often simply estimated by hypothesis (Chhipi-Shrestha et al., 2017). In fact, common applications of reclaimed water such as road cleaning, greenfield irrigation or landscape fountain tend to produce significant amount of aerosols, and people exposed to the aerosols containing pathogens by inhalation can cause illnesses (Douwes et al., 2003; Li et al., 2011; Seidl et al., 2016). Up to now, several significant infectious diseases and epidemiological studies in association with reclaimed water aerosol exposure have been reported. In 2006, a fountain caused an outbreak of giardiasis and cryptosporidiosis in central Florida and finally 40-nine cases were diagnosed (Eisenstein et al., 2008). In 2015, a road cleaning worker was diagnosed as Legionnaires' disease and then *Legionella* pneumophila was detected in the tank of the road cleaning truck (Valero et al., 2017). A study in the USA showed that irrigation workers exposed to the reclaimed water aerosols were about twice as likely to be infected by pathogens as office workers (Camann et al., 1988). Therefore, it is time to pay attention to the risks caused by the reclaimed water aerosols.

Among the waterborne pathogens in the reclaimed water, *Legionella* is one of the most significant opportunistic pathogens, which can be found naturally in ambient water such as rivers, lakes and decorative fountains, with the concentrations ranging from 100 to 200000 CFU/L (Armstrong, 2005; Sales-Ortells et al., 2015). Inhalation of this pathogen is responsible for a serious disease known as legionellosis, which has a case fatality rate ranging from 10% to 50% (Benin et al., 2002). People over the age of 50 are the most vulnerable group, and men are more susceptible than women (ECDC, 2017). Warm water temperature (20°C–45°C), poor water quality and stagnation of water flow are conducive to the growth and propagation of *Legionella* (WHO, 2011). The transmission of *Legionella* is always related to aerosol-generating, and most reported legionellosis cases were associated with whirlpool spa, hot springs, cooling towers, air conditioning system (U. S. EPA, 1999). Researches on *Legionella* during water reuse are rare (Caicedo et al., 2019). The reclaimed water for urban use sometimes has relatively poor quality and usually has a period of storage before use, which could promote the growth of *Legionella* (Sales-Ortells et al., 2015). However, data for modeling the aerosolization of *Legionella* is also very scarce (Hines et al., 2014).

For these reasons, *Legionella* was chosen as the reference pathogen in this study, the objective of which was to evaluate the health risks of *Legionella* through inhalation under three exposure scenarios by QMRA according to typical reclaimed water utilizations: 1) people walk on the sidewalk where road cleaning is taking place; 2) people walk along a greenfield in a park for recreational purpose where irrigation system is on operation; 3) people pass by the ornamental landscape fountain. These scenarios are referred to as road cleaning (RC), greenfield irrigation (GI), landscape fountain (LF), respectively.

2 Materials and methods

2.1 Statistical analysis and simulation tool

Due to a series of assumptions of parameters, models and scenarios (Haas et al., 2014), certain uncertainties and variability exist in the whole process of risk assessment, including natural phenomena such as geography, hydrology, sunshine, temperature, wind direction and wind speed, which will lead to changes in frequency and water-to-air partitioning coefficient of microorganisms, human activities due to the differences in exposure habits and respiration minute volumes of different groups, and subjective factors such as the lack of cognition and understanding of the complex environment system, the unclear assumptions of the model, and the subjective intention of model parameters acquisition and other human factors. Appropriate measures can be taken to reduce the uncertainties. Monte-Carlo simulation minimized the uncertainties caused by objective conditions. As much data as possible were collected to reflect the true distribution of exposure parameters. And for the parameters with insufficient data, fuzzy set theory was used for the simulating calculation, which helps to reduce uncertainties (Zadeh, 1965; Guyonnet et al., 1999; Shakhawat et al., 2006). The fuzzy set theory is generally represented by triangular distributions (TFNs). TFNs can be defined as a three-element array (a, b, c), where the parameters a, b, and c represent the lower 95% value, upper 95% value and the mode of the original distribution. When the samples are insufficient, the mode can be replaced by median.

Fitting distributions and Monte Carlo simulations were performed with MatLab 2018a and Excel 2016. Differences between different groups were assessed using *t*-test or one-way ANOVA, and were considered to be highly significant at $p < 0.01$.

2.2 Quantitative microbial risk assessment

QMRA was undertaken to assess the microbial risk associated with the inhalation of contaminated bioaerosols and articulated in four steps: hazard identification, dose–response assessment, exposure assessment and risk characterization.

As indicated in the introduction above, the hazard was considered to be an infection following the inhalation of *Legionella* bacteria that are present in bioaerosols in different scenario settings. The dose–response model of *Legionella* was an exponential model (Haas et al., 1999; Hamilton et al., 2019), in which the probability of infection per event (P_d) is given as a function of the inhalation exposure dose (d) by Eq. (1):

$$P_d = 1 - \exp(-rd). \tag{1}$$

For *Legionella*, the parameter r of the model is 0.0599 referring to a previous work (QMRAwiki).

2.2.1 Exposure assessment

Exposure assessment was integrated from three separated information: 1) reclaimed water intake volume (V) per event, 2) exposure frequency per year, and 3) concentration distribution of *Legionella* in the reclaimed water and receiving water in China.

Pathogens are spread into the air as aerosols through water-to-air transmission process. The formation of bioaerosols is closely related to the atomization of water, and the atomization of water leads to the increase of humidity. A simple water-to-air partitioning model was employed to calculate the air concentration of *Legionella* by measuring the relative humidity increase as the water quantity in air (see Eq. (2)).

$$C_{air} = k \cdot C_{water} \cdot \omega \cdot (\varphi - \varphi_0) + \mathrm{B}, \tag{2}$$

where C_{air} represents the concentration of a microorganism in air (CFU/m^3); k represents water-to-air partitioning coefficient (non-dimensional); ω represents saturated water vapor content in the air at a given temperature and one atmosphere (the saturated water vapor content at 30°C is 30.3 mL/m^3); C_{water} represents the concentration of the microorganism in the water (CFU/mL), the concentration of *Legionella* in the water refers to the concentration detected in the effluent of China's reclaimed water plant and receiving rivers and lakes (shown in Table S1); φ_0 and φ represent the relative humidity in the air 1.5 m above the ground before and after spraying (non-dimensional), which were actually measured at two close fields without and with water spray; B represents the background level of the microorganism in the air (CFU/m^3).

Inhalation exposure dose was obtained by combining a person's respiratory minute volume (RMV) (Table S2, from the book of Exposure factors Handbook of Chinese Population published in 2013) and the duration (t) of each exposure. The background air concentration of *Legionella* was assigned as 0 CFU/mL. And the dose (d) of *Legionella* per person per exposure can be calculated by Eq. (3):

$$d = k \cdot C_{water} \cdot \omega \cdot (\varphi - \varphi_0) \cdot t \cdot \mathrm{RMV}. \tag{3}$$

To give an intuitive exposure quantity of reclaimed water, the volume of water inhaled by breathing is represented as the volume (mL) of water which generates the water vapor inhaled. Then, the single intake volume (V) can be calculated by Eq. (4):

$$V = k \cdot \omega \cdot (\varphi - \varphi_0) \cdot t \cdot \mathrm{RMV}. \tag{4}$$

Water-to-air partitioning coefficient (k) characterizes the survival rate of microorganism when atomized and distributed from water into the air in the form of aerosol. There are many reasons for the loss of active particles. Perhaps some of the microbes are not atomized, or partial humidity is from the water sprayed on the ground and this part of the water vapor is more difficult to bring out the microbes, and the other part of the loss may be that the microbes are inactivated during atomization because of mechanical damage, surface tension, light radiation damage, etc. (Fisman et al., 2005).

Water-to-air partitioning coefficient (k) of different exposure scenarios may be different, and the k values need to be determined according to specific environmental conditions. From the Eq. (3), water-to-air partitioning coefficient (k) can be calculated by Eq. (5).

$$k = \frac{C_{\mathrm{air}} - \mathrm{B}}{\omega \cdot C_{\mathrm{water}} \cdot (\varphi - \varphi_0)}. \tag{5}$$

Therefore, k can be derived by measuring the concentrations of specific microorganism in the water (Table 1 and Fig. S1) and air and the increase of relative humidity. The total heterotrophic bacteria was used as a substitute of *Legionella* for the measurement of k value, since the test with pathogenic *Legionella* is dangerous and the majority of heterotrophic bacteria in the water is gram negative, so is *Legionella*. Three tests of greenfield irrigation and three tests of fountain were performed in order to get the water-to-air partitioning coefficient k. Taking greenfield irrigation as an example, the background humidity and background air microbial concentration were first measured at a sufficiently distant place (about 100 m), and then the wind speed (testo 425, Germany), humidity (testo 610, Germany), temperature (testo 610, Germany) and air bacterial concentration (SAS ISO 100 Petri, Italy) were measured at random points above the pavement (2–3 m wide, 1.5 m high) of greenfield being irrigated. The measuring data required to calculate k values have been provided in supplementary information.

Table 1 Demographic feature of the questionnaire participants (n (%))

Demographic	Category	RC n = 3181	GI n = 3281	LF n = 4793
Sex	Male	1513	1548	2102
	Female	1668	1733	2691
Age (years)	0–18	506	506	71
	19–44	2445	2513	4205
	45–59	209	220	477
	60–79	14	30	38
	⩾ 80	7	12	2
Education	Middle school	263	278	109
	High school	467	485	424
	College	2194	2241	3905
	Postgraduate	257	277	355
Region	North	338	438	707
	South-east	628	628	878
	Southern	393	393	922
	Western	287	287	499

Notes: RC denotes road cleaning, GI denotes greenfield irrigation in the park during recreational activities, LF denotes landscape fountain. North Regions include Beijing, Tianjin, and Hebei, South-east Regions include Shanghai, Zhejiang, and Jiangsu, Southern Regions include Guangdong, Fujian, and Guangxi, Western Regions include Shanxi, Sichuan, and Chongqing.

2.2.2 Risk characterization

Risk characterization is generally presented as a result of annual probability (P_y), which considers the exposure frequency (f, number of exposures per year) for each subpopulation by Eq. (6). And the exposure frequencies were derived from questionnaire.

$$P_y = 1-(1-P_d)^f. \tag{6}$$

2.3 Questionnaire

A survey of Chinese people was conducted to gain a better understanding of exposure habits of Chinese in different scenarios of recycled water utilization. The survey was conducted between March 21 and November 15, 2020, with 7974 qualified responses from almost all of the provinces and municipalities of China (no data from Macao and Taiwan), 18 years old or older. Questionnaires were distributed and collected through a combination of online and offline methods to obtain the exposure duration and frequency of the population in China. One hundred copies of offline questionnaires were used as an aid to obtain the exposure habits of non-internet users, mainly the elders, while online questionnaires were collected through the online questionnaire tool Wenjuanxing. The demographic feature of the questionnaire participants was shown in Table 1. Questionnaires for samples over 60 years old were difficult to obtain through online survey, so samples over 60 years old were combined into samples 45–59 years old, referred as group over 45 years old. Then there were three categories of age for subsequent risk assessment: “0–18”, “19–44”, “⩾ 45”.

2.4 Data analysis

2.4.1 Exposure duration

2.4.1.1 Exposure duration of road cleaning

Road cleaning is to spurt water on the surface of road through a sprinkler to reduce dust, increase humidity, and cool the street area. The maximum speed of the sprinkler during road cleaning is limited within 20 km/h in Beijing and usually faster than walking speed. So the speed of the sprinkler was assumed as 6–20 km/h. The walking speed of pedestrians is about 4–5 km/h. Further, the sprinkler and the pedestrian on the sidewalk may move in the same or opposite direction, which affects the relative speed and the exposure duration.

When the sprinkler and pedestrians move in the same direction, there is a minimum relative speed (Eq. (7)):

$$v_{\min} = 6-5 = 1(\mathrm{km/h}), \tag{7}$$

when the sprinkler and pedestrians are moving toward each other, there is a maximum relative speed (Eq. (8)):

$$v_{\max} = 20+5 = 25(\mathrm{km/h}). \tag{8}$$

The most common situation is that the sprinkler and the pedestrian move in the same direction. The speed of the pedestrian is 5 km/h, and the mode speed of the sprinkler is assumed as 8 km/h. At this hypothesized scenario, the relative speed is regarded as the mode relative speed (Eq. (9)):

$$v_{\mathrm{mode}} = 8-5 = 3(\mathrm{km/h}). \tag{9}$$

The aerosol radiation range produced by sprinkler was based on the measurement of relative humidity increment (Supporting Information) above the pavement, 10 m before and after the splash point. There was no significant humidity change beyond this distance. Therefore, dividing the aerosol radiation range 20 m by the relative speed can get the exposure duration. The exposure duration of the sprinkler and pedestrian is considered to conform to a triangular distribution with a minimum value of 0.8 s, a maximum value of 20 s, and a mode of 6.7 s.

2.4.1.2 Exposure duration of greenfield irrigation

The exposure duration of greenfield irrigation was obtained by the questionnaire survey. Participants were asked how long it usually took to enjoy their leisure time or do exercise in a park that was being irrigating every time. There was no significant difference for exposure duration between different gender groups, but exposure duration for different age groups have significant difference ($p < 0.01$) by one-way ANOVA analysis. The exposure duration of the age "19–44" group was slightly longer than that of other groups (Table 2). The median of exposure duration was acquired from the data set after 10000 times Monte Carlo simulation.

2.4.1.3 Exposure duration of landscape fountain

Exposure duration of landscape fountain was obtained through a questionnaire survey. Respondents were asked how much time they spent near landscape fountain each time. The location of the fountain was not specified, so it could be anywhere. There is no significant difference for exposure duration between different gender groups, and exposure duration for different age groups has slightly significant difference ($p < 0.05$) by one-way ANOVA analysis (Table 3). The median of exposure duration was acquired from the data set after 10000 times Monte Carlo simulation.

2.4.2 Exposure frequency

As shown in Table 4, age has a much greater influence on exposure frequency than gender. For road cleaning, exposure frequency decreased with the increase of age. The group under 18 years old was the most exposed. One possible reason could be that the time they went to school roughly coincided with the time the roads were cleaning. For greenfield irrigation, the result was just opposite of the road cleaning. Group over 45 years old was the most exposed, which might have something to do with the high park visiting frequency of the older people. For landscape fountain, group under 18 years old was more exposed than the other two groups, which might be due to that they are more easily attracted by the fountains.

3 Results

3.1 Exposure parameters

Monte Carlo simulations were used to calculate exposure doses and risks of *Legionella*, with 10000 values randomly sampled from each distribution input. The dose and risk distributions represent the variability within the data of each model parameter in Table 5. To make the calculation as closely as possible to the real situation, all values were based on the real distribution, except for some parameters (the concentrations of *Legionella* in the water and the time for RC) which were simulated by the triangular distribution due to the small scale of data collection. Fitting distributions and Monte Carlo simulations were performed with MatLab 2018a and Excel 2016.

More details for the calculation process and results of k, ω, C_{water} and RMV are shown in supplementary information.

3.2 Risk characterization

After Monte Carlo simulation, the median of single intake volume for road cleaning was 0.0005 mL. For greenfield irrigation and landscape fountain, the median of single intake volume were 0.2312 mL and 0.0762 mL. The exposure dose of three scenarios was calculated with Eq. (4). For road cleaning, the median of single exposure dose of people exposed to *Legionella* was 0.0718 CFU,

Table 2 Exposure duration distribution of greenfield irrigation for different groups

Demographic	Category	Exposure duration distribution (%)					Median (min)
		< 10 min	10–30 min	30–60 min	1–2 h	> 2 h	
General	Normal	34	42	20	2	1	18
Sex	Male	36	37	23	3	1	18
	Female	32	47	18	2	2	18
Age (years)	0–18	42	32	19	3	3	15
	19–44	32	44	20	2	1	19
	⩾ 45	44	33	19	4	0	14
Education	Middle school	44	16	13	2	5	18
	High school	47	33	15	4	1	12
	College	38	47	12	2	1	16
	Postgraduate	42	41	12	3	2	14
Region	North	34	42	20	2	2	18
	South-east	44	43	11	2	0	13
	Southern	43	43	11	2	1	14
	Western	45	39	13	2	1	13

Table 3 Exposure duration distribution for different groups of LF

Demographic	Category	Exposure duration distribution (%)						Median (s)
		< 30 s	30–60 s	1–5 min	5–15 min	15–30 min	30–60 min	
General	Normal	7	16	37	26	12	2	236
Sex	Male	6	16	40	26	11	1	228
	Female	7	16	35	26	13	2	246
Age (years)	0–18	7	7	21	35	24	6	558
	19–44	6	16	38	27	11	2	237
	⩾ 45	7	15	41	17	20	0	224
Education	Middle school	12	18	33	26	8	3	206
	High school	7	17	38	24	12	2	225
	College	7	17	36	27	11	2	234
	Postgraduate	7	17	36	26	11	3	234
Region	North	7	16	37	26	12	2	236
	South-east	8	17	37	26	10	2	229
	Southern	8	17	46	26	11	2	191
	Western	7	16	40	26	10	1	222

Table 4 Exposure frequency distribution of three scenarios (/year)

Demographic	Category	RC n = 338	GI n = 438	LF n = 707
General	Normal	21 (1–168)	14 (1–72)	36 (1–182)
Sex	Male	21 (2–182)	14 (1–95)	36 (1–182)
	Female	14 (1–152)	13 (1–104)	36 (1–182)
Age (years)	0–18	30 (2–212)	7 (1–103)	36 (1–182)
	19–44	21 (1–151)	14 (1–94)	36 (1–182)
	⩾ 45	21 (1–151)	11 (1–146)	12 (1–182)
Education	Middle school	29 (2–212)	8 (1–109)	36 (1–182)
	High school	28 (2–212)	9 (1–93)	36 (1–182)
	College	21 (1–151)	15 (1–86)	36 (1–182)
	Postgraduate	14 (2–135)	14 (1–102)	36 (1–182)
Region	North	21 (1–182)	17 (1–125)	36 (1–182)
	South-east	14 (1–121)	10 (1–73)	36 (1–182)
	Southern	21 (1–121)	13 (1–82)	36 (1–182)
	Western	21 (2–151)	10 (1–85)	36 (2–182)

Notes: Values outside the brackets are the median, and those inside the brackets are the upper and lower 95% confidence interval values.

that is, people exposed to reclaimed water from road cleaning were likely to inhale an average of one *Legionella* every 14 times. For greenfield irrigation and landscape fountain, the medians of single exposure dose were 15.9593 and 4.3591 CFU. The median of single infection probability of *Legionella* in the road cleaning scenario is 0.0043, that is, when exposed to the reclaimed water used for road cleaning, an average of 43 out of 10000 people will get sick from the inhalation of *Legionella* aerosol. For greenfield irrigation and landscape fountain, the medians of single probability were 0.6156 and 0.2298. The annual probability was calculated with Eqs. (1) and (6). From the perspective of annual probability, the risk of road cleaning was the least, followed by landscape fountain, and the risk of greenfield irrigation in the park was the highest. All the calculation results were shown in Table 6.

3.3 Risk comparison

People of different genders, age groups, educational backgrounds, and regions have different exposure habits, such as exposure duration and exposure frequency, the exposure risks of which were calculated separately according to their actual exposure situations.

As shown in Fig. 1A, when reclaimed water is used for road cleaning, the annual infection risk of women is significantly lower compared to men ($p < 0.01$). It's related to the higher exposure frequency of males than females (Table 4). In the other exposure scenarios, females also show a lower risk than males, but not significant ($p > 0.01$).

When reclaimed water is used for road cleaning and landscape fountains, the annual risk of people under 18 years old have the highest risk (Fig. 1B); as for greenfield irrigation, people aged 19–44 have the highest risk. The various entertainment of different age groups may explain the difference in exposure risk.

When reclaimed water is used for road cleaning and greenfield irrigation, educational background is a significant factor affecting the exposure risk (Fig. 1C). When reclaimed water is used for road cleaning, the higher education level leads to the lower risk; for greenfield irrigation, people with a high school or equivalent degree

Table 5 Exposure parameters summarized for calculation

Parameters	Description	Distribution: Values			Unit
		RC	GI	LF	
C_{water}	concentration of *Legionella* in water	TFNs: (4.52,13.54,172.85)			GC/mL
ω	saturated water vapor	Constant value:30.3			mL/m^3
RMV	Respirator minute volumes	Constant value			L/min
$\varphi - \varphi_0$	relative humidity increments	Random values from Fig. S2	Random values from Fig. S3	Random values from Fig. S3	%
t	time	TFNs: (0.8,6.7,20)	Random values from Table 4	Random values from Table 4	s
r	exponential model Parameter	Constant value: 0.0599			–
k	water-to-air partitioning coefficient	Constant value (Fig. S4)			–
f	Exposure frequency	Random values from Table 4			/year

Table 6 Annual infection probability of groups exposed to *Legionella* for three scenarios

Category	RC	GI	LF
Single intake volume (mL)	0.0005 (0.0002–0.0017)	0.2312 (0.0132–2.5109)	0.0762 (0.0036–0.7963)
Single exposure dose (CFU/GC)	0.0718 (0.0101–0.3492)	15.9593 (0.5659–232.6384)	4.3591 (0.1492–69.7941)
Single probability	0.0043 (0.0006–0.0207)	0.6156 (0.0333–1.0000)	0.2298 (0.0089–0.9847)
Annual probability	0.0764 (0.0032–0.6880)	1.0000 (0.1883–1.0000)	0.9981 (0.0784–1.0000)

Notes: Values outside the brackets are the median, and those inside the brackets are the upper and lower 95% confidence interval value.

are at the greatest risk.

The risks also varied in different regions of China (Fig. 1D). When reclaimed water is used for road cleaning, the exposure risk of people living in south-east region of China is significantly lower than that of people in other areas ($p < 0.01$); when reclaimed water is used for greenfield irrigation, there was no significant difference, people in Guangdong, Fujian, and Guangxi of China took a higher exposure risk than the others ($p > 0.01$). When reclaimed water is used for landscape fountains, people in Shanxi, Sichuan, and Chongqing of China took a higher risk than the others, but not significant ($p > 0.01$).

4 Discussion

In this study, we comprehensively studied the Chinese people's physical characteristics, exposure habits, and the concentrations of *Legionella* in the reclaimed water in China, and calculated the risks under different utilization scenarios. The calculated annual risks (Table 6) are far greater than the acceptable level recommended by WHO (10^{-4} pppy). On the one hand, the results indicate that the health risks should be paid more attention to when reclaimed water is used in these three scenarios. On the other hand, many factors could affect the results of risk assessment.

The most important factor identified in the risk analysis is the concentrations of *Legionella* in the reclaimed water. There are a few methods currently used for *Legionella* monitoring. The main ones include culture and qPCR. There is no consensus on which method is more appropriate. Culture-based methods are considered as the "gold standard" and detect viable *Legionella*, but may underestimate viable but non-culturable (VBNC) cells (Bogosian and Bourneuf, 2001). Quantitative PCR detects *Legionella* more frequently than culture-based methods (Whiley and Taylor, 2016). However, living bacteria are not distinguished from non-viable cell or free DNA (Collins et al., 2015), which might result in an over-estimated result (Hong et al., 2020). In our study, to make a conservative assessment and collect enough amount of data due to the scarcity of environmental pathogen studies in China, the concentrations of *Legionella* were obtained from both culture-based and qPCR methods. And the data were collected from both the reclaimed water plants and the receiving water bodies, because in China, greenfield irrigation sometimes use water from rivers and lakes. The nutrient-rich reclaimed water could support the growth of more bacteria in the receiving water bodies. Concentrations of *Legionella* in effluent were usually only 1/10 of that of the receiving rivers and lakes (Fig. S1). Therefore, the concentrations of *Legionella* used in this study for risk calculation are widely distributed and have a high uncertainty. Nevertheless, this study points out the potential high risk of wastewater reuse. The risk assessment will be more accurate with more pathogen monitoring data in the future.

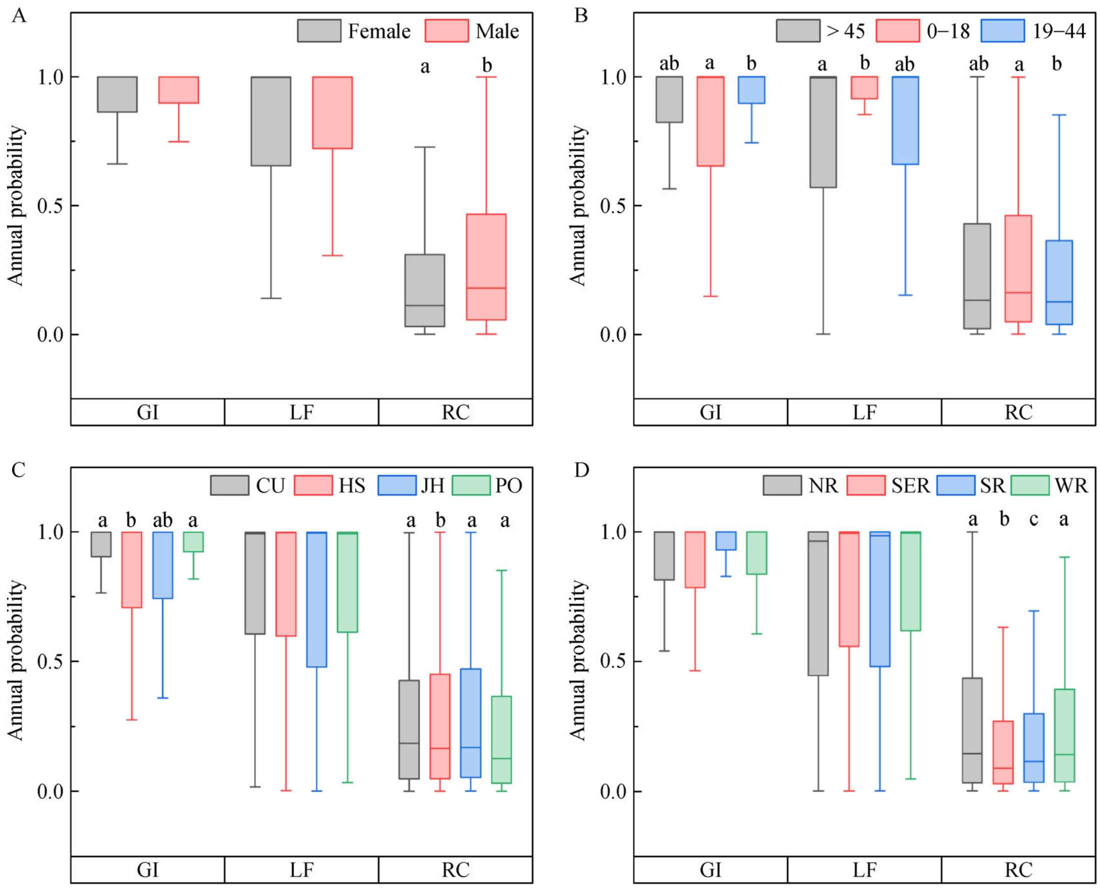

Fig. 1 The risk comparison of different subpopulations (gender (A), age (B), education (C) and region (D)) in the three exposure scenarios, road cleaning (RC), greenfield irrigation (GI) and landscape fountains (LF). NR denotes north region, SER denotes south-east region, SR denotes southern region, WR denotes western region, CU denotes college undergraduate, HS denotes high school, JH denotes middle school, PO denotes Postgraduate. Sharing different letter shows the difference is significant with $p < 0.01$.

Besides the *Legionella* concentration data, the dose–response model is also a main source of uncertainty. The dose-response model for *Legionella* is based on animal model data and guinea-pig is the best extrapolated model for human risk assessment among existing animal inhalation exposure models (Gordon and Read, 2002; Kahn et al., 2002). Researchers exposed guinea-pigs with aerosols containing *Legionella* pneumophila, estimated the doses deposited in lungs, and measured adverse responses in detail. First, there are many different species of *Legionella* with different virulence. The species used for modeling was a known virulent one, while what were detected in the water environment may not be that harmful. This increases the final risk assessment. Second, current dose–response models of *Legionella* include Exponential, Weibull, Approximate β-Poisson, and Logistic. Muller et al. calculated the goodness-of-fit between the various models and found that the exponential model fitted best with a goodness-of-fit of 0.58 (Muller et al., 1983). Therefore, the error from model selection could be significantly large. Furthermore, interspecies differences and dose scaling also lead to errors in the application of the guinea-pig model for human risk. Therefore, all the factors in dose–response modeling may affect the final calculated risks significantly.

The threshold risk value is the acceptable level related to water-borne diseases determined by national policy-makers. WHO set the threshold risk value to 10^{-4} per person per year for water recreation. However, in the short-term, such strict acceptable risk levels might not be achievable or realistic. U.S. EPA had adjusted the threshold risk value to 0.036 for primary contact recreation in fresh recreational waters, when the Recreational Water Quality Criteria was approved (U. S. EPA, 2012). This modification was based on the survey of water-irrelevant infection rate and it has been gradually accepted by academia (Vergara et al., 2016). In fact, according to the US Centers for Disease Control and Prevention, 1 in 44 people in the USA from water-borne pathogens each year. Not anyone infected with *Legionella* will seek medical attention, so the actual number of patients is definitely greater than the record. Therefore, the acceptable risk level could be higher for *Legionella*.

Legionella is spread into the air as aerosol through water-to-air transmission process. As so far no published study was identified with the experimental data of water-to-air partitioning coefficient for *Legionella* species possibly due to its virulence, alternative estimating approaches were developed and applied. The water-to-air partitioning model is a significant contributor to exposure assessments. The ratio of the *Legionella* concentration in the air to its concentration in the water is widely used to estimate the air concentration by modeling exposure pathway-specific water concentrations (Armstrong and Haas, 2007; Hines et al., 2014), but the ratio may vary when bacteria is aerosolized under different conditions, including various devices, sites and bacteria species (Thomas et al., 2011; Hamilton et al., 2019). The cell physical surface characteristics may affect atomization of different bacteria, like different cell wall structures between Gram-negative and Gram-positive bacteria (Jung et al., 2002). Previous studies demonstrated that Gram-negative bacteria like *E. coli* is extremely sensitive to aerosolization mostly due to sublethal injuries of cell wall (Thomas et al., 2011). Therefore, some physical-chemical-biological determinants may inactivate *Legionella* during the atomization process, such as mechanical damage, surface tension, and ultraviolet radiation exposure (Fisman et al., 2005). However, some models do not consider the loss of bacteria during the atomization process (de Man et al., 2014; Sales-Ortells et al., 2015). In this work, total heterotrophic bacteria was used as a substitute for *Legionella* when measuring the water-to-air partitioning coefficient, since the majority of bacteria in the reclaimed water is gram negative as *Legionella*, and heterotrophic bacteria are easily tested without the risk of serious infection. The measurement was also performed on sites. But the difference of this study from Hines' is that the measurement of bacterial air concentration was accompanied with the measurement of air humidity increment. With the humidity data, the water content in the air can be calculated and the air concentration of bacteria can be transferred to the bacteria concentration in the water getting into the air. Therefore, the water-to-air coefficient "*k*" in this study means survival ratio from water to air transmission, while Hines' coefficient does not have a physical meaning. To calculate the water-to-air partitioning coefficient in the Hines' way, the ratio (7.5×10^{-4} L/m^3) for greenfield irrigation (the relative humidity increment was about 5%) in the study is slightly higher than the exposure pathway of shower (3.4×10^{-4} L/m^3) and faucet (5.6×10^{-4} L/m^3) (Hines et al., 2014).

The risk assessment results indicated that actions need to be taken to reduce the exposure and potential transmission of *Legionella* during water reuse. Reclaimed water can be disinfected in storage tank before used for road cleaning and landscape fountains. Reducing the retention time of water in tank could also effectively mitigate the growth of *Legionella*. A warning sign should be set up in a prominent place in the park, when using reclaimed water, considering that the high risks of certain subgroups are mainly due to the coincidence of their recreational activities with the time of reclaimed water utilization.

5 Conclusions

The risk assessment renders a high risk of *Legionella* infection for people who are potentially exposed to the reclaimed water aerosols in China. The risk of infection exceed the recommended threshold by WHO (10^{-4} pppy) for reclaimed water utilization. Reclaimed water used for greenfield irrigation is estimated to pose the greatest risk, followed by landscape fountain, and then road cleaning. The probabilities of annual disease burden are age, educational background, region and gender dependent. Therefore, practical and appropriate methods should be applied to reduce the reclaimed water related infection risk for people in urban area.

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Declaration of Competing Interest There are no conflicts to declare.

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